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Certificate

It is certified that

1) The thesis entitled "**Effect Of Pollutants And Water Salinity On The Productivity And Seed Biochemistry In *Glycine max*"** submitted by **Deepti Shandilya** is the best of my knowledge and belief, original and the result of my own Investigations, except as acknowledged, and has not been submitted, either In part or whole, for a degree at this or any other University.

2) Formulations and ideas taken from other sources are cited as such. This work has not been published.

3) Work evinces the capacity of the candidate for critical examination and Independent judgement.

4) Candidate has put in at last 200 days of attendance.

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Introduction

The **soybean** (US) or **soya bean** (UK) (*Glycine max*) is a species of legume native to East Asia. The plant is classified as an oilseed rather than a pulse . Soybean is known as the "Golden Bean" of the 20^{th} century. It is also popularly called as miracle bean. Soybean is classified as moderately tolerant to salinity, with a threshold of 0.5 S m^{-1}, beyond which growth is markedly reduced **(Maas and Hoffman, 1977)**.Soy varies in growth and habit. Soybeans containing very high levels of protein can undergo desiccation, yet survive and revive after water absorption.Together, soybean oil and protein content account for about 60% of dry soybeans by weight . The remainder consists of 35% carbohydrate and about 5% ash.The oligosaccharides raffinose and stachyose protect the viability of the soybean seed from desiccation. Soybean is considered to be one of the most drought sensitive crop, with approximately 40% reduction of the yield in the worst years.Recent advances made in high-throughput DNA sequencing technologies, emerging omics, such as transcriptome, proteome, interactome and epigenome have been applied to soybean research. Cultivation is successful in climates with hot summers with optimum growing conditions in mean temperatures of 20^0 to 30°C (68 to 86 °F); temperatures of below 20 °C and over 40 °C (68 °F, 104 °F) stunt growth significantly. Soybeans perform nitrogen fixation by establishing a symbiotic relationship with the bacterium *Bradyrhizobium japonicum* and is a model plant for photoperiodism.

It is estimated that about 20% of irrigated land, which yields one-third of the world's food is affected by salinity. Moreover, a significant proportion of recently cultivated agricultural land has become saline because of irrigation (**Munns, 2005**).

Salinity is one of the major constraints limiting plant growth (**Boyer, 1982).** There is about 8.6 mha salt affected soil in the country. Soil salinity is a highly soil condition varying in time and space depending on factors like rainfall and irrigation. Salt stress affects many physiological aspects of plant growth, shoot growth and dry matter.

Medhat (2002) reported that salinity stress induce changes in the ion content of plant's cells, which induce changes in the activity of certain metabolic systems that might have serious consequences for protein.

Specific expression of stress proteins is an important adaptive manifestation in maintaining the integrity, native configuration and topology of cellular membranes components to ensure their normal functioning under salinity stress **(Wahid *et al*., 2007).**

Germination begins with the water uptake of dry seed and ends with the emergence of the radicle. It can be divided into three phases based on the style of water uptake. Phase I is a rapid water uptake phase, in which DNA damage repairing and resuming of glycolytic and oxidative pentose phosphate pathways occur. Phase II is a plateau phase, in which mitochondrial synthesis and translation of storage mRNA occurrs. Phase II is also regarded as a metabolism active phase during which reserves mobilization is initiated. Phase III is the post-germination stage in which the radicle begins to grow **(Muller *et al*., 2006).**

Mobilization of reserves is one of the most critical events in germination, which could provide not only precursors but also energy for the biosynthetic processes. Mobilization of the reserves is crucial for germination efficiency and post-germinative seedling establishment. For cell w**a**ll exp**a**nsion to occur, there must be **a**dequ**a**te turgor inside the cell ,extensibility through re**a**rr**a**ngement or loosening **of** the existing cell w**a**ll synthesis **a**nd deposition **of** newly formed w**a**ll components **(Cosgrove, 1997).** Dry seeds require a period after ripening for the capacity of dormancy to be lost (**Koornneef *et al*., 2002)**. Seed germination is a complex physiological process during which mobilization of nutrient reserves happens, which might be mediated by different regulatory and metabolic pathways. Proteome profiling has been used to construct these pathways. Growth in length and fresh weight of the hypocotyls and roots are completely abolished at higher saline treatment.The deleterious effects of NaCl are mainly due to up- or down-regulation of genes and their corresponding proteins.Generally, Na content is increased under salt stress, but K content does not decrease. Sodium toxicity probably leads to damaging effects.

Plant seeds, especially crop seeds, accumulate abundant reserves such as carbohydrates, oils and proteins during the maturation. These reserves are also important for seed germination and

seedling establishment. Salinity is the major environmental constraints to crop productivity through out the arid and semi arid regions of the world. Between 30 and 40% of the world irrigated agricultural lands are prone to salinity (**Foollad & Yin;1997**). The deleterious effects of salinity on plant growth are associated with low osmotic potential of soil solution (water stress), nutritional imbalance, specific ion effect (salt stress) and a combination of these factors.

Salinity effects may vary with the growth stage ,extent and time of salinity stress. Biological or economic yield reduction is the main effect of salinity at the whole-plant level and is usually attributed to various physiological and biochemical processes at the cellular or molecular levels (**Meloni *et al.*, 2003 ; Nawaz *et al.*, 2010 ; Munns and Tester, 2008**). Germination and young seedling stages are more sensitive to salinity stress than other stages (**Ahmad *et al*;2002**).The cots enlarge, three-fold their original size during germination, getting surfaced by day 3 or day 4.The environmental factors could affect the germination through regulation of biosynthesis and catabolism of phytohormones, such as GA and ABA.

Flavonoid accumulation at the seedling developmental stage provides several important functions, including protection from reactive oxygen species, UV irradiation, pathogens and modulation of auxin transport. **(Murphy *et al*;2000; Brown *et al*;2001**) Several key auxin transporters and MDR/PCP proteins are highly up-regulated before germination (**Ogawa, *et al*. 2003**).High salt imposes negative impacts on growth, nodulation, agronomy traits, seed quality and quantity, thus reduces the yield of soybean. To cope with salt stress, soybean has developed several tolerance mechanisms, including: (i) maintenance of ion homeostasis; (ii) adjustment in response to osmotic stress; (iii) restoration of osmotic balance; and (iv) other metabolic and structural adaptation.

Salinity is one of the most common environmental stress factor. Salinity adversely affects plant growth and development, hindering seed germination (**Dash and Panda, 2001**), seedling growth (**Ashraf *et al.*, 2002**), enzyme activity (**Seckin *et al.*, 2009**), DNA, RNA, protein synthesis **(Anuradha and Rao, 2001)** and mitosis (**Tabur and Demir, 2010).** Plants accumulate and store proteins in protein storage vacuoles (PSVs) during seed development and maturation. Upon seed germination, these storage proteins are mobilized to provide nutrients for seedling

growth. Owing to the complexity of seed germination, -omic strategies, especially proteomic methods have been widely used in study of seed germination. Moreover, post-translational modification behaviours of protein, which could only be studied through proteomic techniques, are also shown to be important for seed germination. Soybean genome has been sequenced . Basically, combating the effects of salinity stress involves two main strategies : one is to improve salt tolerance through genetic breeding and chemical or biological treatment, the other is to avoid or alleviate salinity stress by improving at least part of the root-zone environment. The technologies for combating salinity stress is based on advances in agronomic techniques for managing salinity in the root zone. A comprehensive use of agronomic practices such as suitable cultivars, proper irrigation and fertilization, seed pretreatment, furrow seedling, plastic mulching and induction of unequal salt distribution in the root-zone to combat salinity stress. Further research should focus on exploration and understanding of new products in saline soils like new foliar and specific slow-release fertilizers and commercial plant growth regulators to improve salt tolerance.Soybean in the stress tolerance exhibiting chlorosis or necrosis on leaves in saline growing conditions (**Pantalone *et al*., 1997**). Screening based on the leaf chlorosis score and visual foliar symptoms are considered appropriate for salt-sensitive crops.

During their growth crop plants usually exposed to different environmental stresses which limits their growth and productivity. Among these, salinity is the most severe one (**Kaymakanova, 2009).** The major inhibitory effect of salinity on plant growth and development has been attributed to osmotic inhibition of water availability as well as the toxic effect of salt ions responsible for salinization. Nutritional imbalance caused by such ions leads to reduction in photosynthetic efficiency and other physiological disorders **(Hakim *et al*. 2010**). In arid and semi arid regions, limited water and hot dry climates frequently cause salinity problem that limit or prevent crop production. It has also been reported that under saline conditions, germination ability of seeds differ from one crop to another and even a significant variation is observed amongst the different varieties of the same crop **(Jamil *et al*, 2005).** Salt stress affects many physiological aspects of plant growth.

Soil salinity is recognized as the most important problem involved in crop plant establishment and growth. Transpiration and evaporation from the soil surface, low quality of irrigation water and lack of proper drainage are major causes of area land salinity leading to crop loss. Thus, screening of salt tolerant crop cultivars is of crucial importance (**Okcu *et al*;2005).**

High sensitivity to soil and water salinity is one of the biggest problems with soybean crop.Salt stress severely depresses a wide range of physiological processes such as seed germination, seedling growth and vigour, vegetative growth, flowering and fruit set enzyme activity and protein synthesis..

General symptoms of damage by salt stress are accelerated development, growth inhibition , senescence and ultimate death after prolonged exposure. Growth inhibition is the primary injury that leads to other symptoms although programmed cell death may also occur under severe salinity shock.

Most of the salt stresses in nature are due to Na^+ salts, particularly NaCl . High salinity lowers water potential and induces ionic stress and results in secondary oxidative stress. It severely limits growth and development of plants by affecting different metabolic processes such as CO_2 assimilation, oil and protein synthesis. Water deficiency is one of the most common example of salt stress (**Tabaei-Aghdaei *et al*., 2000), that** results in malfunctioning of the cellular membranes by increasing their ion leakage. Plasma membrane may be the primary site of salt injury (**Mansour; 1997).**

The toxicity problem is especially severe in arid and semi-arid areas where higher evaporation rates are expected but the salt accumulation is also found in many irrigated fields.There are two types of salt response in soybean; includer and excluder. The soybean genotype that translocates Cl to the foliage is called includer whereas excluder stores Cl in the roots.Na^+ is not readily sequestered into vacuoles as in halophytes **(Munns & Tester,2008**). Salt tolerance at germination is easy to measure and performances of many crops are studied at germination stage under saline conditions (**Essa, 2002 ,Hosseini *et al* 2002, ,Mensah *et al* 2006 , Khayatnezhad *et al* ;2010).**

In response to salinity soybean plants resort to various pro-survival strategies, most of which are preceded by specific changes in expression levels of proteins whose biological functions are

related to salt stress tolerance.In the plant life cycle, seed germination and seedling stages are key developmental stages conditioning the final yield of crops. Both are very sensitive to salt stress **(Zhu,2002**) .

Of the 1500 million ha of land farmed by dry land agriculture, 32 million ha (2%) are affected by secondary salinity to varying degrees. Of the current 230 million ha of irrigated land, 45 million ha (20%) are salt affected. Irrigated land accounts for only 15% of total cultivated land, but because irrigated land has at least twice the productivity of rain fed land, it produces one third of the world's food **[Munns & Tester,2008**].

All abiotic stresss reduce plant growth and yield. The products of stress-inducible genes which could be directly protecting against these stresses include the enzymes responsible for the synthesis of various osmoprotectants like late embryogenesis abundant (LEA) proteins, antifreeze proteins, chaperones and detoxification enzymes. Another group of gene products involved in gene expression and signal transduction pathways includes transcription factors, protein kinases and enzymes involved in phosphoinositide metabolism (**Kaur and Gupta;2005**).Plants respond to the diverse protein kinases, including calcineurin β-lime protein-interacting protein kinases (CIPKs) (**Xiang *et al*;2007)**. During response and adaptation to the stresses, many stress-related genes are induced **(Xiong and Yang, 2003**; **Jayasekaran *et al*., 2006**). Plants can initiate a number of molecular, cellular and physiological changes to respond , adapt to stresses, thus enabling them to survive (**Yamaguchi-Shinozaki and Shinozaki, 2006**). Calcium acts as a universal messanger in various signal transduction pathways, including responses to diverse array of biotic and abiotic stresses (**Kim *et al*., 2003a**) and regulation of various cellular and developmental processes. In plants many Ca^{2+} sensing protein kinases have been reported for their involvement in the stress responses. These are protein kinases and Suc non-fermentation-related kinases **(SnRKs).**

Soil salinity affects plant growth and development by way of osmotic stress, injurious effects of toxic Na^+ and Cl^- ions and ,to some extent SO_4^{2-} , Mg^{2+} and nutrient imbalance caused by excess of Na^+ and Cl^- ions. Salinity stress response is multigenic, as number of processes involved in the tolerance mechanism are affected, such as various compatible solutes/osmolytes, polyamines,

reactive oxygen species and antioxidant defence mechanism, ion transport and compartmentalization of injurious ions. Various genes/cDNAs encoding proteins involved in the above-mentioned processes have been identified and isolated. The role of genes/cDNAs encoding proteins involved in regulating other genes/proteins, signal transduction process involving hormones like ABA, JA and polyamines, strategies to improve salinity stress tolerance have also been reported. A host of genes encoding different structural and regulatory proteins have been used for the development of a range of abiotic stress-tolerant plants. Identification of molecular markers linked to salinity/drought-tolerance traits has provided plant breeders a new tool for selecting cultivars with improved drought tolerance (**Sairam and Tyagi;2004**)

Soil salinity may influence the germination of seeds either by creating an osmotic potential external to the seed preventing water uptake or the toxic effects of Na^+ and Cl^- ions on the germinating seeds (**Khajeh-Hosseini**, ***et al*;2003**). Under these stresses there is a decrease in water uptake during imbibitions and furthermore salt stress may cause excessive uptake of ions (**Murillo-Amador *et al*.,2002**) .Seed priming has been successfully demonstrated to improve germination and emergence in seeds of many crops **(Parera and Cantliffe 1994 ,Singh 1995**). Seed priming improves seed germination, seedling emergence and growth under saline conditions. It has been suggested that the cellular Na^+ to K^+ ratio rather than the absolute intracellular concentration of Na^+ determines salt tolerance in plant cells (**Horie *et al*., 2001**). The changes in cation contents are not simply due to the competitive accumulation of Na^+ and K^+. One possibility is that enhanced membrane conductance for K^+ depolarizes the membrane, thereby reducing the driving force for Na^+ influx (**Amtmann and Sanders, 1999**). Another possibility is that high K^+ concentration in the cytosol triggers Na^+ export through up-regulation of nonselective cation efflux systems or down-regulation of nonselective uptake systems (**Bihler *et al*., 1998**).

Comparison of rice subspecies and varieties differing in tolerance to salinity has shown that greater tolerance correlates with the ability to exclude Na^+ from the shoot and maintain a low Na^+/K^+ ratio (**Golldack *et al*., 2003; Lee *et al* 2003; Ren *et al*., 2005**). The salt-sensitive variety

IR29 accumulated Na^+ in leaves at 5- to 10-fold greater concentrations than the salt-tolerant lines BK or Pokkali **(Golldack *et al*., 2003**). Restoring ion homeostasis in plants disturbed by salt stress represents a crucial response. Plant responses in countering ionic stress caused by high salinity include restricting salt intake, increased extrusion, compartmentalization and controlled long-distance transport to aerial parts. Additionally, to avoid cellular damage and nutrient deficiency, plant cells need to maintain adequate K^+ nutrition and a favourable K^+/Na^+ ratio in the cytosol .

The activities of ATPases increase in cells exposed to NaCl and the expression of a number of the corresponding genes is also upregulated (**Tsiantis *et al*;1996,Yamada;1995,Dietz &Arbinger;1996, Niu *et al*;1993**).

Salinity also disturbs the levels of Ca^{2+}, which increases in the cytosol. Increased cytosolic Ca^{2+} functions as a second messenger, resulting in changes in gene expression and metabolism in salt-affected cells. Elevated Ca^{2+} levels return to their original values through active efflux out of the cell mediated by Ca^{2+} pumps (Ca^+ATPase), whose expression increases under salinity. Symbiotic nitrogen (N_2) fixation in root nodules is a major nitrogen source to build essential biomolecules. The number and quality of root nodules determine the nutritional status of the whole plant. Salt stress affects the nodulation of soybean, reduces the efficiency of nitrogen fixation, decreases the number and biomass of root nodules **(Elsheikh and Wood 1995**). Salt stress attenuates the aerobic respiration of nitrogen fixing bacteria, reduces the leghemoglobin content in root nodules and depletes the energy source required for nitrogen fixation (**Delgado *et al.* 1994**). In addition, salt stress strongly inhibits the deformation of root hair in perception of Nod factors and thus hampers the symbiosis process **(Duzan *et al*.,2004).**

High concentrations of Na^+ can replace bound Ca^{2+} in plasma membrane and cell membrane system, when the ratio of Na^+/Ca^{2+} is increased,it finally damages the membrane structural integrity and function. An increase in free cytoplasmic Ca^{2+} impaires cellular metabolism (**Hirayama, 1987**). However, plants can adapt to salinity through osmotic stress tolerance, Na^+ or Cl^- exclusion and the tolerance of tissue to accumulated Na^+ or Cl^- **(Munns and Tester, 2008**). Sodium ion extrusion from the cytosol and partitioning within the vacuole are two main ways to reduce excessive Na^+ in the cytoplasm **(LV *et al*,, 2008**). Sodium chloride stress severely

inhibited root absorption, transportation and distribution of Ca^{2+} and K^{+}. As an essential nutrient, Mg^{2+} participates in the formation of chlorophyll composition. Thus, reduced uptake of Ca^{2+} and Mg^{2+} by soil salinity would have adverse effects on growth and development (**Yeo, 1998**).
In 1995, Monsanto Company introduced Roundup Ready (RR) soybeans that have been genetically modified to be resistant to several external factors was produced through substitution of the *Agrobacterium sp.* (strain CP4) gene EPSP (5-enolpyruvyl shikimic acid-3-phosphate) synthase.

Decreasing protein percentage and content with increasing salinity could be attributed to the disturbance in nitrogen metabolism or to inhibition of nitrate absorption. It has been stated that the reduction in nitrogen under saline conditions might be due to the reduction of absorbed water and decrease in root permeability (**Strogonov *et al.*, 1970).**

Most of the crop species are glycophytes, generally show limited growth and development due to salinity.Nitrogen is the most precious nutrient for crop growth and yield. Nitrification is a major pathway for nitrogen loss .Nitrification results in transformation of the relatively immobile ammonium nitrogen (NH_4^{+}-N) to highly mobile nitrate (NO_3^{-}-N) which promotes N losses through leaching of NO_3^{-}-N as well as gaseous N emission. Nitrification acts as a key process in determining fertilizer use efficiency by crops as well as nitrogen losses from soils. Soybean is the only staple crop that is capable of fixing atmospheric nitrogen through symbiosis with soil-borne microorganisms.

Translocation or long distance transport in plants is achieved by a vascular network that connects and is an integral part of all organs. The vasculature comprises two distinctly different and separate cellular translocation pathways , xylem and phloem. The principal xylem pathway is the transpiration stream that moves nutrients and water taken up by roots to the shoot. This stream also bears products of root metabolism, solutes that reflect features of the internal and external root environment. Phloem provides the means for redistributing xylem-delivered solutes to weakly transpiring organs and phloem distributes the carbon assimilated by photosynthesis in developing seeds. Xylem and phloem fulfill a role in communicating between organs, through

the movement of plant hormones and other signaling molecules.Phloem also transmits pressure/concentration (turgor) information at rates greatly in excess mass flow of solutes (**Thompson and Holbrook, 2004**). Long distance electrical signaling is also thought to be directionally propagated via vascular bundles (**Brenner *et al*., 2006**). These action potential or osmotic signals have a significant regulatory role in terms of phloem function. Phloem also provides a conduit for trafficking macromolecules, some of which may regulate gene expression as a consequence of their translocation (**Banerjee *et al*., 2006; Lough and Lucas, 2006).** Root-derived signals are postulated to regulate shoot processes are in xylem , together with a suite of secreted proteins (**Buhtz *et al*., 2004).**

When nodule development is initiated there is an exchange of signals between the roots and shoots to regulate the number of nodules that develop. A signal derived in the roots is translocated and perceived in the shoot generating a second signal that inhibits further development of nodule primordial. (**Beveridge *et al*., 2007**).

Addition **of a**uxin induces tissue exp**a**nsion correl**a**ted with H^+-**A**TP**a**se **a**ctiv**a**tion **a**nd decre**a**sed extern**a**l pH. Mobilization of lipids and proteins are important for the soybean germination and the following seedling growth. Many developmental and physiological events that occur within the seed compartment are programmed, in part, by the activity of different genes (**Gehring *et al*;2004; Haughn and Chaudhury, 2005**). Seed development, is the result of a mosaic of distinct gene expression programs occurring in parallel in different seed compartments (e.g. embryo, endosperm, seed coat) as well as within specific regions tissues (e.g. embryo proper, suspensor, epidermis).

Reactive oxygen species (ROS) can act as signal molecules to alleviate seed dormancy and promote seed germination.Sugars unloaded from the phloem in seeds may act as regulators of seed development .miRNAs are important regulators of plant development and responses to environmental signals. Majority of their target genes are transcription factors and they play an important role in clearing regulatory transcripts (**Rhoades *et al*., 2002**). miRNAs are expressed in response to environmental conditions and cleave targets to allow the plant to adapt for example in conditions of phosphorus starvation and cleaves the transcript of a ubiquitin-

conjugating enzyme to regulate phosphorus homeostasis **.**

Potassium plays an important role in growth, nutrient distribution and resistance to pests and diseases. Since K^+ and Na^+ have similar physical and chemical structure, Na^+ can partially replace K^+ to promote plant growth in low-K soil (**Zhang *et al*., 2006**). When the Na^+ concentration exceeds a certain limit, Na^+ will be competing with K^+ transport and binding sites, leading to potassium depletion (**Yeo, 1998**). Potassium deficiency in cotton leaves reduced chlorophyll and photosynthesis.

In recent years, phosphorus crisis is emerging at global level, searching for alternative sources and management options for increasing its supply and use efficiency. Increasing cost of cultivation due to phosphatic fertilizers is economically proving prohibitive.Improving phosphorus acquisition , use by crops is critical to economical and environmentally friendly crop agriculture. Restoring ion homeostasis in plants disturbed by salt stress represents a crucial response. Plant responses in countering ionic stress caused by high salinity include restricting salt intake, increased extrusion, compartmentalization and controlled long-distance transport to aerial parts. Additionally, to avoid cellular demage and nutrient deficiency, plant cells need to maintain adequate K^+ nutrition and a favourable K^+/Na^+ ratio in the cytosol.

Molecular , **genetic**, biochemical, physiological, **and** morphological responses **of** plants subjected to P stress have been the subject **of** many recent investigations .Phosphorus (P) is an important plant nutrient for crop growth and crop yield. It maintains biochemical, metabolic functions in plants, maintains soil fertility and soil health. But lower phosphorus use efficiency limits crop yield and economic return. Phosphorus fertilizers are mostly important in India and indigenous rock phosphate having low P concentration, increasing crop production cost. Different phosphatases enzyme activities speed up the conversion process of immobile P to plant available P in soil solution and indirectly enhance plant P uptake. In rhizosphere region many microorganisms play a crucial role to enhance enzyme activities and P solubilization process. But it varies accordingly crop wise, species wise and under different crop cultivation practices.

Plant roots detect water and nutrients by the sensing capacity of the root cap, a small organ housed within the root apex just below the apical meristem. The root cap that senses water,

gravity, touch, other signals and controls the direction of root growth toward positive stimuli such as nutrients and away from deleterious stimuli such as toxins **(Feldman, 1984; Aiken and Smucker, 1996**).The newly synthesized tissue in the region of elongation just behind the root tip is the primary site where infection by nematodes, fungi and bacteria is initiated (**Bauer, 1981; Curl, 1986**). Gibberellic acid (GA) pretreatment of crop seeds can overcome low soil temperature inhibition of seed germination and seedling development. The plant hormone auxin principally indole-3-acetic acid (IAA) has been implicated in the regulation of many aspects of plant growth and root development, including P stress-induced proteoid (cluster) root development. Cytokinins (CKs) regulate several plant growth aspects and developmental processes, including cell division, apical dominance, chloroplast biogenesis,nutrient mobilization, leaf senescence, vascular differentiation, photomorphogenic development, shoot differentiation and anthocyanin production **(Mok and Mok, 2001; Davies, 2004**). Cytokinins also enhance resistance to salinity and high temperature (**Barciszewski *et al*., 2000**). Seed enhancement, seed priming with cytokinins is reported to increase plant salt tolerance (**Iqbal *et al*., 2006a**).

Phytohormones are chemical messengers produced in one part of plant and translocated to the other parts, where they play critical roles in regulating plant responses to stress at extremely low concentration. Phytohormones are natural products and they are called plant growth regulators .They are synthesized chemically. Hormonal regulation is an important factor in the control of nodule development, maintenance and senescence. Auxins also have a role in the regulation of gene expression in many plant tissues. The hormone modulates enzyme activity either by the de novo enzyme synthesis or by activation of preformed dormant enzyme. Auxins stimulate wall bound enzyme like cellulase, glucan synthetase in pea, bean and maize seedlings.

Salinity limits their growth and productivity of plants (**Kaymakanova, 2009**). The major inhibitory effect of salinity on plant growth and development has been attributed to osmotic inhibition of water availability as well as the toxic effect of salt ions responsible for salinization. Nutritional imbalance caused by such ions leads to reduction in photosynthetic efficiency and

other physiological disorders (**Hakim *et al*. 2010**). In arid and semi arid regions, limited water and hot dry climates frequently cause salinity problem that limit or prevent crop production. It has also been reported that under saline conditions, germination ability of seeds differ from one crop to another and even a significant variation is observed amongst the different varieties of the same crop (**Jamil *et al*, 2005**). Salt stress affects many physiological aspects of plant growth. Shoot growth is reduced by salinity due to inhibitory effect of salt on cell division .

Growth of population, massive urbanization, rapid rate of industrialization ,introduction of modern technology in agriculture and animal husbandry led to water pollution, which subsequently results in gradual deterioration of quality of water.Effluents from industries are normally considered as the main industrial pollutants containing organic and inorganic compounds, acids, alkaline, suspended solids and other materials.Among the broad range of organic pollutants contamination soil-water environment, polycyclic aromatic hydrocarbons and pesticides are of great environment concern **(Corgie *et al*;2004).** Seed germination is commonly used method for measuring soil toxicity. The assay also serves as bioindicator response endpoint due to its simple methodology, moderate sensitivity to toxicants and its potential use in situ and ex situ, both. Molecular genetics and functional genomics provide a new opportunity to synthesize molecular and physiological knowledge to improve the salinity tolerance of plants, relevant to food production and environmental sustainability.

BOD is an important indication of the amount of organic matter present in the sewage. The BOD test is a measure of the oxygen requirements of bacteria and other organisms as they feed upon and cause decomposition of organic matter. BOD is normally expressed in mg/l or parts per million for a specified time and temperature, the standard being five days at 20ºC. A high BOD will result in water becoming anaerobic (depleted of oxygen). BOD is therefore a measure of the organic load placed on the treatment facility. Industrial non-organic wastes can also deplete oxygen in the water.This is measured by the chemical oxygen demand (COD) test.

COD is a measure of the oxidisability of waste, expressed as the equivalent amount in oxygen of a strong oxidizing agent consumed by the waste under fixed laboratory conditions (**Kaur *et al*;2014).**

The quality of irrigation water is important in judging its suitability for irrigation. According to specification prescribed by Bureau of Indian Standards (IS : 2490-1974), industrial effluent discharged into and surface waters should not have biochemical oxygen demand (BOD) more than 30 mg/l, pH 5.5 to 9.0, the chemical oxygen demand (COD) and suspended solids not more than 250 mg/l and 100 mg/l respectively. Microorganisms such as bacteria are responsible for decomposing organic waste. When organic matter is present in a water supply, the bacteria will begin the process of breaking down this waste. When this happens, much of the available dissolved oxygen is consumed by aerobic bacteria, robbing other aquatic organisms of the oxygen they need to live. The increasing agriculture reuse of treated effluent serves goals such as promoting sustainable agriculture, preserving scare water resources, and maintaining environmental quality. Use of industrial effluents for irrigation purposes is a highly warranted utility . In India, along with urbanization (26.4%) and industrial growth (5.5%) ,sewage monitoring, treatment and disposal have become matter of great public health significance. Approximately 80 percent of water supplied for domestic use passes out as waste water or sewage. A typical sewage consists of approximately 99.9 per cent water and 0.02 – 0.3 per cent suspended solids and other soluble organic and inorganic substances of water pollutants proposition (**Kalaiselvi *et al*;2010).**

In India, Mushrooming population growth has resulted in the huge quantities of waste generation and sewage waste is one of them. There are many sources of sewage generation such as residential, institutional, commercial and industrial establishments. Potentially toxic element contamination of sewage is generally attributed to discharges from major commercial and industrial premises **(Doyle ;1998).** The chemical constituents present in low concentrations, never the less are extremely important and subjected to variations between communities as well as within community. The most hazardous effect of sewage pollution is bacterial and viral contaminations. Soil is a hostile environment to most bacterial and viral pathogens. Heavy metal pollutant changes the natural balance of the biogeochemical cycles . Soil having higher clay and humus, absorb larger amount of heavy metals due to its better cation exchange capacity.

Application of sewage sludge to cropland could result in soil contamination, phytotoxicity and accumulation of trace elements in the food supply. The magnitude of the problem depends on the interrelationships of a number of factors, such as the composition of sludge, the rate and frequency of applications, soil characteristics of plant species. Sludge type which determine its availability for plant uptake. However, additional plant and soil factors further modify the uptake and the concentration of elements in crops. With the impending constraints on the availability of fresh water, irrigation in agricultural sector will have to depend upon low quality water and polluted water. At present almost 85% of the fresh water is being used for irrigation. This fresh water will not be available for irrigation since the top priority is for drinking, health and sanitation.

Low quality water and polluted water have some inherent constituents which are detrimental to agricultural production, especially sewage water which is highly contaminated with infective bacteria and other micro organisms. They also contain certain colouring materials and toxic metals which are highly injurious to plants. Yet, sewage water also has beneficial constituents which aid to increase agricultural production. There are two main sources of water pollution;point and non point.Point sources are specific discharges from municipalities and industrial complexes. In the surface water-body, non point pollution can contribute to total pollutant loading.

There are spatial and temporal variations in plant injury symptoms when polluted water is used. The symptoms may range from chlorosis to necrosis. The intensity of losses depend upon the pollutant concentration, duration of exposure, climate & edaphic factors, plant species & cultivars. There is reduction in photosynthesis and biomass **(Agrawal, 2005).**

Farmers are using polluted water and sewage for irrigation purposes for growing crops. In many of the cases, such water has accelerated the growth & development processes in cultivated crops but in some, it had inhibitory effect due to the array of different organic ,inorganic solutes and heavy metals .

Salt-affected soils currently account for 8% of the world's total land area (**FAO, 2000**), and the area of salt-affected agricultural land is predicted to double by 2050 for irrigated agriculture and some semi-arid areas (**Pitman and Läuchli, 2002; Rengasamy, 2006).** The area of salt-affected irrigated land, which produces 40% of the world's food, already stands at 20% **(Pimentel *et al*., 2004**). In light of the predicted 70–110% increase in food production that will be needed by 2050 led the rapid growth in global population over the same period (**Tilman *et al*., 2011)** .

India produces annually 5.0-5.4 million tons of soybean, it constitutes nearly 25 per cent of the country's total oilseed production. It is the second largest oilseed in India after groundnut. Commercial production of soybean began in 1971-72 in India.Soybean cultivation was negligible until 1970, but it grew rapidly thereafter crossing over 10 million tones in 2009-10 and become the fifth largest producer of soybean in the world today. Production increased from 5.28 million tones in 2000-01 to this level by having increased area for cultivation from 6.42 million hectares with yield of 822 in 2000-01 to 9.79 million hectares achieving yield of 1076 in 2009-10 . Out of 10.05 million tones, Madhya Pradesh produces 5.85 million tones contributing approximately 59% of total production followed by Maharashtra with 2.76 million tones and Rajasthan with 0.81 million tones.The average yield of soybean in India is about 1000 ha^{-1}, compared with 2300-2800 ha^{-1} in other countries. Nearly 310-320 million tons of oilseeds are produced annually, soybean production alone stands at 170-190 million tons, contributing to over 55 per cent of the global oilseeds production. During the last decade, the production of the commodity grew at the rate of 5.35 per cent at the global level.

Soybean breeding programmes in India have successfully developed 82 improved cultivars since 1969 , through introduction , hybridization of elite cultivars and breeding lines. As few as 9 lines account for more than 65% of the genetic background of cultivated varieties in India**.** Utilization of identified genetically diverse plant introduction, based on molecular markers in crosses for cultivar improvement programmes, have shown more success than conventional selection programmes in producing lines with elite genotypes (**Narvel *et al*;2000).** RAPD, (random amplified

polymorphic DNA) technique is easy , quick, and requires no prior sequence information **(Williams *et al*;1990**) .Markers has been shown to be more polymorphic than AFLP in comparison to RFLPs **(Powell *et al*;1996).**

South Eastern region of Rajasthan is spread over an area of 4.19 million ha, It is characterized by hills of Aravali and Vindhyan ranges, cut rock, up lands and almost flat alluvial plains. Out of 2 million ha cultivated area of the region, about 70% is rain fed. The annual rain in the region is about 800 mm and is mostly (90%) received during july to september in few intense storms . There are soil erosions in the form of sheet, rill, gully & ravines. Soil moisture stress and traditional methods of agricultural production are the major contributors for low crop productivity.

Crop production is dependent upon an intricate relationship between the soil texture and nutrients, climatic elements and irrigational facilities. The indiscriminate and disproportionate use of synthetic fertilizers have not only resulted in the loss of soil fertility but has transformed land in USAR due to soil erosion.

The rainfall dependency, marginalized and poverty stricken farmers, inefficient in-put management and overall knowledge poor farming society is struggling with hand to mouth sustenance by low return of the marketable surplus.Land productivity in areas with conjunctive use of canal and ground water is dependent on several factors including quality and quantity of ground water.

Different strategies are being employed to maximize plant growth under saline conditions.' One of them is to produce salt tolerant genotypes of different crops. Attempts to improve tolerance to salinity through conventional plant breeding methods are time consuming, laborious and depended on existing genetic variability.

The present investigation is a diverse approach encompassing many facets of *G.max* production with special reference to salinity effects-

An attempt has been made to study on the following aspects of *G. max*

(1) Germination of *G. max*., in a wide range of NaCl salinity.

(2) Growth of *G. max* in industrial effluent & sewage, purification of sewage, characterization of physiochemical properties of the industrial effluent/sewage before & after the purification. Bacterial load in pre , post treated effluent and sewage was investigated.

(3)The effect of phytohormone on the growth of *G. max*.

(4)UV spectroscopy of *G. max* grown in saline water & distilled water (control).[◊]

(5)Enzymological study with respect to aspartate transaminase (AST) and alanine transaminase (ALT), Ca++ dependent ATpase, Mg++dependent ATPase & Na+K+ ATpase with respect to duration of germination & levels of NaCl salinity. Characterization of protein & molecular weight, determination of seeds of *G. max* grown in saline environment.

(6) Histological studies to investigate the morphological changes due to salinity.

(7) A survey of the production characteristics & environmental factors in Bundi district.

(8) Pot culture study to investigate the impact of phosphate & potassium fertilizer in saline environment, uptake of Na, K in the soil in several characteristics of plant growth.

Review of literature

Soybean has reasonable tolerance for high levels of NaCl salinity , It showed increase in seed Na content to the extent of 131.1% at 40µg/g ,NaCl , Na^+ and Cl^- ions accumulated in the leaf tissues and other aerial parts of salt tolerant plants . Na absorption and accumulation by ***Glycine max*** is due to high osmotic pressure of the cell sap responsible for effective water absorption. Potassium uptake by the seeds was favoured for significant increase at most of the treatments of the salinity and the heavy metals.A direct relationship was observed between water uptake by seeds and increase of NaCl concentration up to 10 ds/m. When NaCl concentration increase to 12.5 ds/m the water uptake ability decreased in comparison to controls. Increasing NaCl concentration, germination was delayed and decreased germination in all cultivars. Generally root length decreased as NaCl concentration increased. Higher the level of NaCl level, higher root to shoot dry weights was observed. With higher NaCl concentration, a higher Na^+ and K^+ accumulation in

[◊] Additional investigation that approved by SRC of Kota University

seedling roots and shoots was observed. In all cultivars, the Na^+ accumulation was more than K^+ compared to the control. The Na^+ accumulated in shoot was less than roots. In general, total K^+ accumulation in the seeds decreased as NaCl concentration increased. Increasing NaCl concentrations, resulted in delayed cultivars. Increasing salinity concentrations often caused osmotic and/or specific toxicity which may reduce germination percentage (**Saboora and Kiarostami;2006).** Similar declines in seed germination have been reported by others. **(Sharma *et al*;2004**) The osmotic barrier due to NaCl level affected water uptake and mean germination time but not final germination **(Hampson and Simpson, 1990, Neumann 1995**).

Alteration of cell wall extensibility (**Pritchard *et al*., 1991**) and accumulation of salt in the apoplast **(Flowers *et al*., 1991**) were also reported as possible causes of radial plant growth. The rate at which new leaves are produced depends largely on the water 6potential of the soil solution. Reductions in the rate of leaf and root growth are probably due to factors associated with water stress **(Munns, 2002).**

Two cultivars of soybean **(*Glycine max* [L.] Merr**.) were grown in solution up to 100 millimolar NaCl . Leaf solute potential was -1.1 to -1.2 megapascals in both cultivars without NaCl. At 100 millimolar NaCl leaf solute potential was -3.1 to -3.5 megapascals in Bragg and -1.7 megapascals in Ransom. The decrease in solute potential was essentially proportional to the concentration of NaCl (**Ansary & Michel;1986**). All saline treated plants showed a 50% reduction in root biomass at all sampling dates as compared to control .Inhibition of root growth by salinity was less severe than its effect on aerial components (i.e. green , senescent leaves and shoots). Reduced root biomass was particularly evident in plants that underwent stable salinity treatments. Senescence occurred suddenly during stable salinity treatments **(Carolina and Lavado;2011)** .Salinity stress reduced plant height and total biomass production and yield.

Reduced biomass was evident for all vegetative components (**Dabuxilatu and Ikeda 2005b, Essa;2002** and **Shalhevet *et al*;1995).**Increased salt concentration caused an increased in cell membrane injury . Enormity of increase was more intense at 9.4 dS m^{-1} and 14.1 dS m^{-1} as compared to the control .

A study in soybean cultivars was based on visual symptoms of chlorosis at 120 mM NaCl. There were negative, significant correlations between relative shoot dry weight and Cl concentration in

shoot tissue (r = -0.91 p = 0.05), and Cl concentration in shoot was also significantly correlated with visual rating score (r = 0.79 p = 0.05) **(Tamura and Chent;2009***)*. Symptoms of Cl toxicity ranged from burning of leaves and stunting to interveinal chlorosis and premature chlorosis. Symptom severity increased with NaCl concentration.High salt tolerance was associated with Cl exclusion from leaves/shoots .

Valencia *et al*. (2008) found that 120 mM NaCl was the critical NaCl concentration for tolerance selection, which separated includers from excluders at 14 days of Cl stress treatment when interveinal chlorosis on leaves was observed among includers while excluders remained healthy.Under the highest concentration of NaCl (160 mM), both includers and excluders developed thin and dark roots with extremely short and sparse secondary roots. Root development was greatly reduced even at the 80 mM NaCl compared to the control.Includers translocate Cl to shoot tissues; therefore, they had more interference with growth due to applied NaCl solution than excluders. Excluders store Cl in roots, thus the root growth of excluders were hindered by the NaCl solutions by a greater extent than includers. Chloride concentrations in both shoots and roots had positive linear relationships with NaCl concentrations **(Tamura and Chent;2009).**

Roots seem to be more resistant to soil salinity than aerial biomass which could be linked to its low chloride levels.The effect of salinity on aerial biomass were leaf senescence and premature leaf abscission. These processes had been hypothesized to be part of a plant strategy to adapt to high soil salinity **(Maggio *et al*., 2007**).

Salt damage in soybean results from the accumulation of chloride in stems and leaves (**Wang and Shannon, 1999**). Salt levels of 0.4 S m^{-1} or higher resulted in little or no grain production. Senescence occurred suddenly during stable salinity treatments with >70% of the leaf biomass drying out before the second sampling date. On plants receiving a high salinity treatment (0.8S), whole portions of the shoot suffered premature onset of senescence.Seed Na content at NaCl doses was significantly elevated at all the treatment, to the extent of 3.007% at D_1 , 2.69% at D_2 and gradually lowered towards increasing levels of NaCl salinity.

Decrease of tissue water content results in reduction of cellular growth and development. Since root cells have a much less turgor threshold pressure than that of stem cells thus root growth is more than stem growth under salt stresses. Therefore, root is significantly less affected by stress in comparison to stem **(Abd-Ala *et al*; 1998).** Salt stress increase soybean seedling Na^+ content but decrease [K.sup.+] content. Salinity decreased the germination percentage, seedling fresh weight, shoot and root length of soybean cultivars. Overall by increasing the salinity level, potassium content of plant tissues decreased, however sodium content of plant tissue increased. These results has conformity with the **Chippa and Rana results (1995)** ,there is a relationship between potassium decreasing and sodium increasing in seedling tissue with sensitivity to salinity. Sodium affected the cell membrane permeability.Resistant plants to salinity not only have the lower sodium potassium ratio in comparison to sensitive plants to salinity, but also deposite higher sodium in root tissue and therefore it inhibits sodium transmitance to shoot tissue.

Salt stress decreased seedling fresh weight of soybean cultivars. [Na.sup.+]/[Cl.sup.-] toxicity and its effects on seedling growth was the main cause of weight loss. Increasing salinity level caused significant decrease in soybean seedling biomass. Enhanced transpiration is thought to be the major cause of such weight loss under the salt stress .Root and shoot length significantly decreased with the increase of salinity level.

Under optimal conditions, over 98% of salt resistant soybean variety Lee68 (salt resistant) and N2899 (salt sensitive) seeds germinated after a mean of 1.5 and 2.1 d, respectively. When exposed to 100 mmol/l NaCl, the final germination percentage in both cultivars was not affected. However, the mean germination times of Lee 68 and N2899 were delayed by 0.3 and 1.0 d, respectively, compared to the control. In addition, Lee68 and N2899 seeds required 1.2 and 1.5 d, respectively, to reach 50% germination, while seeds germinated in the presence of 100 mmol/l NaCl required 1.5 and 2.5 d, respectively. From imbibition till maximum germination, Lee68 and N2899 took 3.5 and 4.5 d, respectively. Under salt stress this period was 3.5 and 6.5 d, respectively .It was observed that there were many more abnormally germinated N2899 seeds compared to Lee68. Moreover, germination of N 2899 seeds was completely inhibited by

exposure to over 150 mmol/l NaCl. These observations suggested that 100 mmol/l NaCl affected soybean seed germination, especially in N2899 **(Xu *et al.*;2011).**

However, moderate salt stress intensity only delayed germination time, and did not have a severe impact on the final germination percentage of root and shoot length of seedling decreased linearly as the salt concentrations increased (0 to 300 mM). The mean root and shoot length averaged over all varieties decreased from 13.21cm and 11.38cm for 0 mM (the control) to 0.30cm and 0.27cm for 300 mM NaCl respectively .At 180 and 120 mM NaCl all the varieties were able to differentiate into shoot and root. The root/shoot ratio averaged over all varieties for the control was 1.20cm. At 120 and 180 mM NaCl it increased to 2.08cm and 1.95cm respectively. At high salt concentrations of 240 and 300 mM NaCl, it decreased to 0.97 and 0.36 respectively. The overall moisture content in varieties decreased in roots (93.77% to 38.33%) and shoots (93.01% to 29.64%), as the NaCl concentrations increased from 0-300 mM. High salinity induced a decrease in photosynthetic efficiency associated with inhibition of photosystem II (**Kalaji *et al*; 2010**).

Additionally, salinity stress resulted in ion imbalance and hyperosmotic stress in plant system which ultimately leads to the production of reactive oxygen species (ROS) (**Blokhina *et al*.,2003**). ROS such as superoxide (O^{2}_{-}), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-) and singlet oxygen ($^1O_2$) are known to cause oxidative damage to lipids, carbohydrate and DNA which ultimately results in cell death (**Wang *et al*; 2003**). To combat ill effects of oxidative damages caused by ROS, plants have strong stress-protective mechanisms constituted by antioxidants (ascorbic acid, proline, phenols and glutathione) and antioxidant enzymes (superoxide dismutase (SOD), guaiacol peroxidase (GPOX), catalase (CAT) and glutathione reductase (GR) (**Ahmad *et al* 2008,Andre *et al*. 2010).** NaCl stress has been found to enhance the activities of antioxidant enzymes (GPOX, CAT and SOD) in *Adiantum* leaves, which further got improved upon application of EBL.

A reduction in fresh weight due to salinity is probably due to decrease hydraulic conductivity of water from the growth medium (**Ashraf and Bashir;2003**). The reduced dry weight also indicates, decreased photosynthetic activity of seedlings. **El- Tayeb, (2005**) reported that

decrease in photosynthetic pigments due to salt stress may result in decreased plant growth. **Panneerselvam et.al., (1998**) reported that NaCl stress decreased root growth, shoot length and dry matter production of *Glycine max* seedlings. Salt stress significantly reduced the number of flowers, number of pods, 100 seed weight, and number of seed pod-^{1,} The observed decrease in the seed size .Salt treatment may be due to the consequences of increased flowers drop resulting in the reduction in pods plant-^{1} and seeds plant-^{1}.**Boquet *et al.*, (1987**) and **Hussain (1997)** observed differences in seed weight and number of pod plant-^{1} in soybean cultivars.Presence of excessive salts in soil suppressed plant growth and development including inhibition in the weight of seeds and that water and salinity stress affected the seed yield due to plasmolytic effect of water and salinity stress in soybean (**Salinas *et al.*, 1996**). In an experiments, the relative root zone was divided into four layers of equal depth (0-25%, 25-50%, 50-75%, 75-100%). The different layers contributed different amounts of water to the the water requirement of the crop (evapotranspiration : ET) : the top layer (0-25 % soil depth) contributed 40 % (=0.4 ET), the second layer (25-50 %) 30 % (= 0.3 ET), the third layer (50-75 %) 20 % (= 0.2 ET) and the bottom layer (75-100 %) 10 % (= 0.1 ET) to the total ET of the crop. Consequently the amount of soil water available for leaching of salts (LF = leaching fraction) from the upper layers travelling downwards the soil profile into the non-rooted subsoil decreased with soil depth (LF = ECi/ECe : LF0 > LF1 > LF2 > LF3 > LF4. Soybean is very sensitive to $Cl^{-,}$ but not greatly affected by Na+, because of its ability to restrict movement of Na+ to leaves .

A sodic soil contains a high level of sodium relative to the other exchangeable cations (i.e. calcium, magnesium and potassium). A soil is considered "sodic" when the Exchangeable Sodium Percentage (ESP) is 6% or greater. The exchangeable sodium percentage (ESP) is calculated as follows:

ESP = Exchangeable {(Na)/(Ca + Mg + K + Na)} x 100

Further, ESP values as low as 2 can cause soil structure problems if the concentration of salt in the soil solution is very low. This is an issue in irrigated areas when the ratio of soluble sodium

to calcium and magnesium ions in water is high. This ratio is expressed as follows and is called the Sodium Adsorption Ratio (SAR).

SAR = Exchangeable {(Na)/(Ca + Mg) -0.5}

or

$$SAR = Na^{+} / \sqrt{(Ca + Mg)^{++} / 2}$$

where all concentrations are in mmol (+)/litre.

In order to counteract the effect of excessive sodium on the exchange complex and to reinitiate the process of soil aggregation, calcium needs to be reintroduced into soil solution.

(**Richards 1954**) proposed that the sodium adsorption ratio (SAR) of the soil solution adequately defines the soil sodicity problem and is quantitatively related to the exchangeable sodium percentage of the soil.

Adaptation to salt stress requires alterations in gene expression and subsequently the protein profile of the plant and it is very complicated at the whole plant and cellular levels **(Ashraf and**. **Foolad 2007, Parker *et al*.,2006**). Cells of soybean *{Glycine max* L. "Maple Arrow") adapted to NaCl salinity (up to 680 mM) ,which have undergone extensive osmotic adjustment, accumulated Na^{+} and Cl^{-} as principal solutes for this adjustment. The concentration of proline was positively correlated with cell osmotic potential, accumulating to an average concentration of 130.7 mM in cells adapted to 680 mM NaCl and representing about 75% of the total amino acid pool as compared to an average of 1.65 mM and about 17% of the pool in unadapted cells **(Hameda and Kirkwood;1991).**

The *invitro* isolation of glycophytic cells with enhanced salt tolerance has helped facilitate the study of cellular responses to salinity .The intracellular concentrations of soluble sugars increased as a function of the level of adaptation. The magnitude of these changes, however, was

not comparable to that observed for Na^+ and Cl^-. While sucrose levels increased more than 10-fold between unadapted cells and those adapted to 680 mM NaCl, the patterns of carbohydrate accumulation observed in halophytes also indicate that the accumulation of sucrose was favoured over that of glucose in response to salinity **(Briens & Larher 1982). Khajeh-Hosseini *et al*. (2002)** suggested salt stress decrease the germination rate and germination percentage of soybean. They recognized negative osmotic and ionic effects of Na^+ on seedling growth and development as the cause of this observation.

Chippa and Rana (1995) reported that irrigation water with EC 14ds/m decreased the seed germination level of soybean significantly.Salt stress decreased seedling fresh weight of soybean cultivars **.**

Ashraf and Mcneilly (2004) suggested harmful ion like Na^+ increased under salinity stress and decrease growth of *canola*.Root and shoot length significantly decreased with the increase of salinity.Salt stress increased soybean seedling Na^+ content but decreased K^+ content.Sodium affected the cell membrane permeability, deconstructed the cell membrane and destroyed the selectivity property [**Letly ;1993, Makki *et al*;1987**.].

Organic acids accumulated appreciably only in unadapted cells and cells adapted to < 170 mg/l NaCl salinity.Intracellular proline concentration was correlated positively with *tyn;* the relationship was logarithmic for unadapted cells and linear for cells adapted to salt. The relationship between proline concentration and *tyn* for unadapted cells was indicative that proline accumulation is a component of osmotic adjustment and that proline does not accumulate solely in response to osmotic stress.

Water uptake by seed showed to have a direct relationship with increase in NaCl levels. Increasing NaCl concentrations adversely affected shoot dry weight in each cultivar, shoot dry weight fluctuated by varying NaCl concentrations. The root to shoot dry weight ratio changed in different NaCl levels. High NaCl levels caused a remarkable increase in the root to shoot dry weight ratio. Seedling dry matter decreased by increasing salinity. Salinity increased the accumulation of Na^+ and decreased the K^+ content in roots and shoots. The Na^+ content of germinated seeds gradually increased, while the K^+ level diminished. It was concluded that the

delay in germination ws mainly due to higher Na^+ accumulation in the seeds rather than osmotic stress in wheat cultivars (**Akbarimoghaddam** *et al***;2011).**

There are 12 main soluble salts made up of cations (Na^+, Ca^{2+} and Mg^{2+}) and anions (CO_3^{2-}, HCO_3^-, Cl^- and SO_4^{2-}) in saline soil **(Pessarakli, 2001**). Toxicity occurs as a result of uptake and accumulation of certain toxic ions from the saline soil or irrigation water. These toxic constituents include mainly sodium, chloride and sulphate. Although chloride and sulphate are essential elements, their content in the saline soil is far more than required for normal growth of plants. They can reduce crop productivity and eventually cause crop failures (**Nawaz *et al*., 2010**). The salt taken up by plants concentrates in the old leaves over a long period of time eventually results in very high Na^+ and Cl^- concentrations and the leaves die (**Munns, 2005**). The cause of the injury is probably due to rapid accumulation of Na^+ and Cl^- in the cytoplasm and inhibition of enzyme activity. Alternatively, they might build up in the cell wall and dehydrate the cell (**Munns, 2005**). The Cl^- is more toxic than Na^+ in a number of plant species (**Luo *et al*;2002** , **Tavakkoli *et al*., 2010**).

Salt stress (0, 30 and 60 mM NaCl) significantly inhibited the nitrogenase activity, nodule number and dry matter accumulation in plants **(Abd – Allah *et al* , 1998**) Soil salinity >5.72 dS/m markedly affected biological N_2 fixation, nodulation, growth, nitrogen content, water use efficiency and biomass of soybean.Salt stress significantly reduced the number of nodules per plant in both the cultivars. Number of nodules in the cultivars decreased with the increase in NaCl concentration. Nodulation is more sensitive than plant growth to salinity. The addition of NaCl had a negative effect on the initial growth and nodulation of soybean grown in pots (**Tamura, 1992**). Soil salinity decreased root nodulation and N_2-fixation (**Qifu and Murray, 1993).**

Salt adapted soybean cells accumulated both Na^+ and Cl^- as principal solutes for osmotic adjustment. Although Cl^- accumulation increased proportionately with a decrease in *tyn* as did Na^+, the concentration of the latter always exceeded that of Cl^-. Since the concentrations of Na^+ and Cl^- accumulated by the salt adapted cells, particularly at high levels of NaCl, are considered to be inhibitory to cytoplasmic metabolic function, it is likely that vacuolar compartmentation of these ions is occurring (**Wyn Jones & Gorham 1983, Yeo 1983**). Salt stress led to decrease

germination, seedling fresh weight, seedling growth and seedling K^+ percentage in dry matter but increased mean germination time and seedling Na^+ percentage.

Chen *et al.* [1996] reported salt stress led to decreased seedling growth of soybean cultivars. They also reported a positive correlation between Na^+ and Cl^- content of soybean seedlings and susceptibility level to salinity. Cultivars accumulating Na^+ and Cl^- in roots inhibited transportation of these ions to air organs and were more resistant to salt stress. Salinity led to decreased dry weight of shoot and root.

The concentrations of Na^+ and Cl^- did not increase during the period of most active osmotic adjustment which occured in the phase of the growth cycle just prior to and during the initial period of maximum fresh weight gain. The average concentrations of K^+ in the cells adapted to varying levels of NaCl were lower than that of the unadapted cells. The accumulation of K^+ in glycophytes is often inhibited in response to salinity due to a decreased K^+/Na^+ ratio (**Greenway & Munns 1980, Jeschke 1984**). The resulting K^+ deficiencies have been implicated as a contributing factor in the deleterious effects of salinity on plant growth.

Watad *et al.* (1983) observed that salt adapted cells maintain higher concentrations of K^+ in the presence of high external Na^+ concentrations than do unadapted cells. The ability of salt adapted cells to maintain a constant K^+/Na^+ ratio under increasing salinity indicates that the cells were able to preferentially absorb K^+ under conditions of decreasing K^+/Na^+. In soybean, metabolism related proteins were mainly down-regulated with NaCl treatment.

Salt stress due to soil or quality of irrigated water are the main abiotic stresses to which crops are exposed in India. The closure of stomata limits water loss and the integrity of the photosynthesis and carbon fixation is maintained by the initiation of a series of physiological processes **(Horton *et al* ;1996,Long *et al* ;1994**) .

The increase in salt resistance may involve protection of cell organelle membranes, accumulation of some protector components **.**Association between saline environment and endogenous level of water soluble antioxidant enzymes has been reported (**Tsugane *et al*., 1999**).

Some genes, such as embryonic identity genes LEAFY COTYLEDON1/LEAFYCOTYLEDON2/FUSCA3 (LEC1/LEC2/FUS3) and maternal gene

DNA binding with one zinc finger affecting germination (DAG1/DAG2), are involved in signaling of environmental factors or phytohormones and regulate the seed germination. Regulation on reserves mobilization also happens.The germination and germination percentage of the control (considering germination in the control as 100%) was recorded and it decreased as the salt concentrations increased from 0-300 mM NaCl. The mean for germination percentages of the control averaged over all varieties decreased from 79% to 25% as the NaCl concentrations increased from 120 to 300 mM. Salt affected the process of germination as high salt concentrations decreased the osmotic potential of solution creating a water stress in plants. Reduced germination in saline conditions could be a consequence of either the direct toxic effects of salts or the general delay in the germination process caused by osmotic stress **(Munns & Tester ;2008).**

Salinity stress can considerably reduce grain filling duration, grain weight, grains per plant and consequently grain yield per plant in soybean cultivars. These reductions increase with increasing salinity. Oil and protein yields per plant also remarkably decline due to NaCl salinity. Rate of oil accumulation up to maturity was not significantly affected by salinity. With increasing salinity, rate and percentage of protein accumulation, duration of oil and protein accumulation ,oil and protein content per grain decreased, but oil percentage increased. Oil and protein yields per plant decreased as salinity increased.To investigate the effect of salt stress on the K and Na contents in soybean, the concentrations of these ions were measured in root, hypocotyls and leaf of soybean at 0, 20, 40 and 80 mM NaCl after 2 weeks. At 40 mM NaCl, the Na content of the leaf, hypocotyl and root increased, but K content did not change under NaCl treatment. Under stress, plant respiration shifts from aerobic to anaerobic conditions to produce ATP via the fermentative pathway. Alcohol dehydrogenase was up-regulated in the hypocotyl, indicating the main role of this enzyme produce ATP and consume glycolytic products under salt stress. Annexin is important for integrity of the cell membrane and cell signaling, especially under stress conditions, this protein family can help the plant to tolerate the stress and maintain the integrity of the cells **(Cantero *et al* ;2006, Gerke & Moss ;2001**).

Abscisic acid (ABA) promotes seed dormancy and repress seed germination, whereas gibberellic acid functions antagonistically to trigger seed germination **(Kucera *et al*., 2005**).

During the maturation phase of seed development, ABA induces the expression of many maturation-associated genes, such as late embryogenesis abundant *(*LEA*)* genes.Mutants deficient in ABA production or sensitivity fail to enter quiescence, resulting in precocious germination or vivipary, accompanied by reduction of maturation-associated gene expression.During late stages of seed development, plant embryos undergo maturation, acquire desiccation tolerance, and achieve quiescence. VP1, along with ABA and other factors, is required for the regulation of these processes. Maize *Vp1* gene is expressed from before 10 DAP to very late in seed development (**McCarty *et al*; 1989)**

Ethylene is a gaseous hormone that regulates plant growth and development. An ethylene signal transduction pathway has been proposed in *Arabidopsis thaliana* that involves ethylene receptors, CTR1, EIN2, EIN3, and other component**s.** In response to salt stress, abscisic acid content increased, gibberellic acid (GA_{1+3}) and isopentenyladenosine decreased. Indole-3-acetic acid increased in salt-tolerant, but remained unchanged in salt-sensitive. Phosphorus deficiency stress is known to stimulate ethylene production in many plant species, including bean (**Borch *et al*., 1999**) and lupine (**Gilbert *et al*., 2000, Ma *et al*., 2003**). Ethylene regulates the rate of root elongation in both bean and *Arabidopsis* . Under P deficiency stress, ethylene application maintains primary and lateral root elongation but does not affect lateral root density. These results taken together indicate that ethylene production and/or plant responsiveness to ethylene plays a role in root adaptation to P deficiency.Electrolyte leakage in salt-stressed *NTHK1*-transgenic plants and various ethylene-response mutants revealed that relative electrolyte leakage may represented as an indicator for the damage caused by salt stress .In addition to salt stress, ethylene receptor may also play roles in hydrogen peroxide signaling (**Desikan *et al*., 2005).**

El-Iklil *et al*. (2000) have reported that lower ethylene production was associated with salt tolerance. On the contrary, higher ethylene production has been regarded as an indicator for salt tolerance in rice (**Khan *et al*., 1987)**. The effect of endogenous abscisic acid (ABA) and proline contents related to yield in two wheat cultivars sensitive and tolerant to terminal season drought, grown in pots under well watered and water-stressed

condition starting from anthesis until maturity were investigated. Water stress resulted in marked increase in the ABA content of both cultivars. The absolute ABA concentration in tolerant cultivar was more than that of sensitive cultivars. Abscisic acid (ABA) coordinates plant responses to water deficit and the regulation of gene expression plays an important role. ABA plays a vital role in stress avoidance by reducing stomata opening and thus lowering transpiration . Furthermore, ABA elicits the synthesis of proline and proteins, Probably has a role in protecting cellular structures during dehydration (**Smirnoff and Cumbes;1989)** .

Proline has a role as an osmoticum. In particular, because of its zwitterionic solute" , i.e one that can accumulate to high concentrations in the cell cytoplasm without interfering with cellular structure or metabolism . Among phytohormones, BRs and PAs have been widely used to confer salt stress tolerance in plants (**Sharma *et al*;2013).** Protective role of ABA over pigments may be related to stimulation of the non-photochemical quenching imposed to increase the level of xanthophylls. It has an important role in maintaining the integrity of the photosynthetic membranes under situations of oxidative stress (**Munne'Bosch and Alegre;2002).**

The gene *AtNHX1* is ubiquitously expressed in the plant and expression is induced by NaCl and abscisic acid (**Shi and Zhu, 2002**). *AtSOS1* was initially identified as a locus required for salt tolerance and loss-of-function mutations in *AtSOS1* caused plants to be extremely salt sensitive and to accumulate more Na^+ than wild type in the shoots under salt stress (**Wu *et al*., 1996; Shi *et al*., 2000).** *AtSOS1* transcription is specifically up-regulated upon NaCl stress and expression is mainly found in parenchyma cells bordering the xylem and in the epidermal cells surrounding the root tip (**Shi *et al*., 2002).**

Abscisic acid helps to maintain seed dormancy, hence inhibits reserves degradation.The effect of phytohormone Jasmonic acid (JA) and antioxidant ascobin on growth, yield and metabolism of soybean (*Glycine max* L. cv. Giza 111) grown at 0, 50 and 100 mM NaCl was investigated. Growth, yield and metabolic products were most affected by 100 mM NaCl. Treatment with JA or ascobin mitigated the harmful effect of NaCl. The greatest yield (157% of control) was obtained from plants sprayed with JA, while those sprayed with ascobin and irrigated with water

yielded 127% of control plants. Salinized plants (50 mM NaCl) sprayed with JA yielded 146%, while those sprayed with ascobin yielded 159% as compared with nonsprayed plants. The JA or ascobin reduced the salt effects on seed carbohydrates, lipids, proteins, N, P and K. LEA (Late embryogenesis abundance) proteins, which are ABA-inducible group of proteins and originally suggested to be associated with desiccation tolerance during seed maturation are also induced by salinity and water deficit.

Effects of sodium chloride (NaCl) 60 milli Molar (mM) and 80mM on inoculated and uninoculated plants of two cultivars (Williams-82 and its hypernodulating mutant NODI-3) of soybean [*Glycine max* (L.) Merr.] was investigated.

El-Fayioumy *et al*. (1996) showed that soil salinity >5.72 dS/m markedly affected biological N_2 fixation, nodulation, growth, nitrogen content, water use efficiency and biomass of soybean.

Han and Lee (2005) found that salinity in soil decreased plant growth, photosynthesis, stomatal conductance and mineral uptake in soybean plants compared to those from non-saline soil.Phytohormones play a role in initiating and/or maintaining meristematic activity during nodule development.

Davies (1995) presented a model describing the involvement of cytokinin and auxin in stimulating cell divisions in the inner cortex that leads to nodule formation.Salt stress (80 mM) had a significant ($P<0.05$) effect on lAA level in soybean leaves at 48 h after inoculation. The endogenous level of IAA decreased in response to inoculation under salt stress. Major changes in endogenous level of phytohormones occur between 48 to 96 h after inoculation (**Asim *et al*., 2010**). IAA can strengthen the capacity of resistance of the soybean to saline environment **(Wei and Chen, 2000**).Root initiation, adventitious root formation and early development of root are also stimulated by auxin .

Plant species differ in their sensitivity or tolerance to salt stress (**Ashraf and Harris, 2004**). It is thought that the repressive effect of salinity on seed germination and plant growth could be related to a decline in endogenous levels of phytohormones . ABA and JA are increased in response to salinity whereas indole-3-acetic acid (IAA) and salicylic acid (SA) are declined. The exogenous application of PGRs, auxins (**Khan *et al*., 2004**), gibberellins ,cytokinins produces some benefit in alleviating the adverse effects of salt stress and also improves germination,

growth, development ,seed yields and yield quality **(Egamberdieva, 2009**). It has been reported that exogenous application of ABA reduces the release of ethylene and leaf abscission under salt stress in plants, probably by decreasing the emulation of toxic cr ions in leaves .In wheat, seed germination decreased with increasing levels of salinity, while the adverse effect of salinity was alleviated by soaking seed with IAA . In addition, exogenous IAA showed high stimulatory effect on the root and shoot growth of wheat seedling in saline condition (**Egamberdieva, 2009**). Growth and yield parameters of rice were significantly increased in response to application of cytokinin under saline stress (**Zahir *et al*., 2001).**A large number of auxin responsive genes have been identified and characterized from different plant species including soybean and rice (**Hagen and Guilfoyle 2002**). These auxin-responsive genes have been classified into three gene families : auxin/indoleacetic acid (Aux/IAA), GH3 and small auxin-up RNA (SAUR) gene families **(Guilfoyle *et al*; 1993**). The changes in Indole Acetic Acid (IAA) determined it as auto regulatory factor for root nodulation. A decrease in IAA content was found in both the cultivars with salt treatment in inoculated plants. The uninoculated plants showed an increase in IAA content .

CKs retard senescence, having effect on membrane permeability to mono and divalent ions, and localized induction of metabolic sinks (**Letham, 1978).** During stress, a reduction of CK supply from the root alters the gene expression in the shoot and thereby elicits appropriate responses to ameliorate the effects of stress (**Hare *et al*., 1997**) decrease in CK content was as early response to salt stress, but that the effects of NaCl on salt sensitive varieties is not mediated by CKs since the reduction in growth rate preceded any decline in CK levels (**Walker and Dumbroff, 1981**). It is generally accepted that cytokinins are produced in the root tips and developing seeds of plants (**Zahir *et al*., 2001)**. They are translocated to the shoot, by xylem, from roots where they regulate development and senescence processes.

Organic acids like citric acid and oxalic acid has been used to extract labile phosphorus .Oxalic acid plays an important role in the mobilized of soil phosphorus [**Wei *et al*;2009].** Oxalic acid exudation from roots is considered to be one of the mechanisms for plants adapt to P deficiency **[McDowell *et***

***al*;2008**,**Jones & Darrah (1994**]. Several studies have suggested that the exudation of organic acids by plant roots might be a physiological adaptation rather than a passive response of plants to P deficiency [**Ohwaki & Hirata (1992).** It has been postulated that increased exudation was due to increased membrane permeability induced by decrease in phospholipids in P deficient roots , the addition of inorganic P to starved roots results in both depolarization of the plasma membrane and acidification of the cytoplasm by secretion of low molecular organic acids. The concentration of P in plant tissue increased with increased level of applied P in case of P_1. More addition of inorganic P fertilizers at the period of vigorous growth of crop plant directly enhanced the nutrient concentration in plant. Phosphorus uptake by different varieties is affected by genetic potential of the arieties **[Schachtman *et al* (1998)**] as well as concentration of phosphorus in solution. **[Dakora & Phillips (2002)]** transfer of inorganic P from the solution to the plant [**Smeck (1985)]** involves a different set of thermodynamic parameters to those applying to the plasma membrane, mainly because of the millimolar concentrations in the cytoplasm and vacuoles , the interaction between arieties and time interval was also found significant . Maximum P-concentration (0.197%) was recorded after 30 days time interval and minimum (0.129%) after 30 days time interval. Plant P concentration was improved substantially by the addition of P to the nutrient solution. This is due to the fact that increased P uptake rate in accordance with Michaelis and Menten equation.

The response of soybean growth was evaluated in pot experiments with the following treatments: Control (non saline soil), soil salinity level of 0.4 S m^{-1} (0.4S) or 0.8 S m^{-1} (0.8S), and soil subjected to salinity peaks of 0.4 S m^{-1} (0.4P) and 0.8 S m^{-1} (0.8P). The salinity levels were obtained by application of saline irrigation water. Sodium (Na) and chloride (Cl) are the major ions in saline soils. Chloride is phytotoxic, however there is great variability in the sensitivity of plants to it, depending on differences in species and genotypes **(Brinkman, 1988; Xu *et al*., 2000)**. When salinity was a permanent stress factor, regardless of the salinity level (i.e. 0.4 and

0.8 S m^{-1}), biomass production and differentiation of reproductive organs was greatly affected. For 0.8S treated plants, they never reached the reproductive phase. Application of inorganic fertilizers enhances the yield of plants . Excessive use has many ill effects. Cytological effects of two inorganic fertilizers Di Ammonium phosphate (DAP) , Urea and a biofertilizer agrozyme on the root tips of *capsicum annum* L. was investigated, the two applied inorganic fertilizers induced a significant decrease in the mitotic activity while the biofertilizer induced an increase in the mitotic activity. Moreover, the two inorganic fertilizers also induced a range of chromosomal anomalies like stickiness, scattering, precocious movement, bridge etc (**Gupta and Kumer;2008).**

The reduction of mitotic index seems to be common effect of both the chemical fertilizers.Both the fertilizers had mitoclastic and clastogenic properties of the chemical fertilizers, as was evident from the lowering of the mitotic index and manifestation of spindle abnormalities. Mito-inhibition by fertilizers was attributed to blocking of mitotic cycle during interphase that may result from a prolonged G_2 period or to the inhibition of DNA synthesis. The acceleration in the rate of cell division ie AMI in the root tip cells treated with biofertilizer was attributed to the increased rate of biosynthesis of nucleic acid and other substances required for cell division ,the formation of bridge could be attributed to chromosomal stickiness (**El-Khodary *et al*;1990**) , to the chromosomal breakage and reunion . Chromosomal stickiness is defined as chromosomal agglutination of unknown nature which result in a pycnotic, or sticky appearance of chromosome. **Gaulden;(1987**) postulated that the stickiness might have resulted from the defective functioning of non-histone proteins involved in the chromosomal organization, which are needed for chromosomal separation and segregation.The induction of lagging could be attributed to the failure of normal organization and function of the spindle apparatus(**Patil & Bhat;1992**).

Nacry *et al*. (2005) showed that during Pi starvation, IAA concentration increases in the whole primary root and in young lateral roots of *Arabidopsis*. Without IAA, only primary root growth was observed in *Arabidopsis*. .

Bianco and Defez (2009) reported that the IAA-overproducing RD64 strain showed an increased tolerance to 0.5 mol/l NaCl in *Medicago truncatula*. IAA could strengthen the capacity of resistance of the soybean to saline environment (**Wei and Chen, 2000**). The level of IAA in salt-treated Lee68 (a resistant variety of *Glycine max)* was more than two-fold higher than that in the control. The increased IAA in salt-tolerant Lee68 might help seed germination under salt stress.

Different leaf segments of *curculigo orchioides gaertn* showed differential plantlet regeneration response when cultured on MS basal medium supplemented with 0.5 mg/I BAP i.e. , plant regeneration from different leaf segments did not produce same number of shoot buds.Addition of IAA to the medium completely inhibited the growth of leaf explant. Endogenous level of IAA in different segment of the leaf was assayed. It was found that middle part of the lamina had higher amount of IAA which was less in apical and basal leaf segment.It was observed that endogenous level of IAA and number of shoots produced was directly proportional (**Shah and Sharon;2009).**

It was found that all the leaf segments did not produce same amount of shoot buds. Leaf explants failed to respond to IAA, It was presumed that the young leaves may be rich in endogenous auxin. Hence, they did not accept the external supply of auxin for growth . The regeneration capacity of the leaf segment was directly proportional to endogenous level of IAA .Segments from middle part of lamina could regenerate maximum number of plantlets. The endogenous hormonal level play an important role in organogensis .**Skoog and Miller (1957)** reported that the balance between auxins and cytokinins are responsible for caulogenesis and rhizogenesis. Multiple factors determine the amount of endogenous auxin in a particular part of the plant at a given time of growth. Auxin is synthesized in a

relatively large amount in very few localized centers of the plant which are shoot tips and young leaves. Bean seedling were exposed to increasing doses (0.1, 0.2, 0.3, and 0.4 mM) of manganese chloride ($MnCl_2.H_2O$) for 10 days. Elevated Mn levels increased the ABA content in root and leaves of seedlings. An increase of proline and vitamin in leaves of seedling and decrease of chlorophyII (a and b) and total protein contents occurred . Abnormally high level of $MnCl_2$ in soil becomes cytotoxic and can cause injury and create symptoms like brown spots on mature leaves, interveinal chlorosis, early necrotic flecking on stems and growth retardation. Excess Mn induces inhibition of chlorophyll biosynthesis, declines the photosynthetic rate and the amount of chlorophyll (**Arya and Roy;2011**) . Stomatal conductance and water potential of the leaves decreased in relation to ABA content. ABA takes part in the regulation of water status of the plant. It has also been reported that the ABA content is increased in plants when exposed to copper , Ni & Mn. Relative water transport rate is also decreased.

Exogenous application of auxin (IAA) and antiauxin maleic hydrazide (MH) showed differential effect in the activity levels of various plant cell wall degrading enzymes amongst two strains (COG 15 and M 11) of bradyrhizobium spp. (*Vigna)* specific to green gram. Cellulose activity was three times more for strain M 11 than strain COG 15 in unamended control; and with application of IAA upto 50µg/ml , there was gradual increase in cellulose. Addition of MH to the growth medium inhibited induction of cellulose degrading enzymes in both the strains. Combinations of IAA and MH in various concentrations inhibited the cellulose enzyme activity for both rhizobial strains . Pectic esterase enzyme was not significantly influenced by amendment of IAA. MH protease enzyme activity was found to be sensitive to both IAA and MH, and was more for strain COG 15 (**Shendie *et al*;2010).**

The production of hydrolytic enzymes was influenced by different levels of IAA and its inhibitor. Increased cellulase production in response to 50 µg/ml IAA

suggested its role in regulation of enzyme synthesis. Auxin regulation of cellulase activity has been studied in pea epicotyls **(Verma;1975).** There is de novo synthesis of cellulase in response to auxin and it regulates the enzyme synthesis both at transcriptional and translational levels (**Verma;1975, Shendie *et al*;2010).**

The role of cytokinins in root growth and P deficiency stress is not resolved. Traditionally, cytokinins are thought to be negative regulator of root growth while having positive effects on shoot growth . Application of cytokinin inhibits root development and abolishes the auxin effect of increased lateral root development. Plants that overexpress the cytokinin oxidase (*CKX*) genes have reduced cytokinin content and show enhanced root growth due to more lateral and adventitious root formation (**Lohar *et al*., 2004).** Both P and N deficiency result in decreased cytokinin content (**López-Bucio *et al*., 2003),** accompanied by increased lateral root formation. Exogenously applied cytokinin represses the expression of P stress-induced genes in roots **(Martin *et al*., 2000**). In P-stressed lupine proteoid roots, *CKX* gene expression showed a 3- to 5-fold increase in expression . Germination of stressed seeds was partially restored by the addition of exogenous cytokinin (CTK) (**Gidrol *et al*., 1994).**

In response to salt stress, the abscisic acid content increased, and gibberellic acid (GA1+3) and isopentenyladenosine decreased. Reserves degradation is promoted by GA. In cereal seeds, GA is synthesized during germination and induces the expression of alpha-amylase which promotes the degradation of starch. It is not known how different reserves are mobilized and how the mobilization is regulated during seed germination.

Indole-3-acetic acid increased in Lee68 (salt tolerant), but remained unchanged in salt sensitive soybean N2899.Hormone levels are related to seed germination and stress. The IAA content of salt-treated germinated Lee68 seeds was significantly higher than that of the control . Salinity had a stronger effect on the GA1+3 level. The GA1+3 content significantly decreased in both cultivars under salt stress . The GA1+3 content in Lee68 was higher than that in control N2899 .ABA content significantly increased in response to salinity , while the ABA content in salt-treated N2899 was lower than that in Lee68 . The decreased endogenous GA and increased ABA

contents have been observed in salt-stressed soybean. GA3 ameliorates the adverse effects of salt stress and restores normal growth and development of soybean (**Hamayun *et al*., 2010b**). IAA had a major role in regulating plant growth.

Kaur *et al*. (1998) found that GA3 was more effective than CTK in enhancing the reduced germination and seedling growth of chickpea seeds under salt stress. GA synthesis affects not only alterations in protein expression, but also increases in germination rate, promotes root and shoot length during seed germination, and promotes early seedling development (**Gallardo *et al*., 2002; Kim *et al*., 2008**). GA content was significantly reduced and seed germination time was delayed by salt. ABA was involved in responses to salinity (**Jia *et al*., 2002**), and is required by the plant for stress tolerance (**Hamayun *et al*., 2010a). Umezawa *et al*. (2001**) found that the leaf ABA content in Lee (salt-tolerant) increased significantly under salt stress.

A high concentration of salt leads to oxidative damage.Induction of ferritin by ABA has been documented at both transcript and protein levels (**Ravet *et al*., 2009).** ABA and ferritin levels were up-regulated by salt. There is a possible involvement of ABA in ferritin gene regulation.Recent evidence has suggested a role for the ubiquitin-proteasome pathway in CTK, GA, and ABA signalings (**Itoh *et al*., 2003).** Many stress-responsive genes are upregulated by ABA(**Ingram. and Bartel;1996 ;Bray;1997; Rock;2000**). ABA is also a regulatory molecule involved in drought stress tolerance. The main function of ABA is to regulate osmotic stress tolerance via cellular dehydration tolerance genes and to regulate plant water balance.

Most ABA-or PEG-inducible genes were also induced by drought or salt stress, suggesting that OsCIPK genes may participate in the cross talks of signaling pathways for drought ,salt stresses and ABA treatment. Studies have suggested that osmotic stress imposed by high salt or drought can be transmitted through ABA –dependent and ABA –independent signaling pathways

Members of the DELLA protein domain family (in particular RGL2) specifically repress germination **(Lee *et al*., 2002**) and biochemical studies have shown that GA promotes germination by activating the degradation of DELLA proteins via the 26S proteosome pathway (**Fu *et al*., 2004; Tyler *et al*., 2004).**

Application of exogenous GA to the gal-3 mutant that is disrupted in GA biosynthesis and cannot complete germination led to enhanced germination potential and up-regulation of more

than 200 genes (**Ogawa *et al.*, 2003).** Proteomic analysis of *Arabidopsis thaliana* seeds during germination also showed an important role for GA in regulating specific gerrnination-related protein components (**Rajjou *et al.*, 2004**). Experiments at both transcriptome and proteome levels indicate that GA plays a relatively late role in processes associated with germination (**Gallardo *et al.*, 2002; Ogawa *et al.*, 2003).**

Gibberellic acid (GA_3) is involved in the control of mobilization of food reserves from the endosperm to cotyledon . Hence acceleration of the rate of germination by 3000 mg/l GA_3 in the seeds was due to the unhindered entry of GA_3 into the seed. Gibberellic acid has been reported to affect various processes in germinating seeds, including metabolism of amino-acids , respiration and increase amylase content . The response of *trigonella foenum-gracum* to indole-3acetic acid (IAA), gibberellic acid (GA_2) and kinetin (KIN) and their various combinations. Viz. IAA + GA_2 + IAA + KIN, GA_3 + KIN and IAA + GA_3 + KIN were studied. Foliar application of these growth regulators significantly increased nitrate reductase (NR) activity and biomass. Maximum nitrate reductase activity was noticed with IAA + GA_3 + KIN combination.

In general phytohormones, like cytokinin and some synthetic gibberellins delay the loss of chlorophyll whereas ethylene and abscisic acid enhanced nitrate reductase (NR) .It regulates metabolism of nitrate by plants before its assimilation to protein. NR has been suggested as an indicator of nitrogen requirement Plant growth regulators, namely kinetin, 6—benzyladenine and 2—chloroethyltrimethyl ammonium chloride stimulated nitrate reductase activity effectively at 5×10^{-5} M concentration in etiolated and green seedlings .

Application of IAA GA_3 and kinetin promoted shoot length, leaf number, leaf area and plant dry weight. The foliar application of lower concentrations of growth regulators (IAA, GA_3 and kinetin) induced early flowering and delayed leaf senescence and simultaneously enhanced leaf growth till later stages of plants growth.

Table 1. The mechanism of salt tolerance through seed soaking or foliar sparay with chemical or biological agents differ with the type of agents used.Mode of application is shown in table below-

Agents	**Mode of application**	**Effects on stressed seeds or plants**	**Reference**
Kintein	Seed soaking before sowing	Alleviation of salinity effect on seedgermination	**Bozuck 1981**
MCBuTTB	Seed soaking before sowing plus foliar spray at 45 days after planting	Enhancement of seed germination, seedling growth under salinity	**Stark 1991**
Polystimuline K (cytokininanalogue)	Solution culture of seedling	Leading to recovery of damaged PS II centers	**Ganieva *et al*. 1998**

Coronatine (COR)	Applied hydroponically to cotton seedlings at the two leaf stage for 24 h.	Improving the antioxidativedefense system and radical-descavenging activity	**Xie *et al*. 2008**
5-aminol-evelinic acid (ALA)	Foliar spray	Improvement of salt tolerance through reduction of NaCl uptake	**Watanabe *et al*. 2000**
Gycinebetaine	Seed soaking	Allieviation of salt damage	**Li *et al*. 2008**
Calcium sowing sulphate	Seed soaking before	Enhancement of seed germination, seedling growth under salinity	**Javid *et al*. 2001**
Ca^{2+}	Applied hydroponically to cotton seedlings	Offset the reduction in root growth caused by NaCl	**Kent and lauchli 1985**
VAM(vesicular arbuscular mycorrhizal)	Mixed with soil	Increased salt tolerance of cotton seedlings	**Jalaluddin 1993**
Rs-5 strain (Klebsiella oxytoca)	Inoculation through seedsoaking	Enhancement of germination and emergence under salinity stress	**Yue *et al*. 2007**

Rs-5 strain (Pseudomo-nas putida)	Inoculation through seed soaking	Protect against salt stress and promote cotton seedling growth	**Yao et at. 2010**

Gibberellic acid application on to the corolla of *Petunia* hybrid increased anthocyanin synthesis and chalcone isomerase enzyme activity (**Weiss *et al.*, 1990**). The increase in anthocyanin biosynthesis is correlated with the co-ordinated appearance of relevant enzymes such as phenylalanine ammonia-lyase (**Weiss and Halevy; 1989)**, chalcone flavanone isomerase (**Weely *et al.*, 1983**), chalcone synthase (**Rall and Hemleben; 1984**), flavanone 3 hydroxylase (**Dangelmayr *et al.*, 1983**) and 3-**O**-flavonoid-glucosyltransferase (**Gerats *et al.*, 1983**). All of these enzymes are also involved in the biosynthesis of isoflavonoids . Therefore, it is reasonable to suppose that GA application could increase soybean nodulation and nitrogen fixation by increasing the activities of enzymes involved in the biosynthesis of isoflavonoids and also by increasing seed germination ,seedling development under low soil temperature conditions.

A large number of metabolic changes occur during leaf senescence, e.g. increases the activity of proteases, glyoxysomal enzymes and enzymes of chlorophyll catabolism. As a consequence of this increase in enzymatic activities, the contents of protein, RNA and chlorophyll strongly decrease during plant senescence, cytokinins and in some cases gibberellins, delay the loss of chlorophyll whereas ethylene and abscissic acid enhance the rate of chlorophyll loss .

Tryptophan and IAA induced root nodules formation. Addition of extracellular hormones also increased shoot and root dry weight as well as soybean yields. Highest root nodules number and soybean yield were taken from the treatment of 1.0 ppm tryptophan applied in early planting. It seemed that higher concentrations of tryptophan or IAA are required when applied at early planting, presumably because of its concentration decreases prior to root hairs formed.

The existence and effectiveness of root nodules is very impotant for soybean plants. Effective root nodules are able to meet up to 75% of the plant's nitrogen requirement (**Ohyama *et al*., 2009)**, one reason for the low soybean yields compared to its potential are the less effective root

nodule. The formation of root nodule is affected by internal factors of the plant and bacteria, including the ability of plant to excrete tryptophan and the capability of bacteria to oxidize tryptophan into IAA (**Spaepen, 2007**). Auxin plays an important role in early infection process of root nodules (**Spaepen and Vanderleyden, 2010).** Infection process and development of root nodules were induced by auxin **(Spaepen *et al*,. 2007; Spaepen and Vanderleyden, 2010).**
In the normal process, *rhizobium* oxidizes the tryptophan excreted by soybean into IAA. There were some possibilities due to certain factors such as environmental stress, including acidic pH, osmotic and matrix stress , carbon limitation or the presence of plant extracts ,specific compounds (**Spaepen *et al.*, 2007**), the plants may be unable to excrete the amino acid tryptophan, so it does not form IAA and the formation processes of root nodules are inhibited. **Bhattacharya (2006**) reported that heavy metals such as Hg, Pb, Cd and Ba affected IAA production.

Kamal (2000) stated that addition of epibrassinoid on soybean growth media increased root nodules number per plant. The formation of root nodules could also be induced by cytokinin (**Mishra *et al*. 1999). Spaepen & Vanderleyden (2010**) stated that application of exogenous tryptophan increased strongly IAA production in various bacteria. IAA was involved in many processes of nodule formation by rhizobia in legume plants such as founder cell specification, nodule initiation and differentiation, nodule number and nitrogen fixation .

According to **Gregory (2006**), root nodules formation was initiated by cortical or pericycle cell divisions ,their differentiation are controlled by auxin and cytokinin.Plant hormones are central regulators of the dormancy-germination transition.
GABA accumulation in plants is a general response to various biotic and abiotic stresses and can involve both biochemical and transcriptional processes (**Shelp *et al*;1999)** It is known that Ca^{2+} influx into the cell is increased in cold shock, heat shock, salinity, drought , osmotic stress and GABA does accumulate. Stresses that disrupt cellular membranes such as wounding, freezing and heat stress can cause vacuolar contents to be released into the cytosol or inhibit ATPase

enzymes which pump protons from the cytosol into the vacuole or apoplast, causing a concomitant decrease in cytosolic pH and stimulation of GAD activity.

Membranes, their integral and associated components are necessary for the uptake and distribution of ions and solutes. They are considered as determinants in developing stress-resistant crops. Plant cells need to maintain high K^+ levels., 100 to 200 mM to maintain normal metabolic reactions. K^+ also plays a role in maintaining turgor. Na^+ levels on the other hand should be less than 1 mM in cytoplasm.Any excess has to be excluded out of the cell or sequestered in the vacuolar compartment. Addition of Ca^{2+} could protect the membrane structure under salt-stress (**Nilsen and Orcott ;1996**).

The bryophyte *Physcomitrella patens* is unlike any other plant is that it possesses a gene that encodes an ENA-type Na^+-ATPase. The importance **of** having a Na^+-ATPase in plants was determined by conducting physiological analysis **of** PpENA1 in *Physcomitrella*. Maximum induction was obtained after 8 h at 60 mM NaCl or above. No other abiotic stress tested led to significant increases in *PpENA1* expression. In the gametophyte, strong expression was confined to the rhizoids, stem and the basal part **of** the leaf. In the protonemata, expression was ubiquitous with a few filaments showing stronger expression. At 100 mM NaCl, wild-type plants were able to maintain a higher K^+ to Na^+ ratio than the *PpENA1* (*ena1*) knockout gene but at higher NaCl concentrations , no difference was observed. Although no difference in chlorophyll content was observed between *ena1* and wild type at 100 mM NaCl, the impaired Na^+ exclusion in *ena1* plants led to an approximately 40% decrease in growth.

The membrane potential across the plasma membrane is approximately −150 mV for most plant cells.When this negative potential is combined with a difference in pH, the resulting electrochemical potential difference H^+ energizes H^+-coupled transport catalyzed by the many symporters and antiporters in the plasma membrane and hence allows the uptake of essential nutrients, such as K^+ and efflux of toxic solutes, such as Na^+.A gene encoding an AtSOS1 homolog and two ENA-type ATPases has been identified in *Physcomitrella*. The physiological importance of having two Na^+efflux systems in plants is not known, but it is possible that PpENA1, like Na^+-pumping ATPases in fungi, is responsible for the extrusion of Na^+ into the surrounding medium and hence contributes to the high NaCl tolerance of *Physcomitrella*. P-

type ATPases, are used to translocate a diverse set of ions including H^+, Na^+/K^+, H^+/K^+, Ca^{2+} plus heavy metals and possibly lipids (**Axelsen and Palmgren, 1998; Kuhlbrandt, 2004**). This superfamily is divided into five major branches and 10 subfamilies, according to the substrate being transported.

The large (25- to 30-fold) transcriptional upregulation specifically in response to NaCl, implies an important functional role of PpENA1 in salinity tolerance. This up-regulation is much more substantial than the 2- to 5-fold up-regulation of the salt-responsive Na^+/H^+ antiporters observed in vascular plants (**Shi *et al*., 2002; Shi and Zhu, 2002; Yokoi *et al*., 2002;**). Except for osmotic stress, none of the other abiotic stresses tested led to increased *PpENA1* transcript levels, confirming the specificity of the response. A 6-nucleotide element (GT-1) was recently identified in the promoter of a calmodulin from soybean **.** The promoter was not responding to ABA but the presence of this GT-1 element led to strong up-regulation upon salt treatment. An identical element was identified within the first 500 bp of the *PpENA1* promoter suggesting that this element could be the core-regulating cis-element.

*AtNHX1*is up-regulated after exposure to high levels of NaCl or KCl (**Gaxiola *et al*., 1999; Shi and Zhu, 2002; Yokoi *et al*., 2002**). A similar induction **of** *OsNHX1* is observed in rice (*Oryza sativa*) after treating plants with NaCl or with isoosmotic levels **of** mannitol.

AtSOS1 is mainly expressed in the epidermis cells **of** the root tip, the pericycle and the parenchyma cells bordering the xylem, suggesting that AtSOS1 is involved in xylem loading-unloading **of** Na^+ and some **exclusion** into the soil (**Shi *et al*., 2002)**.The correlation between *PpENA1* expression and salt tolerance raises the possibility that heterologous expression **of** *PpENA1* may improve the salt tolerance **of** crop plants. Heterologous expression **of** *AtNHX1* in diverse plants like mustard (*Brassica),* tomato (*Solanum lycopersicum*) and wheat (*Triticum aestivum*) allowed all three crops to grow under highly saline conditions and even produced a higher yield with similar properties to wild-type plants growing under nonstressful conditions (**Zhang *et al*., 2001; Zhang and Blumwald, 2001; Xue *et al*., 2004).** Thus a more efficient system for dealing with Na^+ by changing a single trait is possible and can

confer salt tolerance to crops not only in the greenhouse, but also in field trials (**Xue *et al.*, 2004).**

The H^+-**A**TP**a**se tr**a**nsports protons out **of** the cell **a**cross the pl**a**sm**a** membr**a**ne thus est**a**blishing the proton electrochemic**a**l gr**a**dient th**a**t contributes to the m**a**inten**a**nce **of** the intr**a**cellul**a**r **a**nd extr**a**cellul**a**r pH drives second**a**ry tr**a**nsport **of** ions **a**nd met**a**bolites. **A**s solute tr**a**nsport is directly rel**a**ted to osmotic w**a**ter movement, the H^+-**A**TP**a**se is **a**lso **a** key pl**a**yer in turgor regul**a**tion **a**nd thus regul**a**tes the cell size e.g. during stom**a**t**a**l **a**perture .The H^+-**A**TP**a**se h**a**s **a**lso been proposed to pl**a**y **a** direct role in the regul**a**tion **of** growth **a**nd development. It is regul**a**ted **a**t both the tr**a**nscription**a**l **a**nd post tr**a**nsl**a**tion**a**l levels by **a**uxin .**A** m**a**jor growth hormone h**a**s been proposed to be **a** key pl**a**yer in cell elong**a**tion. The tr**a**nsducing p**a**thw**a**y le**a**ding from **a**uxin to H^+-**A**TP**a**se **a**ctiv**a**tion is unknown. Sodium is highly toxic when it **a**ccumul**a**tes within the cell. Sever**a**l exclusion mech**a**nisms exist to m**a**int**a**in the sodium concentr**a**tion low within the cytosol. One **of** these involves exclusion within the v**a**cuole, which relies on N**a**$^+$/H^+ **a**ntiport energized by the tonopl**a**st H^+-**A**TP**a**se **a**nd pyrophosph**a**t**a**se. **A**nother involves N**a**$^+$ efflux out **of** the cell through **a** N**a**$^+$/H^+ **a**ntiporter (SOS1 in) thought to be **a**ctiv**a**ted by the pH gr**a**dient gener**a**ted **a**cross the pl**a**sm**a** membr**a**ne by H^+-**A**TP**a**se (**Blumwald *et al.*, 2000; Zhu, 2003; Yamaguchi and Blumwald, 2005**). The role **of** the l**a**tter h**a**s been inferred from the observ**a**tion **of** incre**a**sed H^+-**A**TP**a**se **a**ctivity under s**a**lt stress conditions (**Morsomme and Boutry, 2000; Palmgren, 2001)**. More direct evidence w**a**s provided by the observ**a**tion th**a**t **a**n mut**a**nt disrupted in the H^+-**A**TP**a**se *AHA4* gene h**a**s incre**a**sed sensitivity to s**a**lt stress **(Vitart *et al.*, 2001**). A **constitutively a**ctiv**a**ted H^+-**A**TP**a**se incre**a**sed s**a**lt toler**a**nce during the germin**a**tion **a**nd growth **of** seedlings monitored.

Pl**a**nts homozygous for ***A**HA4* disruption showed **a** dr**a**m**a**tic reduction in rosette growth when grown under s**a**lt stress conditions **a**nd this correl**a**ted with **a** 4- to 5-fold incre**a**se in the N**a** to K r**a**tio in le**a**f tissues (**Vitart *et al.*, 2001).**

The C**a**$^{2+}$-dependent phosphoryl**a**tion **of** H^+-**A**TP**a**se **a**nd the resulting inhibition **of** H^+ pumping h**a**ve been reported in root cells **of** o**a**t (***A**vena **s**ativ**a***) **a**nd beet (*Bet**a** vulg**a**ris*) (**Schaller and Sussman, 1988; Suzuki *et al.*, 1992; Lino *et al.*, 1998)**. Ca^+ATPase is located in the endoplasmic reticulum, plasmalemma ,tonoplast, mediates Ca^{2+} sequestration in the

cell.**Guerrero and Crossland (1993)** reported increased expression of genes encoding transmembrane channel proteins.

Transgenic plants expressing the **AHA**3 isoform activated by deletion within its inhibitory C-terminal region displayed reduced growth inhibition when seedlings were grown in vitro at a pH below 5.0 (**Young *et al*., 1998)**. Probably because these young seedlings lacking a cuticle, the alkaline phloem might be more sensitive than other cells within the stem, leaves and roots to the steeper pH gradient across the plasma membrane.Involvement of the proton pump in plant resistance to salt stress has been suggested by the up-regulation of H^+-ATPase genes seen in the presence of salts (**Morsomme and Boutry, 2000; Palmgren, 2001)**. However, direct evidence is still lacking.

In the presence of 200 mM NaCl, germination was delayed. However, ΔPMA4 seeds germinated faster and with a better yield than wild-type seeds. The better salt resistance of ΔPMA4 plants was also shown by the fact that after germination, only plantlets from these lines were able to develop green leaves in addition to cotyledons in medium containing 150 mM NaCl.

Calreticulin is an important calcium binding protein with chaperone functions and plays a pivotal role in regulating calcium homeostasis and protein folding in the endoplasmic reticulum of plants (**Menegazzi *et al*;1993,Wang *et al*;2004)** Calreticulin was down-regulated in rice under osmotic stress (**Zang and Komastu;2007)** .This indicates the main role of calcium as a main secondary messenger for soybean seedlings under salt stress.

Chaperones act to repair the potential damage caused by misfolding of proteins. Most newly synthesized proteins can fold in the absence of chaperones, but a minority strictly requires them (**Horvath *et al*;2008**). The 20-kDa chaperonin is up-regulated protection of proteins by the chaperone in soybean is very important to prevent misfolding of proteins under salt stress. The large 50S ribosomal subunit catalyses the peptidyl-transfer reaction of messenger RNA-directed protein biosynthesis **(Kotusov;1976**) Down regulation of the 50S ribosomal protein indicates the inhibitory effect of NaCl on soybean protein biosynthesis and presumably leads to the consequent reduction in plant growth.

Several proteins have been identified which comprised of stress and defense-related enzymes and proteins including histone H4 (**Patat *et al*., 2004**), chitinase, heat shock and dehydration responsive proteins, reactive oxygen-related enzymes such as lipoxygenase, peroxidase and superoxide dismutase also present were such cytoplasmic markers as 14-3-3 proteins ribosomal proteins and a cytochrome P450.

Farhoudi *et al*. [2007] found salt tolerance of canola cultivars had a direct relationship with Na^+/ K^+ ratio so that the ratio increased with the increase of salinity level but less increase was observed in tolerant cultivars, They concluded that Na^+/ K^+ ratio would be a measure of salt stress tolerance. Abiotic stresses result in transient increases in cytosolic Ca^{+2} either through influx from the apoplastic space or release from the interior (**Knight, 2000, Sanders *et al*.,1999).** Ca^{+2} release is regulated by ligand sensitive Ca^{+2} channels. Inositol polyphosphates, cyclic ADP ribose, nicotinic acid adenine dinucleotide phosphate could act as second messengers. These molecules have been found to be able to induce Ca^{+2} release in plant cells (**Schroeder *et al*;2001).** Calcium has been observed to have a protective effect under sodium stress both in solution culture and in soils that had increased calcium supply. This effect could be due to increased availability of cytosolic Ca^{+2}. When the *Arabiodopsis* ACA4 gene that codes for vacuolar Ca^{+2} ATPase was expressed in yeast, it increased the salt tolerance of the yeast cells (**Geisler *et al*;2000**). An early detectable response to sodium stress is the rise in cytosolic free calcium concentration(**Knight, 2000**). Sodium stress is sensed by an unknown receptor and calcium signal serves as a second messenger. Genetic studies suggested that the sensor protein for this salt-induced calcium signature is the Ca^{+2} binding protein SOS3. A loss of function mutation in this protein renders the plant hypersensitive to salt stress. Sodium extrusion (**Shi, *et al*;2000,Shi *et al*;2002**)is achieved by plasma-membrane localized Na^+/H^+ antiporter SOS1. Mutations in SOS1 rendered the mutant plants sensitive(**Wu *et al*;1996**) to Na. Plasma-membrane vesicles from plants have a Na^+/H^+ antiporter activity,which is enhanced by pretreatment with salt stress **(Qiu *et al* ;2002).**

These channels show high selectivity for K^+ over other monovalent cations and are reputed to specifically mediate K^+ uptake ,transport in plant cells (**Gambale and Uozumi, 2006).** K^+ channels and transport systems play multiple roles in higher-plant processes, including opening

and closing of stomatal pores, leaf movements and ion uptake in roots . AKTl is an inward-rectifying channel for K^+ uptake in roots. Elevated cytoplasmic Na^+ impaired the K^+ permeability mediated by AKTl (**Qi and Spalding, 2004).** The expression of OsAKTl is regulated differently in salt-sensitive and salt-tolerant cultivars of rice (**Golldack *et al*., 2003**). These results suggest the inhibition of K^+ uptake mediated by these channels as a possible cause of toxicity of Na^+. The similarity between OsKAT1 and inward-rectifying K+ channels suggests that OsKAT1 mediates cellular K^+ uptake as KAT1 and AKT1 **(Cao *et al*; 1995).**

P_{1B}-type heavy-metal ATPases (HMATPases) are transmembrane metal-transporting proteins that play a key role in metal homeostasis. Semiquantitative reverse transcription polymerase chain reaction analysis of seedlings showed that *OsHMA9* expression was induced by a high concentration of copper (Cu), zinc (Zn) and cadmium. Through promoter *β*-gluconidase analysis, It was observed that the main expression was in the vascular bundles and anthers. The OsHMA9 green fluorescence protein fusion was localized to the plasma membrane **(Lee *et al*;2007).**

Tolerance in plants grown on metal-polluted soil can be accomplished either by excluding the uptake mechanisms from the roots or by metal efflux, compartmentation and detoxification following that uptake. In dicot species, P_{1B}-type **A**TPases play a role in metal detoxification via efflux **(Williams and Mills, 2005; Andres-Colas *et al*., 2006**).

A K^+-stimulated adenosine triphosphatase (ATPase) has been identified in plasma membrane preparations from roots of several higher plant species (**Hendrix *et al*; 1977**). In each case the enzyme appears to be similar in pH optimum, cation stimulation and substrate specificity.

Fisher *et al*. (1970) suggested that this ATPase mediates energy transfer to a K^+ transport system.The enzyme has been strongly implicated in K^+ transport. K^+-stimulated adenosine triphosphatase was partially characterized in plasma membrane from meristematic and mature soybean root tissue. The substrate concentrations required for maximum enzyme activity (3 mμg Mg.ATP) and pH optimum (6.5) were similar for both systems. Enrichment studies performed to ensure that the membrane vesicle preparations were comparable indicated similar purity levels at selected steps during purification Phospholipid and sterol analyses further substantiated their similarity. The plasma membrane H^+ ATPase generates the potential required to drive in positive ions into the cell . In vacuolar membranes another H^+ -ATPase creates the potential required for

the uptake of K^+ and/or Na^+ into the vacuole by the activity of transporters like a Na^+/H^+ antiporter, which would regulate cytoplasmic Na^+ sequestering it into the vacuole **(Gorham *et al.*, 2009).** The compartmentation of Na^+ into the vacuole, through vacuolar Na^+/H^+ antiporters provides an efficient mechanism to avert the deleterious effects of Na^+ in the cytosol and maintain an osmotic potential by using $Na^+$and chloride accumulated in the vacuole to drive water uptake into cells (**Apse *et al.*, 1999**). Two approaches have been used to increase solute contents in plant vacuoles (**Pasapula *et al.*, 2011**). The first approach involves increasing the activity of a vacuolar sodium proton (Na^+/H^+) antiporter that mediates the exchange of cytosolic Na^+ for vacuolar H^+. The second approach involves increasing the activity of the H^+ pump on the vacuolar membrane to move more H^+ into the vacuoles therefore generating a higher proton electrochemical gradient (DIH^+) that can be used to energize secondary transporters including vacuolar Na^+/H^+ antiporters. Both approaches enhance Na^+ accumulation in the vacuoles and reduce the potential of Na^+ toxicity in the cytoplasm, leading to higher salt tolerance. It is believed that the sequestering of Na^+ in the vacuoles confers two advantages : reduced toxic levels of Na^+ in cytosol and increased osmotic potential of the vacuole and therefore a more negative water potential that aids water uptake by the cells and water retention under high salt conditions **(Lubbers *et al.*, 2007**). The distribution of activity of a potassium stimulated ATPase associated with the plasma membrane was determined in 4 days old soybean roots. Changes in protein based specific activity of the enzyme coincided with developmental changes in the root. Activity was low in the region of the root cap, increased to a maximum in the merlstematic region, decreased to a minimum as cell elongation proceeded, and then increased as lateral root development began. There was a fluctuations in enzyme activity which could be described by a three-phase system and could be approximated by a linear-linear-linear piece-wise regression curve. The need for constructing a biological model to describe plasma membrane development is suggested. A K^+stimulated adenosine triphosphatase (ATPase), thought to be an integral part of the ion transport system in roots of higher plants, has been suggested as a component of the plasma membrane of root cells (**Nagahashi *et al*;1978)** Although the relationship has not yet been proved unequivocally, it is favoured by several lines of evidence (**Leonard &Hotcskss;1976**).

It was reported earlier that differential activity levels for the enzyme in soybean root tissue as related to developmental status (**Travis & Booz;1979**). In vitro acitivity was greater by 2- to 3-fold in vesicles prepared from meristematic root tissue (tip 3-4 mM) than in vesicles prepared from mature tissue (1.5-4 mM behind meristematic zone). Those data were compared with reported distribution of K^+ uptake by roots (**Brown & Cartwright;1953, Scott&Martin;1962).** The observation suggested that the enzyme is apparently a dynamic, not a static, component of the plasma membrane. One major implication of that conclusion is that the developmental status of the plasma membrane of a given root tissue might then be deduced from its level of K-stimulated ATPase activity. There was a significantly greater ATPase activity, per unit membrane protein, in the meristematic region. Mixing experiments indicated that the lower level of activity associated with vesicles from mature tissue was not due to endogenous inhibitor(s).There are Differences in K^+ uptake along successive segments of root tips have been well documented.

Brown and Cartwright (1953), reported that absorption per unit area of root tip was greatest in the segment which was 1.5 to 3.00 mM from the apex.The vacuole in meristematic cells is either absent or quite small relative to that in mature cells.The substrate for the K^+-stimulated ATPase associated with the plasma membrane of oat, corn and soybean roots is Mg^+ ATP have been studied. **Balke and Hodges (1975)** reported that activity was maximum when the Mg to ATP ratio was 1:1. Activity was inhibited by an excess of free ATP or Mg^{2+} .A time course study of enzyme activity determined at 3 millimolar mg ATP indicated linear kinetics for both enzyme systems during the 30-minute assay period.

A Lineweaver-Burk plot of the substrate concentration data showed a typical substrate inhibition curve. Similar results have been reported for oat root plasma membrane ATPase . The apparent Michaelis constant and maximum velocity for the ATPase were estimated from Mg ATP concentrations of 1 to 7.5mM. The Km values were 0.90 for meristematic and 0.7 mg ATP for mature vesicle enzymes. Vmax for the ATPase was significantly higher for meristematic tissue than for mature tissue (33.0 versus 12µ mol Pi/mgprotein.h). Km values was within the range estimated to be available in the cell. The Km of the enzyme can be taken as an indication of

cellular substrate levels (**Segel ; 1975**). It is likely that proportions of the Mg^{2+}-dependent and K^+-stimulated components may have shifted during development.

Most activity in the crude homogenate represents non specific acid phosphatase not associated with the plasma membrane.Since the specific activity of the K^+-stimulated ATPase of vesicles isolated from meristematic tissue was two to three times as great as that from mature tissue. It was interpreted that K^+ uptake is greater in meristematic tissue than in mature tissue. That has been demonstrated for root segments of comparable length from corn (**Brown &Cartwright;1953**)and broad bean (**Scott & Martin;1962**).The distribution of activity of a potassium-stimulated ATPase associated with the plasma membrane was determined in 4-day-old soybean roots. Changes in protein-based specific activity of the enzyme coincided with developmental changes in the root. Activity was low in the region of the root cap, increased to a maximum in the meristematic region, decreased to a minimum as cell elongation proceeded, and then increased as lateral root development began.Study of root anatomy indicated that the soybean root tip was similar to other dicotyledonous species. Section I consisted primarily of root cap cells plus meristematic cells. Section 2 consisted essentially of meristematic cells plus recently derived cells which had not yet begun significant enlargement.Cellular development had progressed to the elongation stage by segment 3.

P-type H-ATPases are active transporters that utilize ATP as an energy source to transport H^+ across the plasma membrane. This in turn, creates an electrochemical gradient that energizes channels and co-transporters **(Duby and Boutry, 2009**). The plasma membrane H-ATPases belong to a large family of pumps P-type ATPases, all of which are energized by ATP and form a phosphorylated aspartyl intermediate during the reaction cycle, therefore the name P-type. The P-type ATPase family is further divided.The plasma membrane H pump is subject to regulation by a number of proteins interacting directly with the pumps.

Pairs of guard cells form stomatal pores regulate gas exchange between plant cells and the surrounding atmosphere. Light (primarily blue) stimulates stomata opening by activating the plasma membrane H-ATPase. Blue light induces rapid and highly sensitive stomata opening correlated with the phosphorylation of a plasma membrane H-ATPase pump and increased H^+

pumping, which results in the activation of voltage-gated K channels by membrane hyperpolarization (**Shimazaki *et al*. 2007**) along with the inhibition of S-type anion channels.

Closure of stomata occurs as response to light to dark transition, high CO_2 levels and the hormone abscisic acid (ABA). Studies of mutants with ABA insensitive stomata have revealed that the plasma membrane H-ATPases in guard cells are important for the ABA induced closure. H-PPases require Mg^{+2} as a cofactor for the formation of the MgPPi complex and the resultant active conformation.Sequences alignments have revealed the existence of only two subfamilies of H-PPase. Type I family members are K-dependent and type II are K-independent enzymes (**Belogurov and Lahti 2002**).K-dependent H-PPases have been found in higher plants. (**Takeshige *et al*. 1988, Docampo *et al*. 2005,Sarafian *et al*. 1992**)

Vacuolar sodium sequestration is a conserved mechanism used by salt tolerant plant species. Overexpression of the type I H -PPase AVP1 resulted in plants with enhanced salt tolerance and drought resistance .The salt tolerant phenotype of these plants was explained by an increased uptake of Na^{+} into their vacuoles. The drought related phenotype was originally attributed to an enhanced vacuolar osmoregulatory capacity (**Gaxiola *et al*. 2001).**Other groups have subsequently demonstrated that overexpression of this and other plant genes encoding for a type I H PPase can increase both salt- and drought-tolerance in heterologous systems . An mutant has been characterized in which energization of vacuolar transport solely relies on the activity of the H-PPase (**Krebs *et al*. 2010).**

Proton gradients are crucial for the transport of ions and solutes across the different membranes in plant cells. Several important developmental processes require a tightly controlled proton gradient across cellular membranes. Of the three primary proton transport proteins: the plasma membraneH-ATPase and the H-PPase are crucial. Plants have two phylogenetically distinct types of H-PPases: type I and type II. Type I H-PPases depend on cytosolic K for their activity and are moderately sensitive to inhibition by Ca^{2+}, and type II H-PPases are K-insensitive but extremely Ca2þ-sensitive. Type I H-PPases have been shown to acidify the plant vacuole. The resulting H and electrochemical gradient is instrumental for the storage of sucrose, organic acids, regulation of hydrostatic pressure through the storage of inorganic ions and cytoplasmic detoxification **(Maeshima ;2001**).

In plants , MAPK constitute a prominent signaling pathway , which relays signals by sequential phosphorylation (**Zhang *et al*;2001**)' . Involvement of MAPK-phosphatase in salt stress response has been suggested. The PTPase is transiently activated under elevated salinity, but reversed by cold treatment**(Xu *et al*;1998).** That a protein specifically related to tonoplast K^+ ion channel is regulated by tyrosine phosphorylation and dephosphorylation . Oxidative stress reversibly inactivates PTPase by oxidative cysteine residue in catalytic region, which is essential for PTPase activity **.** Oxidative stress has profound effect on protein phosphatase during growth and development of peanut seedlings **.** PTPases are associated in counter-acting the effect of environmental stresses such as temperature and oxidative stresses which affect the plant growth and development .

The vacuoles are organelles that fulfill highly specialized functions depending on tissue, cell type, and/or developmental stage. All vacuoles seem to contain vacuolar H-ATPases (V-ATPases) and H-PPases that differ in their function depending on the type of vacuole in which they reside (**Martinoa *et al*. 2007**).Tr**a**nsgenic tob**a**cco (*Nicoti**a**n**a** t**a**b**a**cum*) pl**a**nts expressing either wild-type pl**a**sm**a**membr**a**ne H^+-**A**TP**a**se4 (wtPM**A**4) or **a** PM**A**4 mut**a**nt l**a**cking the **a**utoinhibitory dom**a**in (ΔPM**A**4), gener**a**te **a constitutively a**ctiv**a**ted enzyme .

High levels of Na^+ or high Na^+ to K^+ ratios can disrupt various enzymatic processes in the cytoplasm owing to the ability of Na^+ to compete with K^+ for binding sites. The sensitivity of cytosolic enzymes to Na^+ is similar in glycophytes and halophytes, indicating that the maintenance of a low cytosolic Na^+ to K^+ ratio is a key requirement of plant growth in saline soil(**Apse and Blumwald, 2002**). Specialized transport proteins, in the form of channels, carriers, or pumps, mediate the movement of heavy metals through membranes (**Williams *et al*., 2000).** Several types of heavy-metal transporters have now been cloned from plants **(Williams *et al*;2000; Clemens, 2001)**. The ion pumps in the P-type ATPase superfamily share a common enzymatic mechanism in which ATP hydrolysis aids in transporting ions across the membrane (**Pedersen and Carafoli, 1987).**

Sodium is actively excluded from the cytosol and sequestered in the vacuole, takes allowing water to move into the cell. Na^+/H^+ antiporters mediate the uptake/exclusion of Na^+. The activities of these carriers are coupled to the downhill flux of H^+ leading to generation of H^+ electrochemical gradient across the plasmalemma or tonoplast, which is catalysed by H^+ - ATPase in the plasmalemma (P-ATPase) and tonoplast (V-ATPase), and H-pyrophosphatase in the tonoplast. , Osmotic stress may result in the disturbance of the plant water relations in the uptake and utilization of essential nutrients, and also in toxic ion accumulation (**Gouia *et al*., 1994).** As a result of these changes, the activities of various enzymes and plant metabolism are affected (**Munns, 2002)**. The competition and interactions of soluble salts with mineral nutrients may cause considerable nutrient imbalances and deficiencies (**Rathert, 1982).**

In *Arabidopsis*, a *NAC1* gene was expressed at high levels in root tips and lateral root initiation sites and at low levels in stems and leaves **(Xie *et al*., 2000).** Proteins and photosynthetic pigments did not show any remarkable change under salt stress whereas, treatment of 24-epibrassinolide and polyamines enhanced the titers of protein and photosynthetic pigments of leaves with/or without salt stress. An increase in carotenoid content was observed with salt (150 and 300 mM) stress, which further improved remarkably by supplementation of polyamines and 24-epibrassinolide (**Sharma *et al* ;2013**).

Some salt-inducible genes have been investigated in soybean. A homologue of oxysterol-binding protein was involved in the salt-stress response and cotyledon senescence of soybean (**Li *et al*.,2008).** An acidic isoform of pathogensesis related protein group 5 (PR-5) that is responsive to high salt stress. A leucine-zipper-like protein was induced under salt stress and acted in mature organs of soybean shoots to counteract water potential change, (**Aoki *et al*.,2005)** An acid phosphatase was related to the adaptation of soybean to salt stress, and was involved in reactive oxygen species formation or scavenging or in stress responsive signal transduction pathways (**Liao *et al*.,2003).** The overexpression of a dehydration responsive element binding protein homologous gene (GmDREB2) in soybean caused the accumulation of a higher level of free proline compared to wild-type plants under salt stress; this gene also was an important transcriptional activator and was useful in improving plant tolerance to salt stress **(Chen *et***

***al*.,2007).** These salt stress-induced genes may lead to up-regulation or down-regulation of salt stress related proteins.

Abbasi and Komatsu (2004) studied salt responsive proteins in rice using a proteomic technique, which indicated that an oxygen evolving enhancer protein expressed in the leaf sheath and leaf blade of rice showed a coordinated response to salt stress.The pre-sowing treatments cause initiation of the early metabolic processes and re-drying of seeds arrest, but do not reverse, the initial stages of germination so that on the availability of suitable conditions, the time taken to germinate is reduced (**Bewley and Black ;1982**). During priming, the embryo expands and compresses the endosperm **(Liptay and Zarrifa ;1993**) The compression force of the embryo and hydrolytic activities on the endosperm cell walls may deform the tissues that have lost their flexibility upon dehydration **(Lin *et al*;1993),** producing free space and facilitating root protrusion after rehydration.

The final yield of soybean will be reduced when soil salinity exceeds 5 dS/m **(Ashraf 1994).** It was shown that the average production of 20 soybean cultivars under non-saline conditions (control), 14-15 dS/m, and 18-20 dS/m were 2261.4 ± 438.3 kg/lim^2 (control), 1 073.4 ± 267.1 kg/lim^2 (47.5% of control), and 880.8 ± 259.9 kg/hrrf (38.9% of control), respectively (**Chang *et al*. ,1994).**

High salt imposes damages in the whole life cycle of soybean. The degree of salt tolerance of soybean germplasms varies among different developmental stages. The germination of soybean seeds was delayed in low salt conditions (0.05% and 0.1 % NaCl). The order of salt tolerance in the germination stage is as follows: imbibitions > emergence of radical > growth of radical > growth of lateral roots (**Shao *et al.* 1994b**). High salt tolerance in the germination stage does not infer a similar tolerance in the adult stage . Soybean cultivars “Lee”, “Coiquitt” and “Clark 36”, showed a similar degree of salinity-induced reduction in germination rate. However, deleterious effects of salt on plant height and shoot dry weight were much lower in “Lee” than the other two cultivars **(Essa ;2002).** The seedling stage of soybean is considered to be much more sensitive to salt stress than the germination stage **(Hosseini *et al*. 2002**). The growth of seedings at 220mmol/l NaCl severely declined to 5 % when compared with the unstressed control, whereas stunted growth was observed at 300 mmol/l NaCl. Germination (40%) was still possible even

when the Na^+ concentration in the embryonic axis reached 9.3 mg/g FW (fresh weight), Whereas the growth of seedlings was completely inhibited when the tissue Na^+ Concentration attained 6.1 mg/g FW.

The agronomic traits of soybean could be severely affected by high salinity , including reduction in height , leaf size, biomass , number of internodes, number of branches, number of pods, weight per plant, and weight of 100 seeds **(Chang *et al.* ,1994**). Salt stress reduced the protein contents in soybean seeds (**Chang *et al.*,1994., Wan *et al.* ,2002).**

Although nodulation is a target of salt stress-induced damage, the number of nodules and the efficiency of nitrogen fixation are positively correlated with the salt tolerance capability of both the soybean host and the symbiotic Bradyrhizobia/Rhizobia strains **(Velagaleu and Mursh 1989).**

The early processes involved in nodule formation by soybean were extremely sensitive to NaCl.The supply of photosynthate required by the host for shoot growth, nodule initiation, development, and nodule function is also sensitive to salinity ,(**Jensen;1975**) evaluation of symbiotic processes exposed to stress requires that the processes be independently subjected to the stress.

Introducing NaCl into the rooting medium prior to inoculation reduced nodule formation at every concentration applied.Response of soybean cell culture to NaCl resulted in decrease in protein content and protein molecular weights. A significant negative relation was found between cell membrane stability and total protein content.

Root cells have a much less turger threshold pressure than that of stem cells thus root growth is more than stem growth under salt and drought stresses. Therefore, root is significantly less affected by salt stress in comparison to stem **(Abd-Ala *et al*;1998,Maziah *et al*;2009,Sadeghi;2009)** In the early stages (i.e. day '0' to day 3), all the living cells· showed deep azure B positive tinge around the PBs, which was a shade deeper, in the abaxial layers than in the adaxial layers, it being deepest in the hypodermis. The degree of cytoplasmic stainability for RNA decreased with progress in germination, which could be directly correlated with the degree of fragmentation. In day 9 cots the companion cells in the phloem still showed intense RNA.The neutral lipid appeared as faintly stained (NBC), viscous mass over the PB mass -in the early

stages of germination in most cells of the cot. but discrete oil globules appeared in the cells only when pretreated with 50 % ethanol overnight prior to staining with NBC or in sections of cots of advanced stage of germination. From day 1 to day 5 there was not much qualitative change in the oil globules in most cells but subsequently there was progressive decrease in the oil globule content of the cells.The initial 24 hours period of germination was marked by the physical absorption of water without apparent biochemical changes as judged by the absence of any visible change in the reserves of the cells. There was a definite upward trend in the starch content of the cells in the first 5 days of germination which was followed by a gradual downward trend in the subsequent days as judged by their appearance and sustainability. Some starch synthesis during the early upward trend could be related to the photosynthetic activity in the greening cots, it is also possible that part of it could have originated from sources' other than photosynthetic activity, for several reasons. Firstly, starch grains appeared even in the cells of the cots of dark-germinated seedlings, much in the same way as in those of greenhouse-germinated ones. Secondly, they appeared even in the immature chloroplasts of palisade-like cells of nongreen cots of day 2. Thirdly, they also appeared in the plastids of the interior cells, some of which are apparently amyloplasts. The source of this starch could be from the lipid reserves 'which were abundant in the cots. The conversion of fat to carbohydrate via β-oxidation of fatty acids and glyoxylate shunt is well authenticated in oil-rich seeds during their germination **(Kornberg and Beevers 1957)**. Further, the formation and the activity of the microbodies (i.e. glyoxysomes) during germination of oil containing seeds was also reported .

Judged from the definite topological trend in the fragmentation of PBs, it can be concluded that there was a marked physiological zonation in the cots in so far as the enzyme activity was concerned. Degradation probably started in the peripheral layers, especially on the abaxial side and progressesed towards the interior. A similar trend was noticed for other reserves also. A local hormonal stimulating effect on the enzyme activity in these layers much in the same way as gibberellic acid (GA) controlled α-amylase activity was observed in the aleurone layer of barley GA induced synthesis of protease (**Jacobsen and Varner 1967).** The qualitative trend observed in the oil globule content of the cot cells from day 5 to day 14 suggested their accelerated

depletion during this period. Similar depletion following a peak lipase activity reached on fifth day during germination of soybean seeds **(Holman 1948).** *F.oxysporum* f. sp. glycines is a seed-borne pathogen found frequently in soybean seed lots (**Nasir, 2003**). *F. oxysporum* f. sp, glycines reduces germination by rotting in severely infected seeds of soybeans (**Velicheti and Sinclair, 1989)**. Other related diseases on soybeans are blight or wilt and root rot (**Akinsanmil and Adekunle, 2003)**. It was reported that *F. oxysporum* has ability to colonize seed surfaces and seed coats in soybean seeds (**Velicheti and Sinclair, 1989).** Among fungi different species of *Trichoderma* are popularly used in biological control programs against different fungi (**Kloepper et el., 1992; Gardener and Fravel, 2002).** The site of infections of soybean seeds by *F. oxysporum* f. sp. glycines and effect on seed germination and seedling establishment. Attempt was also made to screen potential antagonists against *F. oxysporum* f. sp. glycines in vitro.

Soybean seeds infected by *fusariun oxysporum* were slightly irregular in shape and appeared whitish moldy. The fungus mycelia colonized the external surface and inner tissues of the seed coat, but not in cotyledon or in embryo. Upper surfaces of seeds showed profuse colonization by mycelia and seed coat tissues became ruptured and distorted in the severely infected seeds. Vigorous mycelial growth was found in the hourglass layer of the seed coat. Effect of artificial inoculation on soybean seed germination and seedling survivability under glasshouse conditions revealed that *F. oxysporum* f. sp. glycines reduced seed germination and seedling survivabtility by 40% and caused pre-emergence damping off of seedlings. *Trichoderma harzianum* isolate UPM40 and *Pseudomonas aeruginosa* isolate UPM13B8 were most effective candidates in inhibiting the mycelial growth of *F. oxysporum* f. sp. glycines in vitro.

Spherical protein bodies (PB) were clumped together as, honeycomb-like mass in the middle of the cells. Each PB presented a hyaline core and deeply stained periphery and they underwent dissolution when treated with crude extract of papain from the fruits of *Carica papaya*. The PBs increasd in size due to swelling or coalescence both by day 4 and the large ones show densely stained core and loose ring around them.

Histopathological studies were conducted to determine the sites of infection in naturally infected

soybean seeds using Light Microscopy (LM) and Scanning Electron Microscopy (SEM). Seeds showing typical symptoms of disease causing *F. oxysporum* f. sp. glycines as well as asymptomatic (healthy) seeds were selected. Different seed components viz. seed coat, cotyledon and embryo separated carefully and cut transversely into 2-3 cm^2 pieces. Histopathological examinations of transverse sections of asymptomatic seeds under LM and SEM were observed. The mature soybean seeds consisted of a seed coat, cotyledon and embryo. The seed coat contained a cuticle and three distinct layers namely palisade cell layer, hourglass cell layer with prominent air-spaces and parenchyma cell (**Carlson and Lersten, 1987**). Transverse sections of infected seeds using LM and SEM showed the presence of fungal mycelia on seed surface and internal tissues of the seed coat. Mycelial growth was more abundant in the hourglass layer of the seed coat where large intercellular spaces were present. Fungal hyphae could be distinguished in the seed tissue based on hyphal morphology. Hyphae of the fungus were hyaline, branched and stained light green with toluidine blue (0.1%). The hyphal breadth ranged from 2.5-3.8 µm (**Sharma, 1992).** Mycelial mat were formed on the seed surface and beneath the seed coats. Severely infected seed coat appeared distorted and empty .Hyphae were not detected in any tissues of the cotyledons or in embryo in the infected seed. Effect of infection was assessed on inoculated seeds from observations of seed germination, pre and post-emergence damping off and seedling survivability under glass house conditions. *F. oxysporum* f. sp. Glycines significantly reduced seed germination and seedling survivability by 40% when compared with uninoculated seeds (control). The germination and survivability of seedlings were recorded at 51% in inoculated seeds and 85% in control seeds. The pathogen also caused pre-emergence damping off was recorded in inoculated seeds. Post-emergence damping-off of seedlings was not observed either in inoculated or uninoculated seeds .

+*Trichoderma harzianum* (UPM40) showed the highest potential ability to inhibit the growth of *F. oxysporum* f. sp. *Glycines.* Resources and space **(Ibrahim**, **2005**). *T. harzianum* was able to overgrow fully the fungal pathogen colony and caused lysis within seven days. When pea (*Pisum sativum*) roots are inoculated with the pea pathogen, *Nectriahaematococca*, most newly generated root tips remain uninfected even though most roots develop lesions just behind the tip

in the region of elongation. The resistance mechanism is unknown but is correlated spatially with the presence of border cells on the cap periphery.

An extensive light microscope study of root anatomy indicated that the soybean root tip is similar to other dicotyledons species. The cots. showed dorsiventral differentiation with cuticle, epidermal layers (i.e, a layer of cubical cells on each surface), two rows of columnar palisade-like cells on the adaxial side, a layer of closely placed, more or less oval hypodermal cells on the abaxial side and large parenchyma cells of varying sizes in the interior. Midrib and a network of vascular strands traversed the parenchyma. They were surrounded by border parenchyma cells. The stomata became visible in day 4 or and 5 cots.There were histological Changes during germination- In day '0' and day 1 cots, most of the cells were devoid of starch grains, although they were seen imperceptibly in palisade-like and adjacent inner cells. The cytoplasm, showed dense PAS positive stainability. The starch grains increased in number size and stainablity (both PAS, and I_2KI) during day 2 to day 5 in almost all cells of the cots. Stained with I_2KI, the grains appeared light blue in the initial stage but appeared deep blue-violet at later stage, apparently due to the increase in their amylopectin component. They appeared as clusters of spherical to oblong concentric ones in the parietally distributed plastids (chloroplasts or amyloplasts). The grains, in each cluster appeared to join to form large, polygonal compound by day 4 to day 5. Beyond day 5, there was progressive loosening of the compound grains into clusters accompanied by decrease in their size and stainability until finally only traces of them were seen in the inner parenchyma cells and to some extent in the palisade-like cells by day l1. This was accompanied by the decrease in PAS positive stainability of the cytoplasm. By this time the parenchyma cell walls were apparently breaking.

The physiological and molecular mechanisms of tolerance to osmotic and ionic components of salinity stress occur at the cellular, organ, and whole-plant level. Plant growth responds to salinity in two phases: a rapid, osmotic phase that inhibits growth of young leaves, and a slower, ionic phase that accelerates senescence of mature leaves. Plant adaptations to salinity are of three distinct types: osmotic stress tolerance, Na^+ or Cl^-exclusion, and the tolerance of tissue to accumulated Na^+ or Cl^-. The *HKT* gene family play a important role in Na^+ exclusion from

leaves. There is a limited molecular understanding of the overall control of Na^+ accumulation and of osmotic stress tolerance at the whole-plant level.

The use of isolated cells does restrict studies of salt tolerance mechanisms to those which are characteristic of individual cells. It does not lend itself to examination of more complex multicellular processes such as root ion exclusion, xylem / phloem ion exchange and redeposition (**jeschke 1984**), or mechanisms involving differentiated highly specialized cells, i. e. salt glands and bladders or transfer cells. It is the intrinsically cellular mechanisms however, which under conditions of extreme salinity ultimately may be the most important to cell survival; they are most readily modified genetically in glycophytic crop species.

Ion exclusion is often considered to be a mechanism used by glycophytes to tolerate NaCl, particularly at moderate levels of salinity (**Sacher *et al*; 1982).** There appears to be genetic variability for the ability to exclude NaCl within glycophytes species with greater exclusion being correlated with greater tolerance . In general, these observations have been interpreted to indicate that mechanisms for ion accumulation and presumably vacuolar compartmentation are not traits which are inherently efficient in glycophytes and may be one of the reasons for their relative susceptibility to saline environments. Although soybean is considered to be a glycophyte, when grown in culture the cells adapt to salinity in a manner similar to halophytes, utilizing Na^+ and Cl^- as primary osmotica and exhibiting a positive correlation between tolerance and intracellular concentrations of Na^+ and Cl^- (**Hasegawa *et al*. ;1987**) reported that the water content of cells declines during adaptation to salinity, resulting in as much as a 5-fold reduction in cell volume, even though increased water content is a common response when glycophytes are exposed to salt. Dryland salinity affects soils when groundwater is brought to the surface by capillary action; evaporation removes water and leaves salt at the soil surface. Water uptake by plants can also increase soil salinity. Water percolating through the ground has salts dissolved in it. Plant roots work by taking in water while excluding salts and other non-nutrients. The excluded salts will gradually build up around the roots.

The halophyte *Mesembryanthemum crystalinum h*as emerged as a model system for understand in the molecular response to salt-stress. This plant switches from C3 photosynthesis to

crassulacean acid metabolism (CAM) in response to salt or drought stress. Organic acids, oxalate and malate are important osmolytes in plants. Application of salt to plants brings about a major change in the protein profile. Salinity is a quantitative trait, and arrays of salt-induced genes have been isolated.

Under osmotic stress an important consideration is to accumulate osmotically active osmolytes in order to lower the osmotic potential which do not apparently interfere with the normal cellular metabolism. These molecules are not highly charged, but are polar, highly soluble and have a larger hydration shell. Mannitol functions as a protector or stabilizer of enzymes or membrane structures that are sensitive to dehydrations or ionically induced damage. Sorbitol is found in a variety of plant species, usually as a constituent of seeds. It functions as a trans-located carbohydrate and is also reported in vegetative parts in the halo-tolerant *Plantago marituma(***Ahmad *et al*;1979)** . It may contribute to the desiccation tolerance of the mature embryo.

Polyamines have recently gained importance in the escape of seedlings from the abverse effect of salinity. Suppression of polyamine biosynthesis by cyclohexylamine has been reported to result in increased ethylene synthesis as well as seed germination **(Gallardo *et al*;1995).** This suggests a cross-linking of pathways of polyamine and ethylene brosynthesis. **Lin and Kao (1995)** reported an increase in the level of spermidine under salinity. Polyamines such as spermine and spermidine are derived from methionine and ornithine. Under salinity and drought conditions polyamines as well as their corresponding enzyme activities are substantially enhanced **(Lefevre and Lutts;2000).**

Water source with an EC of 1.0 mMho/mM, is a quality suitable for irrigation of most crops. Salts accumulate in the rootzone by two processes : the upward movement of a shallow saline-water table and salts left in the soil due to insufficient leaching. Leaching is the process of applying more water to the field than can be held by the soil in the crop rootzone such that the excess water drains below the root system, carrying salts with it. The more water that is applied in excess of the crop water requirement, the less salinity there is left in the rootzone despite the

fact that more salt has actually been added to the field. The term leaching fraction (LF) is used to relate the fraction or percent of water applied to the field that actually drains below the rootzone.

Anuradha and Rao (2001) observed that Brassinoids (BRs) could significantly reduce the inhibitory effects of salt stress in rice plants by improving the level of nucleic acid and compatible solutes. Exogenous application of Putrescine (Put) and Spermidine (Spd) were able to ameliorate salt stress in barley seedlings by maintaining root tonoplast integrity (**Zhao and Qin;2004)** .Salt stress induces the synthesis of abscisic acid which is responsible for stomata closure. Consequently, photosynthesis gets affected and photo inhibition and oxidative stress occur **(Zhu *et al*;2007)** Moreover, ROS are also produced under stress conditions.

Sirhindi et. al;(2012) reported that BRs enhanced the activities of APOX, CAT and GR in *B. Juncea* under salt stress conditions. Application of putrescine had also been shown to ameliorate NaCl stress in chickpea plants through elevating the activities of CAT, GPOX, GR and SOD **(Sheokand *et al*;2008).**

Besides antioxidants and antioxidant enzymes, certain compatible solutes such as proline, sorbitol and glycinebetains also get accumulated that are actively involved in NaCl stress amelioration. The response of soybean seeds to salt stress during germination was investigated at both physiological and proteomic levels.

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A cascade of complex events involving several interacting components required for initial recognition of signal and subsequent transduction of these signals to the physiological response is triggered. The cascade of events is called signal transduction,which normally acts through second messengers that can trigger the molecular events leading to physiological response, often by modification of gene expression (**Trewavas and Malho;1997**).Many genes that are induced or upregulated by osmotic stresses have been identified (**Ingram and Bartel;1996,Bray ; 1997) .** Gene expression profiling using cDNA microarrays or gene chips has come up as an important tool in identifying many more genes that are regulated by drought or salt stress. (**Seki *et al* 2002 , Seki *et al* 2004**) Plant cells are connected with neighbouring cells by means of plasmodesmata which could allow signaling molecules to pass directly from cell to cell. **(Zambryski and**

Crawford 2000) .The products of stress-inducible genes are classified into two groups **(Seki *et al*.;2004)**. (i) Those which directly protect against stresses, and these are the proteins that function by proecting cells from dehydration. They include the enzymes responsible for the synthesis of various osmoprotectants like late embryogenesis abundant (LEA) proteins, antifreeze proteins, chaperones and detoxification enzymes. (ii) The second group of gene products includes transcription factors, protein kinases and enzymes involved in phosphoinositide metabolism. This group of gene products regulates gene expression and signal transduction pathways. Stress-inducible genes have been used to improve the stress tolerance of plants by gene transfer **(Hasegawa *et al*; 2000 , Shinozaki *et al* 2000 , Thomashow;1999)** . The signal transduction pathways in plants under environmental stresses have been divided into three major types **(Xiong *et al*;2002).** (i) osmotic/oxidative stress signaling that makes use of mitogen activated protein kinase (MAPK) modules; (ii) Ca^{+2} –dependent signaling that leads to activation of LEA-types genes such as dehydration responsive elements (DRE)/cold responsive sensitive transcription factors (CRT) class of genes, and (iii) Ca^{+2} –dependent salt overly sensitive (SOS) signaling that results in ion homeostasis. Two mutants for salt tolerance during growth at seedling stage of Arabidopsis were isolated. In one pst 1 mutant, it was shown that salt tolerance was due to enhanced scavenging of reactive oxygen species (ROS) . The transcriptional factors identified from mutations in the ABA signal transduction pathway seemed to be mainly functioning in seed development (**Tsugane *et al* ;1999**) . Molecular genetic analysis using stress-responsive promoter driven receptors was suggested as an alternative approach to genetically different types of abiotic stress signaling pathways.

On exposure to water deficit or salinity stresses, plants lower the osmotic potential of the cell cytosol and accumulate compatible osmolytes**(Xiong *et al*;2001**) . In glycophytes, the capacity for sodium compartmentalization and osmolyte biosynthesis is limited; however, an increased production of compatible osmolytes such as proline, glycine, betaine and polyols can reduce stress damage to plant cells. This is an adaptive strategy and transgenic plants with increased osmolyte production or decreased degradation showed improved salt and drought tolerance, **(Nanjo *et al*;1996**). Salt tolerance was also increased in transgenic plants engineered to produce new osmolytes absent in the parental lines or in plants that overexpressed the genes whose

products limit the production of these osmolytes **.**Upregulation of protein biosynthetic genes such as pyrroline-5-carboxylate synthase (P5CS) has been reported under salt or drought stresses (**Yoshiba *et al*; 1999) and (Xiong *et al*; 2001**). The transgenic plants overexpressing ROS scavenging enzymes such as superoxide dismutase, catalase and glutathione S-transferase (GST) showed increased tolerance to salt stress. The MAP kinase pathways are intracellular signal modules that mediate signal transduction from the cell surface to the nucleus. These kinases seem to be widely used as osmolarity signaling modules. The environmental signals are first perceived by specific receptors that upon activation initiare a cascade to transmit the signal intracellularly and in many cases activate nuclear transcription factors to induce the expression of specific sets of genes. MAPKs are activated in response to drought and other environmental stresses **(Agrawal *et al*;2003).**

Ca^{+2} dependent signaling leads to activation of LEA-type genes -The most common and widely reported genes that are stress regulated are the LEA or LEA-like genes. These are highly expressed in seeds during desiccation stage and in vegetative tissues in response to water deficit. **(Baker *et al* ;1988**),**Xu *et al;*1996)** Overexpression of individual LEA genes has been reported to confer stress tolerance in transgenic rice, (**Xu *et al*.,1996).** One group of genes in *Arabidopsis*, e.g. RD (responsive to dehydration) is strongly induced by salt and drought stresses. The enhanced expression of transcription factors that regulate the expression of these genes increased the tolerance of transgenic plants to drought and salt stress. It showed the protective effect of these proteins, which could be due to the prevention of denaturation of key proteins by acting as chaperones. (**Kasuga *et al*; 1999).**

To help understand the maintenance of elongation in the apical region of roots growing under water deficit conditions, **Spollen and Sharp (1991**) measured the spatial distribution of turgor pressure and found the values were iniformly decreased by over 50% throughout the elongation zone of water stressed compared to well watered roots. Water stress results in an increase in longitudinal cell wall extensibility in the apical region. In contrast, cell wall extension properties are inhibited in the basal region of the elongation zone in water-stressed compared to well-watered roots (**Fan and Neumann, 2004; Fan *et al*., 2006**).

Increased apoplastic ROS in water-stressed Roots which has a -Potential Role in Enhanced Cell Wall loosening. One large group of CWPs that showed changes in abundance under water stress are related to ROS metabolism ROS, including superoxide radicals, H_2O_2, and hydroxyl radicals, are normally produced in various cell compartments including the apoplast **(Mittler *et al*., 2004**). Increased ROS production often occurs under abiotic and biotic stress conditions, including water deficit, and can be associated with oxidative damage (**Iturbe-Ormaetxe *et al*., 1998**). Thus, it is possible that the changes in proteins associated with ROS metabolism may play a role in scavenging ROS and thereby preventing oxidative damage to the root cell walls and plasma membrane under water stress conditions (**Apel and Hirt, 2004**). The six proteins in this category that increased in abundance included two putative oxalate oxidase/g ermin proteins and a superoxide dismutase, which contributed to H_2O_2 production. Generation of hydroxyl radicals from H_2O_2(by either the fenton reaction or peroxidase activity) can play a direct role in cell wall loosening via polysaccharide cleavage (**Liszkay *et al*., 2003**). There is evidence that salinity-induced inhibition of leaf expansion in maize is associated with reduced apoplastic ROS production (**Rodriguez *et al*., 2007).**

The risk of photo-oxidation occurs under conditions such as salt and drought stresses, wherein internal leaf CO_2 concentration declines as stomata close to prevent water loss, electron consumption by photosynthesis is reduced, and over-reduction of the electron transport chain occurs . Photorespiration rises in plants under salt stress, as indicated by a higher CO_2 compensation point (**Fedina *et al*;1993**). Experiment were conducted to determine the effects of salinity, variety and maturation rate on soybean emergence and seedling growth. Field plots were salinized with sodium chloride and calcium chloride salts prior to planting, The soybeans were irrigated with furrow irrigation which redistributed the salts towards the tail ends of the field plots. Elevated soil salinity near the tail ends of the field significantly reduced soybean emergence rate, shoot height and root length. No significant reduction was found for emergence or seedling growth when the electrical conductivity of soil solution extract (ECe) was less than 3 ds m-1. Soybean emergence and seedling growth was significantly reduced when soil ECe reached about 11 dsm-^{1}.(**Wang &Shannon;1999).**

Transport of soluble proteins to lytic vacuoles in plant cells is a receptor-mediated process involving a protein family termed vacuolar sorting receptors (**Neuhaus and Paris, 2005).**

To investigate the response of soybean seed germination to non-lethal concentration salinity levels, salt sensitive and nonsensitive seeds were exposed to 100 mmol/L NaCl until the radicle protruded from the seed coat. Eighteen protein spots showed more than two-fold reproducible differences in abundance as a result of salt stress in both cultivars.

During seed development and maturation, newly synthesized storage proteins are usually transported and stored in a specialized compartment, termed protein storage vacuole (PSV), which is defined by the presence of α- and δ-tonoplast intrinsic protein (TIP) in their tonoplasts (**Jiang *et al*., 2000**).Upon seed germination, storage proteins, oil body, and starch are degraded and used for seedling growth (**Poxleitner *et al*; 2006)**. A Cys protease, termed sulfhydryl-endopeptidase (SH-EP), is synthesized de novo in the cotyledons of germinating *Vigna mungo* seeds.VSRs are being newly synthesized, the total amounts of VSR proteins in germinating seeds, as detected by western-blot analysis with VSRat-1 antibodies, gradually decreased as germination proceeded from day 1 to day 3 (**Wang *et al*;2007) .**

Aleurain and SH-EP were newly synthesized during seed germination, they play a role in mediating protein degradation in germinating seeds at a later stage.

The MVB membrane-located VSRs may represent the recycling receptors during early stages of seed germination, whereas the internalized VSR inside MVBs are targeted for degradation upon delivery to vacuoles. Because VSRs are known to be recycled from PVC/MVB to Golgi for a further round of cargo binding and selection whereas proteins delivered to lytic vacuoles from PVC are degraded (**Oliviusson *et al*; 2006**), VSR proteins may perform dual functions in plant cells.

Results indicated that VSR proteins might have distinct functional roles in developing and germinating seeds.VSR proteins were mainly found inside the lumen of MVBs in day 3

germinating seeds, which indicates that the membrane-localized VSRs at steady state may represent the population of recycling receptors, whereas those VSRs inside the lumen of MVBs are destined for degradation, presumably after fusion of the PVCs/MVBs with the vacuole (**Jiang *et al*;2002**; **Jiang and Rogers, 2003).**

Comprehensive comparisons were also carried out between rice and soybean germinating seeds. 764 proteins belonging to 14 functional groups were identified and metabolism related proteins were the largest group. Lipids were degraded through lipoxygenase dependent pathway and proteins were degraded through both protease and 26S proteosome system, and the lipoxygenase could also help to remove the reactive oxygen species during the rapid mobilization of reserves of soybean germinating seeds. The differences between rice and soybean germinating seeds proteome profiles indicated that each crop species had distinct mechanism for reserves mobilization during germination. Different reserves could be converted into starches before they were totally utilized during the germination in different crops seeds.The most abundant group was the storage proteins in soybean and metabolism related proteins in rice. These proteins accounted for 42.8% of the total proteins in terms of abundance. The major storage proteins in soybean seed are 11S globulin (beta-conglycinein) and 7S globulin, whereas, those in rice are glutelin and globulin family proteins. It has already been reported before that the degradation of these storage proteins might help to nourish the germinating soybean seeds and young seedlings .There were 36 redox regulation proteins identified in germinating soybean seeds. To better understand the characteristics of reserves mobilization during soybean germination, these metabolic proteins were further categorized into 17 sub-groups based on the metabolic pathways they were involved in. These sub-groups include photosynthesis, major carbohydrates metabolism, glycolysis, fermentation, gluconeogenesis and glyoxylate cycle, phosphate pentose pathway, tricarboxylic acid (TCA) cycle, mitochondrial electron transport/ATP synthesis, cell wall metabolism, lipid metabolism, nitrogen metabolism, amino acid metabolism, secondary metabolism, cofactor and vitamin metabolism, tetrapyrrole synthesis, nucleotide metabolism and C1 metabolism. There were 5 proteins that could be sorted into 2 or 3 different metabolic pathways. Compared with the metabolic proteins identified in germinating rice seeds, proteins

involved in phosphate pentose pathway, S-assimilation, and poly-amine synthesis were absent in soybean seeds .

There were 10 major carbohydrates metabolism related proteins in germinating soybean seeds. Some starch catabolic enzymes such as beta-amylase, alpha-glucan water dikinase, endo-1,3-beta-glucanase and UDP-glucose 6-dehydrogenase were identified. Whereas, only one starch biosynthesis related enzymes ADP-glucose pyrophosphorylase was identified. There were 9 starch biosynthesis related proteins identified in germinating rice seed (**Xu *et al*;2011**). This difference were ascribed to different reserves biosynthesis during seed filling in rice and soybean. In rice, starches were rapidly accumulated during seed maturation. So the enzymes were synthesized and could still function during germination. As for soybean seed, it mainly synthesized proteins and lipids during seed maturation, so very few starch biosynthesis enzymes could be detected. These enzymes might be synthesized at the late stage of seed germination, because of the accumulation of starch granules after 2 days imbibitions. 36 redox regulatory proteins were identified in the germinating soybean seeds.

The proteins extracted from germinated seeds were separated using two-dimensional gel electrophoresis (2-DE), followed by Coomassie brilliant blue G-250 staining. Ferritin and 20S proteasome subunit β-6 were up-regulated in both cultivars. Glyceraldehyde 3-phosphate dehydrogenase, glutathione S-transferase (GST) 9, GST 10, and seed maturation protein PM36 were down-regulated in Lee68 by salt, but still remained at a certain level. However, these proteins were present in lower levels in control N2899 and were up-regulated under salt stress. The results indicate that these proteins might have important roles in defense mechanisms against salt stress during soybean seed germination.

The proteomic approach, based on reproducible two-dimensional gel electrophoresis (2-DE) and powerful mass spectrometry (MS) analyses, offers the possibility of identifying those proteins The proteomics of soybean in response to abiotic stress have been studied (**Cheng *et al*., 2010; Nouri and Komatsu, 2010)**

Ferritin, a class of iron-storage proteins, is composed of at least two different subunits and its level decreases gradually during soybean germination (**Masuda *et al*., 2001).** A proteolytic complex involved in recognizing and catabolizing ubiquitin-protein to remove abnormal proteins

(Sassa *et al*., 2000; Smalle and Vierstra, 2004) and also operates in the stress response by removing abnormal proteins . 20S proteasome α subunit A was up-regulated in soybean under osmotic stress **(Toorchi *et al*., 2009).** The up-regulation of the 20S prote0some in both cultivars after stress could be associated to the degradation of oxidatively-damaged proteins caused by salt stress.

Approaches to demonstrate DNA binding of legume TFs include electrophoretic mobility shift assays (EMSAs), hybridization of labeled DNA to TFs on filters, DNase-I footprinting, and yeast one-hybrid assays. In one example, **Bastola *et al*. (1998**) identified cDNA encoding Alfin1, a protein with a putative Zn-binding domain, by differential screening of salt-tolerant alfalfa cells.

Further evidence of TF activity has been provided using transactivation assays. Some groups have demonstrated in vivo transactivation in cell culture and transient transformation systems, including particle bombardment of bean cotyledons (**Chern *et al*.,1996b)** polyethylene glycol-mediated transfection of *Arabidopsis* protoplasts , and in vitro transcription activation in rice (*Oryza sativa*) cell extracts .TFs interact physically with other proteins, in addition to the RNA polymerase complex itself, to effect changes in gene transcription (**Lee and Young, 2000).**

Transcription factors (TFs) are sequence specific DNA-binding proteins that interact with other transcriptional regulators, including chromatin remodeling/modifying proteins, to recruit or block access of RNA polymerases to the DNA template. Plant genomes devote approximately 7% of their coding sequence to TFs.Extensive sequencing of cDNA and genomic DNA indicates that legumes encode upwards of 2,000 TFs per genome.

TFs likely play crucial roles in plant development and differentiation.Plant development and differentiation are programmed primarily at the level of gene transcription, which is controlled by TFs and other proteins that either recruit or block access of RNA polymerases to the DNA template. TFs play crucial roles in agriculturally important processes in legumes, such as symbiotic nitrogen fixation (SNF).Recent transcriptomic studies, using arrays of cDNA or oligonucleotides to measure transcript levels, have identified thousands of legume genes that are differentially expressed during various types of plant-microbe interactions development and differentiation , and in response to abiotic stress (**Buitink *et al*., 2006**).

TF families are generally defined by the types of DNA-binding domain contained by proteins in the family. An important feature of legumes that sets them apart from plants in other families is their ability to form nitrogen-fixing symbioses with soil bacteria, called rhizobia. These bacteria take up intracellular residence nodules, that develop on roots and stems . Many TF genes have been found to be expressed during nodule development and differentiation.

Three TFs have been implicated in nodule development or function in this way.

Five legume TFs have been implicated in abiotic stress tolerance. One of these, alfalfa *Mszpt2-1*,was found to be induced in roots by salt treatment. Inhibition of *Mszpt2-1* by antisense RNA resulted in increased sensitivity of transgenic plants to salinity . Overexpression of CAP2 and Alfin1 TFs in transgenic plants conferred salt tolerance and increased growth (**Shukla *et al.*, 2006).**

Viviparous1 (*Vp1*) encodes a B3 domain-containing transcription factor that is a key regulator of seed maturation in maize (*Zea mays*). Expression of *Vp1*decreased after culture in hormone-free medium, but was **induce**d by salinity or osmotic **stress**. Application of exogenous **abscisic acid** (ABA) also **induce**d transcript levels within 1 h in a dose-dependent manner. Sequence analysis of the *Vp1* promoter identified a potential ABA-responsive complex, consisting of an ACGT-containing ABA response element (ABRE) **and** a coupling element 1-like motif. Electrophoretic mobility shift assay confirmed that the ABRE **and** putative coupling element 1 components specifically bound proteins in embryo nuclear protein extracts.

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protruded from the seed coat. Eighteen protein spots showed more than two-fold reproducible differences in abundance as a result of salt stress in both cultivars.

Soybean (*Glycine max*) storage proteins were characterized by sedimentation and by polyacrylamide gel electrophoresis under dissociating (8 M urea) and nondissociating conditions.Three sedimenting classes of proteins were found, with sedimentation coefficients of 2.2S, 7.5S, and 11.8S. The coefficients were related to the bands obtained by electrophoretic separation. The results support the idea that relatively few proteins make up the bulk of the seed protein.

Three prominent peaks were found, with maxima in fractions 10, 17, and 22. The approximate sedimentation constants for these peaks were determined to be 2.2S, 7.5S, and 11.8S.The soybean proteins with the highest relative mobility in this electrophoretic system are composed largely if not completely of proteins with a sedimentation coefficient of about 2.2S.The 7.5S protein separates into three bands with electrophoretic mobilities indistinguishable from those of bands 1, 2, and 3 obtained by electrophoresis of the total protein extract.In contrast to the 7.5S protein, the 11.8S protein gave a single diffuse band in the non-dissociating buffer system.

Ethylene signaling plays important roles in multiple aspects of plant growth and development. Its functions in abiotic stress responses remains largely unknown.Alteration of ethylene signaling affected plant salt-stress responses. A type II ethylene receptor homolog gene *NTHK1*(*Nicotiana tabacum* histidine kinase 1) from tobacco (*N. tabacum*) conferred salt sensitivity in NTHK1-transgenic (*Arabidopsis thaliana*) **plant**s as judged from the phenotypic change, the relative electrolyte leakage, and the relative root growth under salt stress has been isolated.

Overexpression of the *NTHK1* gene or the receptor gain-of-function activated expression of salt-responsive genes *AtERF4* and *Cor6.6* have also been reported. In addition, the transgene *NTHK1* mRNA was accumulated under salt stress, suggesting a posttranscriptional regulatory mechanism. These findings imply that ethylene signaling may be required for plant salt tolerance. *NTHK1* increased salt sensitivity of the transgenic plants and ACC could suppress this sensitivity.Ethylene has been proposed to negatively regulate its receptor activity **(Hua and Meyerowitz, 1998).**

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The H^+-**A**TP**a**se might be involved in **a**nother w**a**y, **a**s **a**cidific**a**tion **of** the extr**a**cellul**a**r sp**a**ce is expected to f**a**cilit**a**te tr**a**nsport **of** the proton**a**ted form **of a**uxin into the cell **a**nd so further stimul**a**te the cell response (**Leyser, 2005)**. However, whether the effect **of a**uxin on cell elong**a**tion **a**ctu**a**lly requires cell w**a**ll **a**cidific**a**tion by H^+-ATPase is still a matter of controversy **(Kutschera, 2006).**

Young *et al*. (1998) an**a**lyzed tr**a**nsgenic pl**a**nts expressing **A**H**A**3 with **a**n **a**ltered C terminus. When grown in vitro, the tr**a**nsform**a**nts were more resist**a**nt to **a**cid medium, suggesting **a** role for this pl**a**sm**a** membr**a**ne H^+-**A**TP**a**se in cytopl**a**smic pH homeost**a**sis.

Genomic and Genetic Control of Phosphate Stress in Legumes has been a subject of recent study.Phosphorus (P) is taken up by plants as **phosphate** (Pi), but Pi is unevenly distributed **and** relatively immobile in soils. As a result, more than 30% **of** the world's arable l**and** requires the application **of** P fertilizers for cropping (**Vance *et al*., 2003)**. Unfortunately, P fertilizers are manufactured from non renewable resources that are increasingly becoming more costly **and** less available.

Plants have evolved a variety **of** adaptive strategies to improve their acquisition, use, **and** remobilization **of** P (**Vance *et al*., 2003; Hammond *et al*., 2004;Lambers *et al*., 2006**). Plant responses to P stress conditions involve changes in root morphology **and** architecture (**Liao *et al*., 2001**; **Lynch and Brown, 2001**, **Beebe *et al*., 2006**; **Ochoa *et al*., 2006**), as well as changes in shoot **and** flower development .

Recent studies showed that P stress delayed: (1) leaf development **and** leaf expansion along the main **and** axillary shoots; (2) axillary shoot emergence **and** elongation, resulting in stunted plants;**and** (3) timing **and** frequency **of** flower emergence .P stress research is moving toward an exciting phase centered around signal transduction, regulation **of** developmental plasticity, gene function, **and**increased efficiency **of** use. Sustainable cropping practices require that plant researchers identify **and** discover mechanisms in plants that improve P acquisition **and** exploit these P stress adaptations to make better plants

that are efficient at acquiring Pi. Efforts that improve soil P availability to plants contribute greatly to the practice **of** economical **and** environmentally friendly crop agriculture.

Genetic variability with contrasting degree **of** root architecture responses to P-limiting conditions has been known for a wide range **of** plant species.As a result, P stress tolerance **and** adaptation have begun to be analyzed in common bean **and** through the identification **of** quantitative trait loci (QTL) approach (**Beebe *et al.*, 2006; Ochoa *et al.*, 2006; Reymond *et al.*, 2006).** Water stress induced changes in large number of the proteins and were localized in cell walls, these were putative oxalate oxidases and probable germin protein 4s, α-t-arabinofuranosidase, α-1,4-g1ucan-protein synthase,β-galactosidases, putative chitinases, endo-1,3;1,4-β-d-glucanases, putative α-galactosidase preproteins, β-1,3-g1ucanases, XTHs, xylosidases, 1,2 β-d-glucosidases, and peroxidases. Most of the identified proteins (69%) had a putative N-terminal signal peptide that may lead to protein targeting into the secretary pathway (**Nielsen *et al.*, 1997**). The root elongation zone was shorter in the water-stressed roots.The changes in abundance of CWPs due to water deficit were predominantly region specific (**Zhu *et al*;2007).**

Laboratory DNA-RNA hybridization experiments revealed that approximately 14,000 to 18,000 diverse mRNAs are present in soybean embryos at different developmental stages **(Goldberg *et al.*, 1981b, 1989)**.Small numbers of mRNAs, including those encoding storage proteins, are regulated quantitatively at specific developmental stages (**Goldberg *et al.*, 1981a**). **Murai *et al.* (1983**) demonstrated that the common bean phaseolin seed storage protein gene could be transferred to sunflower (*Helianthus annuus*) cells and expressed.

Research in rice suggests that an important step in regulation**of** gene expression during plant stress appears to be the transcriptional activation or repression **of** genes (**Chen *et al.*, 2002; Wu *et al.*, 2003**).

In microarray analysis, a transcription factor *WRKY75* gene was found to be up-regulated during Pi starvation (**Misson *et al.*, 2005**).MicroRNAs (miRNAs) are one such small RNA, about 18 to 32 nucleotides in length in plants, which function as posttranscriptional negative regulators or

repressors through base pairing to complementary or partially complementary sequences to the target mRNA cleavage (**Reinhart *et al*., 2002; Fujii *et al*., 2005Rhoades *et al*., 2002;Bartel, 2004**; **Sunkar and Zhu, 2004**).miR399, first identified in **and** rice was shown to be induced by P stress after 24 **and** 48 h **of** P starvation . Phosphate starvation triggers distinct alterations of genome expression in Arabidopsis roots and leaves.Increased transcript abundance **of** several genes coincides with P starvation in white lupine **and** *Medicago*. Abiotic stress treatments, such as nitrogen (N) starvation, aluminum toxicity, or addition **of** naphthylacetic acid, did not show reporter gene enzyme activity in transgenic plants containing *LaPT1* **and** *LaSAP1* genes.

Expression **of** P stress-induced *LaPT1*, *LaSAP1*, **and** *LaMATE* in cluster roots was reduced in girdled plants. Moreover, when P-stressed plants grown in a 16-/8-h photoperiod were placed in the dark for 24 h, P stress-induced gene expression in roots was abolished but recovered upon 16-h reexposure to light .

Stress Enzyme Activities -The activities of stress responsive enzyme (LOX, PAL CAT, POD and SOD) were reported in bacteria treated soybean plant together with negative and positive control plants. Bacteria treated seeds showed higher enzymatic activity in leaves in comparison with positive and negative control.

Beside LOX, the activity of plant POD, SOD, CAT and PAL enzymes are known as stress indicators, POD participates in the cell wall polysaccharide processes such as oxidations of phenols, suberization, and lignification of host plant cells during the defense reaction against pathogenic agents the high peroxidase activity detected in treatments are linked to lignification and generation of hydrogen peroxide that inhibit pathogens directly or generate other free radicals with antimicrobial effects .

The existence of a complicated transcriptional regulation system involved in plant responses to Pi starvation is well documented **(Franco-Zorrilla *et al*. 200**4). A rice TF (OsPTF1) involved in the response to phosphate starvation has been reported (**Yi *et al.* 2005**). OPTF1 is expressed in

phloem cells of the primary root, leaves, and lateral roots. Overexpression of OsPTF enhances rice tolerance to Pi starvation. Interestingly, microarray data on this OsPTF transgenic rice plants showed a concomitant enhanced expression of H-PPases (**Yi *et al*. 2005).**

In *A. thaliana* Pi starvation triggers increases in transcript and protein abundance of both AVP1 and the plasma membrane H-ATPase.

Overexpression of the H -PPase AVP1 in results in increased cell division at the onset of organ formation, root, and shoot hyperplasia as well as increases in auxin transport. The salt tolerant phenotypes triggered by the overexpression of the H-PPase are consistent with its residence at the tonoplast. Sucrose must be actively transported from mesophyll cells to companion cells via a sucrose/H symporter that depends on the proton gradient generated by the plasma membrane H-ATPase (**Srivastava *et al*. 2008).** In order to have an adequate ATP supply for the maintenance of this transmembrane proton gradient, a percentage of the incoming sucrose must be cleaved into fructose and UDP-glucose by sucrose synthase (**Lerchl *et al*. 1995).**

A series of immuno-gold studies with phloem tissue of R. communis seedlings prompted the suggestion that the H-PPase could be involved in sucrose transport **(Long *et al*. 1995; Robinson *et al*. 1996**). These authors suggested that both H-pumps are required for sieve element membrane energization to maintain high sucrose, K, and amino acid concentrations.

Proteins changed in soybean leaves by salt stress show that photosynthesis related proteins are mainly down-regulated and suggest that NaCl affects photosynthesis and leads to energy reduction inside the plant and consequent reduction in plant growth. Calreticulin is an important calcium-binding protein with chaperone functions and plays a pivotal role in regulating calcium homeostasis and protein folding in the endoplasmic reticulum of plants.

Glyceraldehyde-3-phosphate dehydrogenase was down-regulated at both the mRNA and protein levels in response to NaCl treatment, suggesting that it plays a role in salt stress and can be used as a target gene in soybean seedlings. Improved salt tolerance of potato by transfer of the glyceraldehyde-3 phosphate dehydrogenase gene.

At the step catalyzed by glyceraldehyde-3-phosphate dehydrogenase in glycolysis, one NADH is produced from NAD^+. It is indicated that the ATP production will be reduced by down-

regulation of glyceraldehydes-3-phosphate dehydrogenase and consequently there will be a decrease in plant growth under salt stress.

Na^+ is the main toxic ion in saline soils for most plants, including the major cereals (**Tester and Davenport, 2003**). Many cytosolic enzymes are inactivated by Na^+ and high Na^+ concentrations are detrimental to cellular metabolism. In addition, excess Na^+creates hyperosmotic stress, causing a range **of** effects, such as secondary oxidative damage . To survive in a saline environment, plants have evolved a range **of** protective mechanisms. Recent studies, which have identified and characterized key enzymes in these protective pathways, especially in salt-tolerant species, have allowed the engineering **of** crop plants with increased salt tolerance. Several strategies for improvement **of** salt tolerance have been employed (**Chinnusamy *et al.*, 2005**). The initial strategies were to increase the production **of** small osmolytes like mannitol, Glyce betaine, Pro, and trehalose (**Tarczynski *et al.*, 1993; Kishor *et al.*, 1995; Xu *et al.*, 1996; Sakamoto and Murata, 2002).** A more recent strategy has involved the overexpression **of** vacuolar and plasma membrane ion transporters, increasing Na^+ **exclusion** from the cytosol. Overexpression **of** two Na^+/H^+ antiporters, NHX1 and SOS1 (for salt overly sensitive), has conferred salt tolerance to transgenic plants by sequestering Na^+ in the vacuole or transporting Na^+ across the plasma membrane out **of** the cell, respectively (**Apse *et al.*, 1999; Shi *et al.*, 2002, 2003).**

In plants, maintaining nontoxic levels **of** Na^+ appears to rely solely upon Na^+/H^+ antiporters. A surprising exception was the recent identification **of** two Na^+-ATPases (PpENA1 and PpENA2) in the bryophyte,*Physcomitrella patens* . PpENA1 was shown to act as a Na^+ pump when expressed heterologously in yeast and complemented a salt-sensitive yeast strain deficient in Na^+ and K^+ efflux .Heterologous expression **of** membrane transporters can alter the properties **of** the transporter e.g. the ion selectivity **of** the transporter.

To determine over what time frame and under which salt concentrations PpENA1 plays a physiologically important role, quantitative reverse transcription (qRT)-PCR was performed on protonemata exposed to various NaCl concentrations (5–400 mM) for different times (30 min to 3 d). Exposing protonemata to low NaCl concentrations caused a 3- to 8-fold increase in

expression. However, increasing the NaCl concentration to caused a much higher, 30-fold, induction in *PpENA1* mRNA levels after 8 h.

To determine whether the induction **of** *PpENA1* was specific to Na^+ stress, protonemata were exposed to NaCl, osmotic stress, cold, oxidative stress, or ABA. The induction **of** *PpENA1* expression under Na^+ stress was due to a combination **of** osmotic and ionic effects.One **of** the reasons Na^+ is toxic to plants is that Na^+ can replace K^+ in certain enzymes, rendering them nonfunctional.

In vascular plants, the salt-specific response is also post translationally coordinated via the SOS pathway consisting **of** a Ser-Thr kinase (SOS2) and a calcium-binding protein SOS3;(**Shi *et al.*, 2000).**

The steady-state level **of** Na^+ and K^+ions will ultimately reflect the sum **of** the many transporters involved in uptake, efflux, and compartmentation. To determine the physiological advantage **of** having Na^+-ATPase in planta, the K^+-to-Na^+ ratio was determined at different NaCl concentrations. Research showed that wild-type *Physcomitrella* was able to maintain a higher K^+-to-Na^+ ratio at 100 mM NaCl than the *ena1* mutant. Growth impairment correlated well with the intracellular K^+-to-Na^+ ratio; when the ratio was 3 or above, growth was similar to the nonstressed control, but at 2 or below, growth was impaired.
One **of** the reasons for Na^+ toxicity is that Na^+ can replace K^+ in essential enzymes and thereby inactivate them. Under severe Na^+ toxicity, metabolism and photosynthesis will be affected, leading to decreased levels **of** chlorophyll.

It appears that, although *ena1* plants contain more intracellular Na^+, it is able to protect the sensitive components either by sequestering the Na^+ in the vacuole, synthesizing compatible osmolytes, or up-regulating levels **of** protective proteins. Up-regulation **of** a dehydrin-like protein essential for the recovery **of** *Physcomitrella* after salt stress and **of** an enzyme involved in protection against reactive oxygen species has recently been shown **.**

When *PpENA1* apparently gives *Physcomitrella* a clear selective advantage under moderate salt stress, it is intriguing that Na^+-ATPases are absent in vascular plants. The presence **of** both a Na^+ K^+ATPase and a SOS1 homolog in the red alga, *Porphyra*, living in the sea at high salinity and alkaline pH also suggests that having both types **of** pumps is important.It will be **of** great interest to see whether the expression **of** *PpENA1* in, for example, roots, where Na^+ can be extruded to the large volume **of** growth medium, will be able to improve salt tolerance **of** vascular plants.The **a**ccumul**a**tion **of** s**a**lt in germin**a**ting pl**a**ntlets is cruci**a**l **a**nd th**a**t **a**voiding s**a**lt stress during this e**a**rly st**a**ge might help the pl**a**nts to cope with highly s**a**line medi**a**.

High H^+-**A**TP**a**se **a**ctivity could be detriment**a**l for pl**a**nt cells **a**nd th**a**t counter selection **of** pl**a**nts showing high ΔPM**A**4 **expression** possibly occurred during tr**a**nsform**a**tion or regener**a**tion.

Alteration of Plant Development

Activ**a**ting the H^+-**A**TP**a**se not only incre**a**ses the tr**a**nsmembr**a**ne pH difference, but is **a**lso expected to incre**a**se the tr**a**nsmembr**a**ne potenti**a**l difference. This might be import**a**nt when considering cell exp**a**nsion, since, besides w**a**ll loosening, it **a**lso requires sust**a**ined osmotic pressure, which depends, in p**a**rt, on **a**ctive ion **a**nd nutrient tr**a**nsport. Some tr**a**nsport, such **a**s K^+ through ch**a**nnels, depends more on the tr**a**nsmembr**a**ne potenti**a**l difference th**a**n on the tr**a**nsmembr**a**ne pH difference.

Proteins, such **a**s exp**a**nsins **a**nd endotr**a**nsglycosyl**a**ses, **a**re required to we**a**ken the w**a**ll (**Van Volkenburgh, 1999**).While **a**uxin is known to stimul**a**te H^+-**A**TP**a**se, H^+-**A**TP**a**se **a**lso h**a**s **a** direct effect on **a**uxin tr**a**nsport. **A**uxin is **a** we**a**k **a**cid **a**nd therefore H^+-**A**TP**a**se **a**ctiv**a**tion, which results in **a** lowering **of** the extern**a**l pH, should f**a**cilit**a**te upt**a**ke **of** the proton**a**ted **a**uxin into the cell.

Pollen form**a**tion is **a** critic**a**l developmen**a**l st**a**ge **a**nd m**a**le sterility h**a**s been observed when PM**A**4 **expression** is prevented by cosuppression (**Zhao *et al*., 2000).**

Sodium is highly toxic when it **a**ccumul**a**tes within the cell,several exclusion mech**a**nisms exist to m**a**int**a**in the sodium concentr**a**tion low within the cytosol. One **of** these involves exclusion within the v**a**cuole, which relies on N**a**$^+$/H^+ **a**ntiport energized by the tonopl**a**st H^+-**A**TP**a**se **a**nd

pyrophosph**a**t**a**se. **A**nother involves N**a**$^{+}$ efflux out **of** the cell through **a**N**a**$^{+}$/H^{+} **a**ntiporter (SOS1 in) thought to be **a**ctiv**a**ted by the pH gr**a**dient gener**a**ted **a**cross the pl**a**sm**a** membr**a**ne by H^{+}-**A**TP**a**se **(Blumwald *et al.*, 2000; Zhu, 2003; Yamaguchi and Blumwald, 2005**). The role **of** the l**a**tter h**a**s been inferred from the observ**a**tion **of** incre**a**sed H^{+}-**A**TP**a**se **a**ctivity under s**a**lt stress conditions (**Morsomme and Boutry, 2000; Palmgren, 2001**). More direct evidence w**a**s provided by the observ**a**tion th**a**t **a**n mut**a**nt disrupted in the H^{+}-**A**TP**a**se *AHA4* gene h**a**s incre**a**sed sensitivity to s**a**lt stress (**Vitart *et al.*, 2001**). A **constitutively a**ctiv**a**ted H^{+}-**A**TP**a**se incre**a**sed s**a**lt toler**a**nce during the germin**a**tion **a**nd growth **of** seedlings.

Thiols have ubiquitious distribution and euphoretic activity. Thiols apparently contribute their metabolic and biological functions due to the property that their-SH group is readily oxidized. The nucleophilicity of a thiol ion Is affected by the extent of the ionization of-SH group, the pK and the pH. The-SH groups are implicated yet by an unsolved mechanism through which the free energy (F) from electron transport is harnessed to drive phosphorylation of adenosine-diphosphate (ADP) by inorganic phosphate. The-SH group may also be involved directly in the catalysis of the enzyme system. Oxygen uptake is enhanced in presence of various TCA cycle substrates and their role in synthesis of ATP redox state is well established. Both Na^{+} and K^{+} affect interaction of ouabain wih the enzyme. Sodium potentiates inhibition by ouabain **(Matsui and Schwartz, 1966; Schatzmann, 1965).** Although Na^{+} and K^{+} are both required to activate the enzyme high concentration of either ion inhibited activation by the other. Optimal enzyme activity has been observed at Na^{+}/K^{+} ratio between 10.1 and 5:1. The requirement for Na^{+} is absolute but numerous other ions can substitute for K^{+} and do so with varying degree of efficiency (**Britten and Blank, 1968; Skulskii *et al.*, 1973).**

A Na^{+} -ATPase activity inhibited by K^{+} at low level of ATP has been reported to indicate that two different enzymes with different affinities for ATP contribute to the total hydrolytic activity Two kinds of cation binding sites existed on the Na^{+} K^{+} dependent ATPase molecule from porcine kidney. One of the binding sites was for three moles of Na^{+} per mole of ouabain binding site. Na^{+} ions exhibited cooperativity with a hill coefficient of 2.5 to 3.00. One mole of K^{+} could displace 3 moles of Na^{+} ions. The membrane bound Na^{+} K^{+} ATPase was actively involved in

the translocation of Na^+ and K^+ ions across the plasma membrane. **Matsui *et al,* (1977**) reported that 2 moles of Na^+ and 2 moles of K^+ ions were bound alternatively per mole of ouabain binding site of the ATPase. The amount of Na^+ binding was in proportion to the enzyme concentration. The amount of Na^+ binding in the absence of 100 mM KCl increased markedly with an increase in the Na^+ concentration trom 0.05 to 0.4 mM.

The rate of hydrolysis is also influenced by the type of the cations. divalent cations being more effective. Further, the ionic ratio of the divalent cation also affected the dephosphorylation. Various divalent cations with an ionic radius ranging from 0.65 -0.99 A^0 were tested with myosin at Optimum concentration of the metal ion in the presence of 2.5 mM ATP and pH 7.6 All the cations with a ionic radio in the range 0.65-0.99 A^0 were potent stimulator of ATPase activity of this actomyosin **(Melchior; 1954, Weber ;1959, Kitagawa and Tonomura, 1960)**. Whether such an analogus situation exists in plants is no certain.**It was** reported that bovine seminal plasma Na^+ K^+ ATPase by using ouabain inhibition method. Inhibition of Mg^{2+} Na^{2+} K^+ by ouabain was non linear as with respect to ouabain concentration.Ouabain sensitivity to Mg^{2+} + Na^+ + K^+ ATPase is well documented in other tissues **(Albers *et al*., 1968; Tobin and Sen 1970).** The degree of inhibition depends upon time, temperature and concentration of the ligands in the incubation medium. Na^+ ions increase ouabain inhibition. Ouabain generally has a 50 per cent inhibition at a concentration ranging from 10^{-7} to 10^{-6} M but wide variation in the sensitivity of different tissues and species has been reported .

The positive relationship between the onic radii and activity was observed irrespective of whether it was mono or divalent cation. In an enzyme substrate complex, in the presence of cation, the free energy of hydration would be proportional to the values of Z/re where, Z is the cation charge and 're' is its effective ratio of solubilization (**Powell and Latimer, 1951**), Smaller cations, such as Mg^{2+} forms weaker complexes than a large similarly charged cation such as Ca^{2+} because of repulsions between ligand groups, which lead to the smaller ions performing partial coordination with many ligand centres which may account for the bimodal dip in the cationic effect on the enzymic activity .

The property of the chelates is based on the assumption that univalent cations bind through 'S'

orbital and bivalents through dsp^2. The relative bond distance is almost three times greater in bivalent cations as compared to monovalent .

Transaminases (AST and ALT)

The enzyme L-Aspartate : 2-oxoglutarate amino transferase; AST (Glutamateoxalo acetate transaminase, GOT) EC 2.6.1.1. catalyzes the the transfer of amino group of aspartic acid to α-keto glutaric acid with the formation of glutamate and oxalo acetic acid. The enzyme α -alanine: 2-oxoglutarate amino transferase (ALY) EC 2.6.1.2 catalyzes the transfer of amino group of alanine to α-keto glutaric acid with the formation of glutamate and pyruvic acid.

Assimilates and nutrients

In legumes Suc is the predominant sugar (**Zimmermann and Ziegler, 1975)**, and among nitrogenous solutes, amino acids, principally the amides Gln and Asn predominate, both in xylem and phloem (**Atkins, 1991**). In addition nodulated legumes also translocate unique solutes formed as result of the symbiosis with rhizobium and that can influence plant development. These include very high levels of cytokinin (**Upadhyaya *et al*; 1991**), a bioactive product of riboflavin hydrolysis, lumichrome (**Matiru and Dakora, 2005**). Legumes have provided an ideal model in this area particularly for studies of translocation of photoassimilates and nitrogen compounds to seeds (**Zhou *et al*., 2007**). Translocation of inorganic nutrients ,specific amino acids **,** plant growth regulators **,** and quinolizidine alkaloids.

Recent research has indicated that cytosolic GLYR1 and plastidial GLYR2 reduce both glyoxylate and SSA (succinic semialdehyde) to glyoxylate and GHB (γ-hydroxybutyrate) respectively (**Simpson *et al*;2008;Hoover *et al*;2007)**

Aldehydes such as glyoxylate and SSA can accumulate in plants under stress, and react with DNA, oxidize membrane lipids, modify proteins or influence the transcription of stress-related genes, there by causing cellular and developmental problems (**Kotchoni ;2006).** Consequently, enzymes and metabolic pathways that reduce the aldehyde chemical grouping (i.e. H-C=O) in these compounds to its corresponding alcohol are probably essential for maintain plant health.

Glyoxylate and SSA are best known as intermediates in the metabolism of glycolate and GABA (γ-aminobutyrate) respectively.

ROS (Reactive Oxygen Species) production is influenced by NADPH/NADP ratios in chloroplasts and mitochondria and NAD(P)H oxidases are key players in ROS generation at the plasma membrane. ROS consumption partly depends on glutathione and ascorbate pools, which are fundamentally maintained by NAD(P)H. NAD(P) plays other defensive and signaling roles, such as production of NO and metabolism of reactive aldehydes.

The ATP-dependent phosphorylation of NAD(H) is catalysed by NADK (NAD kinase). The enzymes are divided into NADKs or NAD(H) ks depending on their substrate preference for the oxidized and/or reduced forms of NAD (**Strand *et al*;2003,Berrin *et al*;2005).**

Glutamate can be metabolized via a short metabolic pathway that bypasses the NAD-dependent 2-oxoglutarate dehydrogenase and the ADP-dependent Succinyl-Co A ligase steps of the TCA cycle and allows the glutamate carbon backbone to enter the cycle as succinate (**Shelp;1999,Fait *et al*;2008)**.The decarboxylation of glutamate is catalysed via a cytosolic Ca^{2+}/Ca-dependent GAD (glutamate decarboxylase) with the consumption of a proton (**Shelp**;1999). The catabolism of GABA apparently involves mitochondrial pyruvate- and glyoxylate-dependent, but not 2-oxoglutarate-dependent, GABA-T (GABA transaminase) activities (**Van Cauwenberghe *et al*;2002,Clark *et al*;2009**), resulting in the production of alanine and *glycine r*espectively.

Malate synthase is a characteristic enzyme of the glyoxylate cycle. In germinating oilseeds, the glyoxylate cycle has a key role in converting acetyl-coenzyme A produced by fatty acid β-oxidation into oxaloacetate, and subsequently into sugar**.**The expression and regulation of genes under salt stress is a complicated process, and is affected by experimental materials, salt concentration, treatment methods, and stress time.There is a strong correlation between increased cellular proline levels and the capacity to survive both water deficit and the effects of high environmental salinity. Although proline can be synthesized from either glutamate or ornithine, glutamate is the primary precursor in osmotically stressed cells. Transcripts corresponding to both cDNAs accumulate in response to NaCl treatment. There is evidence that degradation of proline in the mitochondria is directly coupled to respiratory electron transport system and ATP Production.

The effect of organic contaminants : anthracene and chlorpyrifos on the seed germination of *Lolium lmultiflorum* (rye grass) showed that there was no inhibitory effect of anthracene as compared to the control whereas significant reduction and delay in seed germination was observed at the higher chlorpyrifos concentrations of 75 and 100 mg/kg. The level of seed germination was found to decrease with increasing concentrations of chlorpyrifos in the soil **(Korade and Fulekar;2010). It was** observed a slight reduction in the dry matter production in soybean due to irrigation with paper mill effluent as such or diluted (50%), when compared to that obtained from irrigation with river water. However seed weight per plant, number of seeds per plant, and hundred seed weight did not differ significantly. **Shrivastava, (1991),** Evaluated the paper mill and chloralkali plant effluent (CAP) on seed germination of healthy seeds of radish and onion in different dilutions of effluents and revealed that the percent germination was faster with lesser concentrations of the effluents when seeds treated for one to five days all. In the case of radish, at 10% concentration of the effluents, there was a important reduction in mean root length, shoot length and secondary roots as compared with control, while no secondary root could appear in 100% concentration of CAP effluent. Low dissolved oxygen linked with high mercury and residual chlorine content in effluent affected negatively the germination and later growth of seedlings.

Dixit (2003) Experimented bioassay studies to Evaluate the toxicity of raw and diluted distillery effluent on seed germination, seeding growth and pigment content of sugar beet by collecting effluent samples from Sri ganganagar sugar mill factory, in Rajasthan. Seeds kept moist in different dilutions (1 , 5 , 10 , 20and 30%) of effluent solution double distilled water, served as the control , that top concentration (>5%) of effluent was found to be toxic, however, the effluent was usable for irrigation purposes after suitable dilution. Biological Oxygen Demand (BOD) is a measure of the oxygen used by microorganisms to decompose this waste. If there is a large quantity of organic waste in the water supply. There will also be a lot of bacteria present working to decompose this waste. In this case, the demand for oxygen will be high (due to all the

bacteria) so the BOD level will be high. As the waste is consumed or dispersed through the water,BOD levels will begin to decline.

By comparing the BOD of incoming sewage and the BOD of the effluent water leaving the plant, the efficiency and effectiveness of sewage treatment can be judged. For example, in a typical residential city raw sewage has a BOD value of around 300 mg/L. if the effluent from the sewage treatment plant has a BOD of about 30 mg/L, the plant has removed 90 percent of the BOD.

If water of a high BOD value flows into a river, the bacteria in the river will oxidize the organic matter, consuming oxygen from the river faster than it dissolves back in from the air.

The chemical oxygen demand test procedure is based on the chemical decomposition of organic and inorganic contaminants, dissolved or suspended in water. The result if a chemical oxygen demand test indicates the amount of water-dissolved oxygen (expressed as parts per million or milligrams per liter of water) consumed by the contaminants, during two hours of decomposition from a solution of boiling

potassium dichromate. The higher the chemical oxygen demand, the higher the amount of pollution in the test sample. For the contaminants that can be oxidized biologically, the biological oxygen demand (BOD) method is used.

It is also obvious that soil pH could increase when alkaline effluent is irrigated to the soil. A larger amount of suspended and dissolved solids increases the BOD and COD of the soil. Direct use of effluent water to the crop result in significantly poor germination, lesser seedling growth and vigour index. This might be due to the presence of greater amounts of Ca, Mg and other solids materials in the effluents.

Sharma *et al*, (2002), studied the effect of fertilizer factory effluents (0, 1, 2, 5, 10, 25, 50 and 100%) on seed germination of tomato cultivars . The percentage

germination step by step decreased with rising concentration of effluents. Germination increased at 25% effluent concentration. Higher concentration (50 and 100%) showed adverse impact on germination**(Soundarrajan and Pitchai ; 2007) .** Tannery effluent at different concentration (25, 50, 75 and 100%) inhibited on germination by 25 and 50% effluent and fully dormant by 75 and 100% effluent in *Oryza Sativa* (**Rajannan *et al*;1998**). Even the chlorophyll and protein contents of plant were reduced with the effluent concentration of 75 and 100%.

Seeds of pre-treatment sets were imbibed for their full-imbibition period in the industrial effluent. For control set seeds were imbibed in distilled water for their whole imbibitions period. There after, seed were washed with water and transferred to distilled water moistened filter paper in petriplates for germination in dark. Seeds were allowed to germinate at temperature in laboratory conditions. The seeds with 10 mM length of radicle were considered as germinated seeds. The imbibition period for *Hordeum vulgare* was 12hours.

Selected effluents from different sites were used for the study of seed germination.The inhibition in seed germination in effluent site -A, site –B and site C was inhibited with varying intensities respectively in Hordeum vulgare .

For post radical emergence treatment seed were firstly imbibed in distilled water for 12 hours, After the emergences of radical, seeds were transferred to the effluent of various sites –A, B and C for seedling growth studies , seedling were dissected into plant i.e. radical and coleoptiles after 6^{th} day and their length were measured , it was observed that with increasing effluent concentrations there was a decrease in length of seedling parts-maximum inhibition in seedling growth was observed in the site – A effluent treated set. Pots experiments were made to investigate the role of effective microorganisms (EM) in improving phytoextraction of metals (cd^{+2} and Mn^{+2}) and growth of soybean plant in industrial waste water polluted soil. Waste water application to soil were made in four different dilution (i.e. 25%, 50%, 75%,

and 100%). Effective microorganisms were added into waste water prior to application. Plant height significantly increased with all treatment except at 25% waste water treatment. Plant dry biomass and oil contents in seed significantly increased with all treatment compared to control but were higher at low concentration of waste water. Waste water treatment significantly increased the Cd ++ and Mn accumulation in plant while inoculation of EM further enhanced the metals accumulation(**Ali *et al*;2012**).The reduction in growth of seedling was attributed to the greater amounts of Ca, Mg and solid materials in the effluents. **Dhevagi *et al.* (2000**) studied the effect of paper mill effluent on spermosphere microflore of maize, sunflower, greengram, blackgram, soybean and groundnut with different dilution levels. Among these crops studied, groundnut recorded the maximum microbial population (104.1 X 10^{6-1} g of soil), followed by soybean (50.7 X 10^{6-1}g of soil). In case of blackgram, greengram, and soybean up to 100 per cent effluent concentration, the total microbial population was higher than the control.

Rajaram and Janardhanan (1988) conducted germination studies and early seedling growth of soybean, cowpea, rice and sorghum by germinating seeds in pertridishes containing equal volume of different concentrations (1, 2.5, 5, 10, 25, 50 and 100%) of distrillery effluent germinated seed in distilled water served as control. The germination percentage and early seedling growth of soybean was markedly suppressed as the concentration of effluent increased. It was considered that high total dissolved solids value would retard seed germination by enriching the salinity and conductivity of the solutes which were being absorbed by the seeds prior to germination.

Sodium in soybean seeds at D_1 (40 ug/g) of NaCl rising to extremely high level, 3.007% ($p<1$) was elevated upto 131.1% but the interactive effects of all the concentrations of NaCl-Zn brought significant depressions of Na between 9.2% and 11.5%. Na accumulated to higher levels in seeds than in roots at all the levels of the applied heavy metals. K uptake in soybean seeds increased upto 0.476% at D_5 (25ug/g) of copper and 0.316% at the lower concentration, D_4 (25 ug/g) of

the same metal in root exhibited almost similar levels enhancements., 155.9% and 155.2% respectively (**Singh *et al*;2011**).

Mechanism of heavy metal ion stress tolerance varies form species to species in different ways-Excretion of salts into the cell vacuole, precipitation in the protoplast and retention in the soluble but non-ionic and non-toxic chelated form. The specific mechanism depends on the chemical nature of the protective substances developed by the plant (**Levitt;1980)** The resistant species have been found to accumulate high amount of heavy metals in their roots in comparison to non-resistant herbaceous plants which have high amount in their leaves.

Doses of NaCl salinity, copper, zinc and iron each were applied on µg/g dry soil basis . Plants were harvested along with roots at the seed maturation stage; Seed and root components were analysed for Nitrogen and Potassium. Three replicates for each of the two elements were considered for all the treatments.

Potassium in root under NaCl was significantly increased from 80 to 120µg/g its peak levels being 0.254% .At 200µg/g NaCl was most effective to favour K absorption and accumulation to the extent of 0.31% (P<1) over 0.123% of the control.

The salinity of the heavy metals exhibited quite different responses of seeds and root. Potassium in seeds increased with increasing doses of the salinity and the metals. K in root was favoured by low concentrations of the salinity and the heavy metals, Na and K mobilization in seeds and root of soybean varied considerably under different treatment The salinity had a significant role in increasing Na in seeds upto 131.1% at NaCl 40 µg/g . K absorption and accumulation was favoured to higher extent than Na in soybean (**Singh *et al*;2011**).

Heavy metals accumulate in the top soil resulting into increase in their absorption and accumulation in plant, the anthropogenic use of heavy metals either through

sewage-sludge or through fertilizers is due to enrichment of heavy metals in soil. Cadmium (Cd) toxicity in plants cause leaf roll, chlorosis and reduced growth of both root and stem. The levels of Cadmium(Cd), Chromium (Cr), Nickel (Ni), and lead (Pb) have been observed above the critical toxic level in plant leaves . In general, the concentration of cd in plant decreases in the order; Root>fruit>seed. The pH-induced cd immobilization in soil is attributed to various reasons such as increase in adsorption due to increase in surface negative charge, greater affinity of hydroxyl species ($Cd\,OH^{+}$) for adsorption sites than Cd^{++} and precipitation of Cd as $Cd\,(OH)^{2}$. Cadmium is also an effective inhibitor of photosynthesis (**Vassilev *et al*;2005**)

Cadmium was most hazardous, the combined application of Cd and Pb was also harmful while the individual effect of Pb was found least harmful in relation to biomass production, bioaccumulation of heavy metals, sugar content and vitamin C content in the dietary vegetables.(**Mani *et al*; 2012**)

There are spatial and temporal variations in plant injury symptoms when polluted water is used. The symptoms may range from chlorosis to necrosis. The intensity of losses depend upon the pollutant concentration, duration of exposure, climate & edaphic factors, plant species & cultivars. There is reduction in photosynthesis and biomass (**Agrawal, 2005**).

Farmers are using polluted water and sewage for irrigation purposes for growing crops. In many of the cases, such water has accelerated the growth & development processes in cultivated crops but in some it had inhibitory effect due to the array of different organic & inorganic solutes and heavy metals .

In-plant treatment of waste water is not being practiced in most of the cities and almost practically nothing is being done in Bundi. Agricultural utilization of waste water offers a low cost alternative. The sewage water contains significant amount of nitrogen, potassium sulphate and number of bacteria, fungi, ameba, even parasites, virus and coliforms etc.The faunal and microfloral components of soils are altered when sewage sludge is added [**Mitchell *et al*, 1978**].

A change in soil pH can result from application of sewage sludge. Increased soil pH occurred when municipal sewage sludge was added to soils [**Silviera and Sommers, 1977**].

Addition of relatively high rates of sludge increases the cation exchange capacity (CEC) of soils [**Soon, 1981; Mitchell *et al*;, 1978**]. This increase in CEC results in additional cation binding sites which retain essential plant nutrients within the rooting zone. Possibly the CEC increase causes more complexing of heavy metals in an unavailable form for plant uptake [**Kladivko and Nelson, 1979b**]. Less than 17 percent of the total amount of Cu, Zn, Pb, and Cd in sludges and approzimately 22 percent of Ni are in the sorbed and exchangeable fractions, that are readily available to plant. The remainder of the metals is present in forms which require conversion to water-soluble, exchangeable, or sorbed forms before uptake by pants. It is suggest that different chemical forms of a metal after incorporation into the soil may not be similar for different sludges [**Sommers, 1977**].The chemical and physical properties of soils that receive sludge influence metal conversions [**Stover *et al*., 1976**]. Soil properties, such as CEC, pH, organic matter content, sesquioxide content, redox potential, texture, and presence of other elements affect plant uptake, solubility, and mobility of these metals [**Soon, 1981].**

Comparisons between crop species show wide variations in their ability to absorb potentially toxic trace elements from the sludge-soil system. Cereals and legmes accumulated less Cd in shoots than did leafy vegetable, like curlycress. (*Lepidium satival* L.) lettuce (*Lactuca sativa* L.), and spinach (*Spinacia oleracea* L.) [**Bingham *et al*., 1975].** Sensitivity of plants to metal toxicity can be associated with the tredency to accumulate the metal in shoots. Soybeans, lettuce, and curlycress were injured by soil Cd levels of 4 to 13, ug/g soil. These plants tended to accumulate Cd in shoots [**Boggess *et al*., 1978; Bingham *et al*. [1975**]. The status of $CaCO_3$ and soil pH are two crucial factors in terms of availability of these heavy metals in sewage-irrigated soils (**Laetitia *et al*;2002**) . The sewage-irrigated soils have been found relatively low in Ca content (**Tiwari *et al* ;2003)** however, it contains vaiable amount of heavy metals like Cd, Cr, Cu, Pb and Zn etc **(Kaushal *et al*;1993). Samras et al (1998**) have reported a strongly negative correlations of pH and $CaCO_3$ with heavy metals. . Organic matter promoted the availability of these heavy metals by supplying complexing agents that interfered with the fixation of these

metals (**Chitdeshwari *et al*;2002**). Probably due to the increased microbial activity and redox potential of the surface soils.

There is high affinity of lead, copper, cadmium and zinc to soil OM under natural conditions. Soils with high OM and clay minerals, alongwith significant amount of carbonate minerals may be suitable media for the immobilization of an eventual heavy metal contamination. The immobilizing effect of a soil decreases with increasing number of polluting metals, and a very small pH change (one unit) can result in loss of an important part of this capacity.

Stark and Clapp [1980] showed that crop yield and N uptake increased with sludge application as compared to treatments that did not receive sludge.

Mineralization is the conversion of an element from an organic form to an inorganic state as a result of microbial decomposition. Mineralization is important since plants absorb most of their N in the forms of NH^{4+} and No^{3-}. Mineralization has been reported to occur in most sludges [**Hsieh *et al.*, 1981].**

Barley, wheat and oat produced high protein seeds in sewage irrigated fields but digestible nutrient content in seeds were not affected. There are sufficient amounts of colouring matters in sewage effluents. These dyes impart toxicity and impede light penetration in receiving water bodies. Adsorption on charcoal is an efficient method. Biosorption acts by sequestering organic and inorganic species including metal, dyes and odour causing substances. Absorption on activated charcoal is represented by an equilibrium isotherm, which is the plot of the quantity of sorbate, retained on biosorbent as a function of the equilibrium concentration of the sorbate in liquid phase. According to Langmuir adsorption isotherm, biosorbents have high monolayer saturation capacity and can accumulate dye more than its weight.

Lal & Swarup (2005) studied the grain fodder production systems irrigated with sewage & tube well water. Application of sewage resulted in higher biological activities as compared to tube well water irrigation.The growth and yield of wheat were markedly improved with the use of

sewage water and was maximum with the application of recommended dose of N and P. Gain in grain & straw yield of wheat was 21 and 24% respectively, with application of sewage water. Contents of N and P in grain and straw of wheat were found more in sewage irrigated as compared to tube well irrigated. The grain yield of rice was improved by 19% with sewage as compared to tube well water.The effluents may be treated by bacterial system, fungal system, chemical method and combination of chemo + biological methods in order to bring down the lethal or harmful effect to the **eco system .**

Jothimani and Elayarajan (**2003)** conducted a study to know the effect of effluent treated by different methods on germinability and growth of blackgram and greengram. Results suggested that in both the crops, the higher germination percentage, root length, shoot length and vigour index were observed in the effluent treated with bio system and chemo + biological combined system, while chemically treated effluent inhibited the germinability and seedling growth. This was attributed to the presence of higher amouts of salts in the chemically treated effluent which might have restricted root growth by increasing soil osmotic pressure. The reason attributed for higher germinability under chemo + biological treatment may be due to more nutrients availability in the effluent.

It is a well known fact that with the increase in concentration of soluble salts, the electrical conductivity of the soil treated with undiluted effluents would be maximum. It is also obvious that soil pH could increase when alkaline effluent is irrigated to the soil. A larger amount of suspended and dissolved soilds increases the BOD and COD of the soil. Direct use of effluent water to the crops results in significantly poor germination, lesser seedling growth and the vigour index. This might be due to the presence of greater amounts of Ca, Mg and other materials in the effluents. Analysis of the sewage disposed in the soil showed that total metal concentrations of Pb. Cu and Cd in the soil ranged from 11.85 ± 0.20 to 29.27± 0.69, 8.83± 0.11 to 26.22± 0.40 and 32.75 ± 0.30 to 56.19 ± 0.50 µg/g, while those in plants ranged from Nd to 16.76 0.36, Nd to 15.50 0.23 and Nd to 56.30 0.25 µg/g, respectively. Significant correlations were observerd between the soil Pb, Cu and Cd. Extraction of Cd and its transfer in shoots was significant. The highest content of all these metals were noted in *N. hindostana* with the maximum accumulation

for Cd. It is advocated that *N. hindostana* may prove effective in Cd removal from sludge and waste substrates (**Tripathi and Mishra;2012).**

The primary heavy metals present in sewage, include Zn, Cu, Pb, Cd, Ni, Cr, Sn and As (**Stephen ;2009).** The long-term use and disposal of sludge can cause heavy metal accumulation in soils and can constitute risk to environment and organisms when contaminant levels reach up to limit **[Alvarenga *et al*;2008)].** Most heavy metals accumulate in the top soil and in the long term augment their concentration increasing their absorption and accumulation in plants (**Nabulo *et al*;2008)**. As a matter of fact, all plants do not accumulate heavy metals as the level of concentration of heavy metals varies with, the type of metal, soil conditions and type of plant variety (**Rosselli *et al* ;2003).** There are numerous studies on phytoextraction and bioaccumulation of heavy metals in plants (**Yoon *et al*;2006), (Lai *et al* ;2010).**

Phosphorus has low availability in the plant root rather than low phosphorus concentration in the soil. The chemical composition of the soil organic matter differs significantly among clays, silt and soil size classes. Particulate organic matter (POM) is a fertility indicator due to their sensitivity to management and association with N-supply. POM contents are also positively correlated with the amount of soil derived N-taken up by crops as well as with the amount of fertilizer - N retained in the soil.

The specific field requirement of phosphorus is governed by the soil type, crop and agriculture conditions. Phosphorus is necessary for cell divisions, meristemetic growth, root , seed and fruit development. Potassium is involved in the opening and closing of the stomata which are essential for photosynthesis and nutrient transport. It reduces lodging and imparts disease resistance.

Any inorganic phosphorus (P) added to the soil becomes labile after application but is transferred to active P to stimulate soil P sorption and stimulate P buffering which is governed by an equilibrium constant .

Using x-ray computed microtomography (x-ray CT) to observe differences in moisture around fertilizer in P granules with the application of minimum phosphate, it was observed that mass

flow of water toward the granule had a restricted diffusion. There is reduced lability of granular phosphorus .Nuclear magnetic resonance spectroscopy has shown that the chemical composition of soil organic matter differs significantly among clay, silt & sand sized classes .

Under deficiency of P, plants develop stunt growth, reddish purple coloration in nodes and internodes due to increase in sugar content and show formation of anthocyanin. Phosphorus need (Kg P_2O_5/ha) of crops to produce optimum yield on low or deficient soils is 20-40 for oil seeds.

Plants have evolved a variety of adaptive strategies to improve their acquisition, use and remobilization of phosphorus (P). Plants response to P stress conditions involved changes in root morphology and architecture.

A multiple regression coefficient has been worked out to predict the best crop yield.

$$\hat{y} = a \times 1 - b \times 2 + c \times 3 - d$$

where $\hat{y}$ is the expected best crop yield, x1, x2 & x3 are the precipitation, temperature , sunshine and a, b, c & d are constants.

A study was conducted on the quality of irrigation water in Kota . The main aquifers of the area included shale, sand stone, lime stone and basalt. The survey revealed that the chemical character of irrigation water varied widely. The river water had EC 210 μ mhos/cm. The ground water varied considerably from fresh (EC 210 μ mhos/cms) to highly saline (EC 660 μ mhos/cm).

The excess of soil water depletion that leads to reduction in the rate of transpiration, depends upon the evaporative condition of the atmosphere. Greater soil water depletion can be tolerated without any adverse affect on growth at low evaporative potential of the atmosphere. The maximum evapo-transpiration in soybean ranges from 2mm on day 2 to 0.80 on day 10 when crops are grown in well stored soil water. Fertilizer application increases crop yield not only by correcting nutrient deficiency but by also enhancing water use.

Rise and fall of civilization in ancient India and development in agriculture, environment and meteorology

The review presents an extremely brief glimpse of the developments in agriculture environment and meterology from the PREHARAPPAN civilization up to the Gupta period (2300 BC to 647 AD). A PREHARPPAN civilization flourished in Rajasthan around 300 BC which was deserted later due to reasons which are still speculative.

The review has been presented in two parts. The first part deals with the chronological developments from PREHARPPAN to GUPTA period.

The second part presents the research findings and statements from the prodigies.

Preharappan township was found at the mound of Kali banagn and Pilibangan in Rajasthan. Kalibangan is on the left bank of the dry bed of the Ghaggar (probably the ancient Saraswati river) in the Ganga Nagar district. Below the Harappan citadel are the remains of Pre Harappan small township which represents a different culture. 14C-dating determination show the date between 2450 to 2300 BC for the pre Harappan level. Some of the houses had oven and cylindrical pits lined with lime mortar used for the storage of food grains –a pattern which persist in Rajasthan even now.

The desertion of the Kalibangan and other sites in the Saraswati Valley took place owing to a change in the course of the river about 1800 BC to 1700 BC. Faulted strata and ruptured walls were revealed in the excavated pre Harappan settlement which were probably destroyed due to earth quake. It was rehabililated and subsequently flourished for about 600 years. Some of the customs and adoration of ornaments still prevails in Rajasthan. Marwari women cover their entire arms with silver bangles like the dancing girl of Mohan Jodaro.

A Banasian culture (named after Banas river and its tributaries) has been excavted at Ahar situated on the bank of Banas. The excavated mound is called Dhulkot. A hill girt valley was chosen by the people of Ahar. Some cereals like wheat were ground into flour and made into

chapaties. Jiwari (a kind of millet) were found mixedwith clay in making potteries. The prehistoric Aharians certainly ate rice. Rice was of long seeded strains, perhaps the ancestors of fragrant Basmati rice. Ahar culture has been called the copper age culture. They used copper tools and weapons made from the copper smelted from the deposits in the Aravalis.

The Banasian culture was distinctive in its absence of stone industry and presence of numerous copper objects. The Aharian culture started around 1999 BC to 2144 BC. The Banasian culture might have extended from 1800 to 1400 BC.

The period from 2800 BC to 1800 BC, there was rise and fall of Pre Harappans & Harappan cultures especially along the Ghaggar valley where the urban settlement flourished due to availability of water eg. at Kalilbangan. The dune records **(Thomas *et al* 1999**) reveal that the civilization was established during a phase of continued Aeolian activity and not during a period of good rain fall as is customarily believed. The Harappan settlements in the desert, therefore, appears to be more a case of human adaptation to declining rainfall than that of improved hydrological/ precipitation events as is evident from their water harvesting methodologies and emphasis on the winter crops.

Aridity started in western Rajasthan about 10,000 cal B.P. and was probably the reason for the origin of Thar Desert. The Ghaggar-Hakra river possibly dried up. It was once the water source for the Indus Valley Civilization. Remote sensing studies have revealed that late quarternary climatic changes and neotectionics played a significant role in modifying. The drainage which is evident by the presence of Paleo channels even this day.

There are controversies whether the river Ghaggar is actually the river Saraswati (**Nair et. al., 1999)** It was earlier thought that the origin of Saraswati was Glacial. However investigations based on Sr and Nd isotopic composition of the Alluvium of Ghaggar refutes this theory **(Tripathi, 2004).**

Isotopic compostion (2H, 18O, 3H, and 14C) on the ground water in the Kishangarh – Ghantiyali Ghotaru sector of Jaisalmer District show, that possibly river Saraswati charged this aquifer in Ancient times. **(Rao, 2003).**

A Rigvedic description mentions the sudden appearance of the river following breaking up of mountains, distinctly pointing out tectonics being responsible for the birth of river Saraswati. (**Rao, 2005).**

Rice cultivation in Gangetic plains of India had started as early as 8500 cal. B.P. (late Holocene) Recent findings are based on modern techniques Phytoliths found in a Holocene lake fill succession of Ganga plain has been described by **Saxena *et al*. (2006**). They have identified phytoliths of orizae tribe of oryzoidaea subfamily and were able to distinguish between wild and domesticated rice.

Phytoliths are microscopic opal particles that occur when monosilicic acid are carried into the plants with ground water and gets precipitated in the cells & between cells of living plant tissues. After the decay of plants, phytoliths. remain in the soil and sediment of earlier existing ecosystem. Rice leaves produce mainly two types of phytoliths, the balliform & grass silica short cell phytoliths. Rice phytoliths can also be distinguished on the basis of shape, size and ornamentation. Based on these information's, the rice phytoliths can be identified in genera & species. The discrimination between phytoliths of wild and cultivated rice is based on the number of scales on the edges of the fan; the cultivated rice phytoliths show large number of scales (**Lu *et al*., 2002**). Based on these criteria, it was observed that while rice phytoliths were present around 10300 cal yrs BP, the cultivated rice (Oryza sativa) began around 8000 cal yrs BP. This was also evidenced by the presence of diatoms. Diatoms are found in paddy fields. They can withstand highly fluctuating water levels of paddy fields. Excavation of archeological sites have yielded remains of domesticated rice (O. Sativa), wild rice (O. rufipogon) and millet (Setaria sp). The radiocarbon dating was associated it with an age of 7247 cal yrs BP. It appears that cultivated rice is the culmination of the effects of hybridization among the wild species and

the selection of better ones. Thus breeding experiment in cultivation of rice was started as early as 7247 cal yrs BP in Holocene period.

The grain size analysis was done by normal sieving method followed by determination of salt and clay fractions. In this experiment, radiocarbon dates of total organic matter was utilized. At a depth of 2.75m, radiocarbon dating method indicated that the probable date of 10425 cal yrs BP was the period of rice cultivation. The rate of sediment deposition was 2.8 mM/100 yrs. **(Saxena et.al, 2006)**

Reconstruction of paleoclimate and paleo vegetation in the Quaternary was inferred from several proxies such as pollen, soil, organic matter, microfoconal assemblage, carbon isotope data, geochemistry, magnetic minerals and phytoliths (**Bremond, *et al*., 2004**). In subtropical-tropical climate with prolonged seasonal droughts, preservation of organic matter and pollen spores is usually poor. However, in such areas, phytoliths are preserved in discrete particles.

Holocene period has seen marked changes in climate which forced people to adopt themselves to the changing environment and modify their food requirement. Agricultural practices were transformed to produce adaptable grains.

The Vedic Age (1500 B.C. to 1000 B.C.)

One must realize that there was complete absence of instrumentation in ancient period and whatever observations were made were purely based on intuition, instinct, reasoning and logics. They used clumsy bamboo pipes of various diameters to observe the astronomical changes. It was only during the reign of Emperor Vikramaditya that studies in meterology and astronomy were at its zenith.

There are 21 references to agriculture and ploughing in the Rig-Veda, the bulk of which are in Books I and X. Books I and X, which account for a good bulk of the Rig-Veda, are admittedly

late, both from the point of view of style and the nature of material culture they reveal. Most references to field agriculture are confined to these mandalas, and evidence furnished by them shades off into post Rig-Vedic period from around 1000 B.C.

The Aryans' land of Northern India is well-watered and has seven rivers. There is reference to craftsmen (ribjus) who led forth the rivers. (R., IV, 4.1.7). The reference is to irrigation by channels taken from the rivers. There is also reference to soil erosion by rivers. "Rivers, the corroders of their banks, like armies destructive of their foes" (R., IV.2.9.7).

Wells were in use for irrigation. "Tie the ropes tight to the water pots, Let us draw water from the unfalling well. (R. × 9,219) indicating that wells are inexhaustible souble of water.

Measuring-rods of presumably standard lengths were used for measuring fields. "Like a field measured by a rod" (R.,I.16.5.5).

Harvesting began with a prayer. The harvesting tool was the sickle. Thus: "I take the sickle also in my hand with a prayer to thee" (R., VIII.8.9.10). "May the crop swell at my prayers; let the sickles cut down the heavy crop of grain" (R., V. 6.12.9.10) " May there be abundant food, may the grain fall ripe towards the sickle. (R., X. 9.2.3). Some form of container or measure was used for which the word urdara is used.

Harvesting was both by cutting down the crop at the level of the ground and also by cutting the earheads. As barley is harvested by separating the earheads from the stalk" (Y., 122.1). Thou milkest the nutritious grain from the humid stalk" (R., II. 2.2.6).

Later Vedic Period (1000 B.C. to 600 B.C.)

From 1000 B.C. to 600 B.C., a number of Vedic texts were complied in western Uttar Pradesh. They include the Samhitas and Brahmanas. The collections of the Vedic hymns or mantras were known as the Samhitas. The Rig-Veda Samhita is the oldest Vedic text, on the basis of which has been described the early Vedic age. For purposes of singing, the hymns of the Rig-Veda were set

to tune, and this modified collection was known as the Sama-Veda Samhita. In addition to the Sama-Veda in the post-Rig-Vedic times, two other collections were composed, viz. the Yajur-Veda Samhita and the Atharva-Veda Samhita. The Yajur-Veda contains not only hymns but also rituals which have to accompany theire recitation. The rituals reflect the social and political milieu in which they arose. The Atharva-Veda contains charms and spells to ward off evils and disease. Its contents throw light on the beliefs and practices of the Aryans.

Generally, in the Vedic period, two harvests a year were gathered. The number of references pertaining to agriculture found in the Vedic literature indicate that the cultivator in the Vedic period possessed a fair knowledge of the fertility of the land, selection and treatment of seeds, seasons of sowing and harvesting, rotation and other cultural practices of crops, manuring for increased production of crops, and the like. The Vedic farmers knew the method of improving the fertility of the soil by using the method of rotation. The Taittiriya Samhita mentions that rice would be sown in summer and pulses in winter on the same field.

The Atharva-Veda provides us with a considerable number of spells to avoid blight and secure a good harvest.

Buddhist Period (6th century B.C.)

By the sixth century B.C. the iron age was well established in Uttar Pradesh and Bihar. Spear-heads, arrow-heads, axes, daggers and knives of iron were manufactured in substantial numbers. Iron ploughshares and sickles of iron made farming more efficient.

The Magadhan Empire (543 B.C. to 491 B.C.)

Magadha emerged as the most powerful State in eastern India in the sixth century B.C. Bimbisara (ruled 544 B.C. to 492 B.C.), the king of Magadha, conquered Anga. Bimbisara made Magadha the paramount power. He was succeeded by his son Ajatasatru, who ruled from 492 B.C. to 460 B.C. Ajatasatru began the construction of fortifications of Rajgir. He was succeeded

by udayin (460-444 B.C.), who made his capital at Pataliputra, the present-day Patna. In 413 B.C., the house of Bimbisara was overthrown by Nanda. Mahapadma Nanda conquered Kalinga and Kosala. The Nandas of Magadha used elephants on a large scale for their warfare.

In 325 B.C., the Nanda dynasty was overthrown by Chandragupta Maurya, whose chief adviser was Kautilya (322 B.C.), also called Chanakya, the author of the Arthashastra. Megasthenes was sent on an embassy to Chandragupta Maurya, by Seleucus Nikator, king of Bactria. There were all round developments in agriculture, irrigation, administration and financial managements entirely due to the efforts of Kautilya observed Megasthenes.

In the ring wells, instead of bricks, terracotta rings were used. Ring wells appeared in India in the sixth century B.C. and continued till about the second century of the Christian era. The ring wells of Ropar are dated from fifth to fourth centuries B.C. to the beginning of the Christian era. Ring wells can be seen even at present in the south and in Orissa and Bengal. Their present use is for drinking water. In ancient times, they were mostly used as soakage or sullage pits. In some places, undoubtedly, they also must have been in use for drinking-water. In ancient times, they appeared often in clusters. In Ropar, there were five of them in a cluster, their depth being different from one another..

Agriculture in the Mauryan Age

The Greeks noticed in India two annual harvests-the winter and the summer ones- and the sign of an astonishing soil fertility. Aristobulus described the cultivation of rice in enclosed sheets of water.

In the Asthashastra, we find a mention of the suitability of different lands for the cultivation of different crops, viz. lands that are beaten by foam, e.g. river banks, etc. are suitable for growing pumpkin, gourd and the like. Lands that are frequently flooded with water for long are suitable for pepper, grapes and sugarcane; those in the vicinity of wells for vegetables and root crops; moist beds of lakes, etc., for green crops; and the marginal furrows between any two rows of

crops are suitable for the plantation of fragrant plants, medicinal herbs, khus-khus roots, and the like.

The seeds of grains are to be exposed to mist and heat for seven nights; the seeds of kosi are treated similarly for three nights.

The sprouts of seeds, when grown, are to be manured with a fresh haul of minute fishes and irrigated with the milk of snuhi (Euphorbia anti-quorum).

The Kama Jataka speaks of a Brahmana clearing the jungle for cultivation and making little embanked squares for water. We also hear of the rivers being dammed for the purpose of irrigation. Says the Kunala Jataka, 'The Sakiya and the Koliya tribes had the river Rohini, which flows between the cities of Kapilavasthu and Kolia, confined by a single dam, and by means of it cultivated their crops. In the month of Jetthamula when crops began to droop, the laboureres from both the cities assembled.

Kautilya also refers to sluice-gates of tanks and enjoins that 'persons letting out the water of tanks at any other place than their sluice-gate shall pay a fine of six panas; and persons who obstruct the flow of water from the sluice-gate of tanks shall also pay the same fine.' It is further laid down that 'the water of a lower tank, excavated later on, shall not irrigate the field already irrigated by a higher tank and the natural flow of water from a higher to a lower tank shall not be stopped, unless the lower tank has ceased to be useful for three consecutive years.'

Plant Diseases : In the Kallavagga, we find the Buddha pointing out that when the disease called 'mildew' falls upon a field or rice, that field of rice cannot last long; neither does a field of sugarcane continue long if the disease called 'blight' falls upon it.'

The use of kiln-burnt bricks became popular in the Khshan period (40 A.D. to 220 A.D.). In the sites which have been excavated in northern India, the use of kiln-burnt bricks for floors and tiles for floors and roofs is common. The Kushan bricks from Sanghol in the Punjab are large, 32×20 c.m., and 6 to 8 c.m., thick. An agricultural innovation which can be attributed to the Kushans is the construction of brick-wells, which were used for irrigation.

Iron technology made great progress in the age of Satavahanas and Kushans. Indian iron and steel weapons and cutlery were exported to western Asia where they enjoyed high esteem. In India it led to the manufacture of sturdy agricultural implements. A number of iron agricultural implements were recovered from the Bhir mound at Taxila. These include a variety of hoes with length varying from 18 to 30 centimeters, seven spuds, 15.5 to 18 centimeters in length, and five sickles. One sickle had a curved blade and the other a straight blade and a curved handle. The length of the blades varied from 12.5 to 18 centimeters. True spades were also discovered which are superior in design to those currently in use in rural India. The workmanship of these iron agricultural implements indicated the high level of iron technology in India during 300 B.C. to A.D. 100.

The Age of Guptas (A.D. 320-544)

Our sources of information about the life of the people and their agriculture and horticulture in the Gupta age are Vatsyayana's Kamasutra, Varahamihira's Brhatsamhita, and Amarsimha's Amarakosha. Vatsyayana's Kamasutra also provides information on gardens. Varahamihira was an astronomer, astrologer, and encyclopaedist. He flourished in the period A.D. 505-587. His Brhatsamhita provides information on agriculture, botany and zoology, apart from astronomy, medicine, metallurgy and geography. It describes specific characteristics of animals and the treatment of plant diseases. The Brhatsamhita, and the Puranas, particularly the Agnipurana, incidentally deal with the selection of land, manuring, cultivation, collection and the treatment of seeds sowing, planting, reaping and grafting.

The Amarakosha of Amarasimha, a scholar in the court of Chandragupta II (A.D., 380-415) contains information of soil, irrigation and agricultural implements.

Soil classification and Land use

The Amarakosha describes 12 types of land in the chapter on Bhumivarga, depending upon the fertility of the soil, irrigation and physical characteristics. These are : urvara (fertile), usara (barren), maru (desert), aprahata (fallow), sadvala (grassy), pankila (muddy), jalaprayamanupa

(watery), kaccha (land contiguous to water), sarkara (land full of pebbles and pieces of limestone), sarkarvati (sandy), nadimatrka (land watered from a river), and devamatrka (rain-fed). In the Vaisyavarga are mentioned different kinds of soils and their suitability for the cultivation of specific crops, e.g., ksetram-rice and corn; yavya-barley; tailinam-sesamum; maudginam-green-gram, etc. There are also different names for lands ploughed once, two times and three times, and at several stages.

Use of Manure

The Brhatsamhita prescribes that seeds which have been properly treated are to be sown with the addition of pork or venison into the soil (where previously the sesame crop was raised, dug up and trodden) and sprinkled daily with water mixed with milk (ksira). It says further, 'To promote inflorescence and fructification, a mixture of one adhaka (64 palas) of barley powder, one tola of beef thrown into one drone (256 palas) of water and standing over seven nights should be poured round the roots of the plant. 'To ensure sprouting, and to promote the luxuriant growth of the stem ant the foliage, the seed should be soaked in an infusion made of paddy power, urad, sesame and barley mixed with decomposing flesh, and the whole mass steamed with the addition of turmeric.

According to the Agnipurana, a tree becomes laden with flowers and fruits by manuring the soil with powdered barley, sesamum and the offal of a goat mixed together, and soaked in washings of beef for seven consecutive nights. A good growth of these is secured by sprinkling the washings of fish on them.

Irrigation :

There is enough evidence to indicate that due recognition was given to irrigation. Land known as nadi-matrka depended on irrigation from river water. Water in tanks and pools was used for irrigation in the central and southern parts of India.

The Naradasmtri states that the erection of a dyke in the middle of another man's fields was not prohibited in view of the fact that it would be advantageous for irrigation, whereas the loss is

trifling. It states further that a man with the permission of the owner can restore a decayed dyke, although without the owner's consent he cannot use it. Narada classifies the dykes into kheya (which is dug into the soil to drain off excess water) and bandhya (which is constructed to prevent the water from flowing out)'.

Harshavardhana (A.D. 660-647)

Agriculture : Hiuen Tsang (629-647 a traveler from China) mentions the characteristic products of the regions visited by him. From his account, it appears that cereals like wheat, rice and millets and fruits were extensively cultivated. He specifically mentions a dozen states which were remarkable for their fertile soil, good farming and rich crops. In Poonch and Mathura, fruits were grown in orchards adjoining homesteads. Paryatra (Bairat) produced a variety of rice and which was ready for harvesting in sixty days while Magadha grew another variety with large grains of extraordinary fragrance which was called 'rice for grandees.'

Shaman Hwuii, The disciple of Huen Tsang mentions of a rice variety as large as the bean; like no other rice at all. "It grows only in Magadh and no where else."

Bana Bhatt's Harshcharita provides a vivid picture of Indian society, climate and crops. These are based on the inside knowledge of a native with a keen sense of observation.

Bana thus describes autumn, "when the rains stop and paddy ripens." "It is the beginning of the autumn, when the clouds are thinned". The range of mud diminishes, young sand isles bud is formed in the Priyangu blossoms and reed grass smiles with flowers".

Bana describes the products of the Thaneser Tract (Present day Haryana) "unbroken lines of sugarcane seem besprinkled by the clouds, throughout; it is adorned with rice crops extending beyond their fields."

During the Vedic period, Rivers were considered holy and respected with reverence. Polluting the rivers, ponds, lakes and wells was considered abhorrent in the Purans, A list of such acts have

been mentioned which must not be committed at such places. The following Shlok in "Taitariya Aranyak" specially commands not to perform such acts which would pollute the river Gangas.

गंगा पुण्यजलां प्राप्य त्रयोदष विवर्जयेत् ।

षौचमाचमनञचैव निर्माल्यं मलघर्शणम् ।

गात्रसंवाहनं क्रीडां प्रतिग्रहमथो रतिम् ।

अन्यतीर्थरतिं चैव अन्यतीर्थप्रषंसनम् ।।

वस्त्रत्यागमथाघातं सन्तारं च विषेशतः ।

नाभ्यङ्गितः प्रविषेञच गंगायां न मलार्दितः ।।

न जल्पन्न मृशा वीक्षन्त वदन्ननृतं नरः ।

"Thou shall not defecate or urinate in the holly Gangas. Thou shall not brush or wash your mouth, rub your body, throw dirty clothes, make percussions, swim perform sexual act, leave adorned clothes, shall not accept donations, shall not use anoinments, bathe in dirty clothes and shall not use vulgar languages."

Use of contaminated water was considered as sin. (Bhavishya Puran chapter 3- Nirnaya sinahv), After bathing, one was required to irrigate the plants.

In Raghuvansh, written by Kalidas, it is mentioned that King Raghu asks Rishi Kavras whether the water he is using is polluted and affects his daily chores as is described in the shlok given below :

निवर्त्यते यैर्नियमाभिशेको येभ्यो निवापाञजलयः पितृणाम् ।

तान्युञछशश्ठांकितसैकतानि षिवानि वस्तीर्थजलानि कच्चित् ।।

The details about meteorology is described in the book Guru Samhita probably written by Acharya Brihaspati. There are 452 shlok in the samhita. Since agriculture depends on rain fall

and rain fall is governed by the clouds, the book describes various types of clouds and their shapes. By closely watching the types of clouds it was possible to profess the possibilities of rainfall or otherwise. The possibility of rainfall by observing the lightening and velocity of wind could be professed. It also gives the description of 6 types of weathers. In ancient period, agriculturist designed their operations based on this book. It also describes the science of rainfall and its occurrence in all the 12 months right from October to the next cycle.

(कार्तिक से अष्विन मास) ''गार्भिके... सम्पत्तिरुपम्'' (Guru Samhita 1)

"The colour of the clouds are white, yellow, black, or of copper colour. मार्गषीर्श आदि पाँच मासों के षुक्लपक्ष में यदि किसी तिथि का क्षय होता है तो दुर्भिक्ष होता है (The observations are so prefect that even modern historical research data show that several internecine war were fought between adjoining states during the time of famine. The possibility of a war during famine have been described in the shloka.

मार्गादिञचमासेशु षुक्लपक्षे तिथिक्षयः ।

दुर्भिक्षं छत्रभंगो वा जायते राजविग्रहः ।। ;ळनतनॅंउीपजं 27द्ध

मार्गषीर्शे यदा मासि सप्तमी नवमी दिने ।

ईषानादिसमाश्रित्य दृष्यते मेघमण्डलम् ।।

स्तोकं वर्शति पर्जन्यो•थवा वातमादिषेत् (Guru Samhita 28-29)

If there is occurrence of cloud in the north eastern direction (ईषान दिषा) on seventh or ninth day, it may lead to scanty rainfall or there is staring breeze.

गर्जते वर्शते पौशे विघुद् वायुष्च षोभनः ।

अतिवृश्टिं विजानायाद् धान्यं याति महर्घताम् ।। (Guru Samhita 53)

If there is excessive rainfall with thunderous cloud , lightening and pleasant wind velocity then it may lead to excessive rainfall and food commodities will become very costly.

माघामासे तु संक्रान्तौ वर्शते माधवो यदा ।

बहुक्षीरप्रदा गावो बहुषस्या वसुन्धरा।।

" If there is rainfall in माघ–मास संक्राति (January-February) then the cows will give more milk and the land will give good agricultural production.

आशाढ़मासे प्रथमे च पक्षे

निरभ्रदृष्टे रविमण्डले च ।

न विघुतो गर्जति नैव वृश्टि –

र्मासद्धयं वर्शति नैव देवः ।। (Guru Samnita 236)

"If there is no cloud in the first fortnight of आशाढ़ मास (June-July) there is no thunderstorm and no rainfall, then it should be known that there will be no rainfall in proceeding two months."

पूर्वस्यां यदि सन्ध्यायां मेघसञछादितं नभः ।।

केचिदृश्ट्रसमा मेघाः केचित्कुञजरसन्निभाः ।

केचिद्वै षूकरमुखाः केचिन्महिशसन्निभाः ।।

केचिद् वै पर्वताकारा केचिद् वृशभसन्निभाः ।

एतद्वर्णाष्च ये मेघा वर्शन्ते नात्र संषयः ।। (Guru Samhita 355-356)

"If during the evening time, the sky is profusely covered by clouds and are having shapes like camel, elephant, mouth of a pig, hills or bullock then it is certain that there will be a heavy rainfall".

Acharya Parashar has written a book Krishi Parashar. Another very famous contribution is by Acharya Varahmihir the Brihit Samhita wherein he has described it in detail about the meteorology and instructions about agricultural activity.

In the 10th chapter of Manu Smriti, it has been said that ploughing results in destruction of many organisms, worms , insects and according to the shastra, it is not good to kill living organisms. However, Acharya Parashar says that agriculture is must for survival. Therefore, it is necessary to perform agricultural production activities. He says that agricultural activity by an agriculturist is a religious phenomenon, has sanctity and a lifeline for survival. As described in shloka Krishi Parashar 1/8.

कृशिर्धन्या कृशिर्मेध्या जन्तूनां जीवनं कृशिः ।

हिंसादिदोषयुक्तो•पि मुच्यते•तिथिपूजनात् ।।

He further says that agriculture is basically dependent upon rainfall and the rainfall is also the essential requirement of mankind. Hence, he must have detailed knowledge about the rainfall.

वृश्टिमूला कृशिः सर्वा वृश्टिमूलं च जीवनम् ।

तस्मादादौ प्रयत्नेन वृश्टिज्ञानं समाचरेत् ।।

[Krishi Parashar 2/1] (Page no – 16)

Parashar states that in a plough, 8 bullocks should be used. The bullocks should not be overburdened. However, if a farmer uses 2 bullocks in a plough then the ploughing work should continue for 3 hours from sunrise, with 4 bullocks upto noon, with 6 bullocks upto afternoon and with 8 bullocks till evening. (Parashar Smriti 2/10)

There are several samhitas which describe in detail regarding astronomy, meteors and general meteorological observations. These have been described in detail by Acharya Garg in Garg Samhita, Narad in Naradiya Samhita. Brihit Samhita by Varahmihir have described in detail regarding natural disasters like earthquake and seismic disturbances. The observations and predictions by Varahmihir were so precise that the present day geologists of Madras University have confirmed the observations of Varahmihir.

1, When earth, sun and moon revolve and come in same line (1-180º) then the gravitational force on the earth increases tremendously.

2. Consequently, there is alteration in the revolution of earth which leads to displacement of tectonic plates.

3. It has been concluded that the earthquake based on the richter scale is mostly dependent on the state of the planets.

Acharya Garg has proposed following principles of seismic disturbances. He states that "On full moon night and no moon night and the next two days are important is relation to earthquake." The present investigations have shown that 55.8% earthquakes have occurred on these days. On the first day and the fourteenth day 33.5%. earthquake occurred . 17% earthquake occurred on full moon and the first day after full moon.He further states that distance between saturn and equator and the position of planets give a precise idea about the occurrence of earthquake. Varahmihir has described the orbitals in 4 categories : (a) Windy Circle (b) Fire Circle

(c) Rainy Circle (d) Oceanic Circle

The samhitas states that the sun masters the weather cycle. When the sun enters the आर्दा नक्षत्र, the rainy season commences.

The colour of lightening also depicts the type of rain. The following shloka describes the affect of type of lightening.

वाताय कपिला विद्युदातपायाति लोहिता ।

पीता वर्शाय विज्ञेया दुर्भिक्षाय सिता भवेत् ।।

Grayish lightening brings about storm, red lightening causes sunshine, yellow lightening results in excessive rainfall and white lightening results in famine.

Acharya Varahmihir has described about the rainfall in the following verse.

प्रायो ग्रहाणामुदयास्तकाले समागमे मण्डलसङ्क्रमे च ।

पक्षक्षये तीक्ष्णकरायनान्ते वृश्टिर्गते•र्के नियमेन चार्द्राम् ।।

{Brihit Samhita 28/30}

“ When a planet is appearing or disappearing, when two planets unite, when moon enters in to another orbit, on full moonnight or no moonlight (Amavasya), when sun enters आर्द्रा नक्षत्र, all these denote the possibility of rainfall”.

In Brihit Samhita 28/22, Varahmihir describes some observations regarding excessive rainfall. They are : water becomes tasteless, salt melts, clouds disappear, sun shines, sharpy ants rush with their eggs, frogs, rabbit, fish rush out of their places, birds bath in the sand, peacock scream in a flock.

Kautilya has described the effect of rainfall on agricultural productivity specially based on the intensity of rainfall in different months. He states that if 1/3rd of the rainfall occurs between july to october and if there is half of the rainfall between august and november then it is extremely good for agricultural productivity. When Jupiter enters from Aries to Taurus, then it leads to fog, rainfall and clouds.

Research methodology

The present study focused on the responses evoked by *G.max* under conditions of salt stess.The lay out of the study is presented below.

In an experiment, a control is a group or an object that is not altered. The control is used to study the differences that occur on the group or object being experimented on. Each treatment was replicated three times. Percent seed germination, mean root length, mean shoot length and mean cotyledon length were measured on day 5 and day 8 .

Some where NaCl concentration is given in normality (N)

- **The experimental design was arranged** – Effect of 2M, 1M, 750mM ,500mM and 100 mM respective electrical conductivity on the germination of *Glycine max* has been determined by Sand method and Rolling towel method.
- Control- Seeds germinated in deionized water .
- Seeds germinated in a solution of 2M, 1M, 750 mM, 500 mM NaCl ,10 mM ,20 mM,50 mM &100mM.
- Seeds germinated in Industrial effluent & Sewage water.

Sand method- Soybeans (*Glycine max*) were sterilized by immersing in 0.1% cupric sulfate solution, washed with water and immersed in water overnight at 28°C. Soybeans were germinated on quartz sand at 28°C in the dark and harvested after 5 day and 8 day. Sterilized water and instruments were used during the germination**.**

Paper Towel Method

The seeds were germinated between two layers of paper . Hundred seeds of the representative sample were counted, , Seeds were placed in rolled towel paper. The rolls of towel paper were then placed inside the germinator in an upright position and kept at room temperature for 5-7 days . Experiments were replicated thrice. The rolling towel beds were changed after three days in order to avoid salt accumulation. The effect of effluent on the germination in the seed was also studied.

Seeds of *G. max* were germinated in industrial effluent, raw sewage & sewage treated with activated charcoal.Sewage was treated with activated charcoal, thoroughly shaken for its adsorbtion capacity & repeated several times.Activated charcoal treated sewage was centrifused at 5000 rpm for 5 minutes & filtered. The supernatant was used for the germination of *G.max* seeds.Seed development was monitored on day 5 & day 8.Length of root, shoot & cotyledon were measured.

Salinity is a measure of the amount of dissolved particles and ions in water. There are several different ways to measure salinity; the two most frequently used analyses are described below :

Electrical Conductivity (EC) : The ability of an electric current to pass through water is proportional to the amount of dissolved salts in the water–specifically, the amount of charged (ionic) particles. EC is a measure of the concentration of dissolved ions in water, and is reported in µmhos/cm (micromhos per centimeter) or µS/cm (microsiemens per centimeter). A µmho is equivalent to a µS.The electrical conductivity was measured in an electrical conductivity meter.

Relationship between EC and TDS

TDS = 0.64 EC units is used to calculate salinity .However this relationship between EC and TDS varies with the concentration of salts in the water and the proportions of the various salts present.

The most common test for estimating industrial wastewater strength is the Chemical Oxygen Demand (COD) test. This test essentially measures the chemical oxidation of the wastewater by a strong oxidizing agent in an acid solution. The value for the COD test is always greater than the BOD test and is not always a good indication of BOD values for the same waste.

COD was measured by the formula-

$$COD\ ,\ mg/L = \frac{(A\text{-}B) \times M \times 8{,}000}{Volume\ of\ sample,\ mL}$$

Where:

A = ml of titrant used for sample

B = ml of titrant used for blank

M = normality of ferrous ammonium sulfate .

COD determination

The dichromate reflux method is preferred over other methods using other oxidants such as potassium permangnate because of:

a.its superior oxidising ability,

b.applicability to a wide range of wastes, and

c.ease of use (**Kaur *et al*;2014)**

BOD determination

The test consists taking the given sample in suitable concentrations in dilute water in B.O.D. bottles. Two bottles were taken for each concentration and three concentrations were used for each sample. One set of bottles was incubated in a B.O.D. incubator for 5 days at 20°C; the dissolved oxygen (initial) content (D_1) in the other set of bottles was determined immediately. At the end of 5 days, the dissolved oxygen content (D_2) in the incubated set of bottles was determined.

$$\text{mg/l B.O.D.} = \frac{(D_1 - D_2)}{P}$$

where,

p = decimal fraction of sample used.

D_1 = dissolved oxygen of diluted sample (mg/l), immediately after preparation.

D_2 = dissolved oxygen of diluted sample (mg/l), at the end of 5 days incubation. **TSS (**

Total Suspended Solids)

Glass fiber filters were first soaked in distilled water, dried at 103^o C, weights were recorded. The dried, weighed glass fiber filter were placed in a filtering flask and pouring into the water. The volume of water filtered was recorded. The increase in weight represented TSS. Total solids includes both total suspended solids, the portion of total solids retained by a filter and total dissolved solids, the portion that passes through a filter . TSS was calculated by using the equation below. **(American Public Health Association, 1998).**

TSS (mg/L) = ([A-B]*1000)/C

Where A = End weight of the filter

B = Initial weight of the filter

C = Volume of water filtered

Gram staining -

Gram stain was used to distinguish between gram-positive and gram-negative bacteria.In the Gram stain, the cells were first heat fixed and then stained with a basic dye, crystal violet, which was taken up in similar amounts by all bacteria. The slides were then treated with an I_2-KI mixture (mordant) to fix the stain, washed briefly with 95% alcohol (destained), and finally counterstained with safranin. Gram-positive organisms retained the initial violet stain, while gram-negative organisms were decolorized by the organic solvent and hence showed the pink counterstain. The difference between gram-positive and gram-negative bacteria lies in the ability of the cell wall of the organism to retain the crystal violet.

Germination test is conducted under most favourable conditions of moisture, temperature and light to know the germination (%) of seed and is calculated as under .

Seed germination (%)=(Number of seeds germinated/ Total number of seeds used)x100

Bacterial studies-Following three samples were analysed for bacterial population-

1. Sample 1: Sewage water as such.

2. Supernatant of Sewage + activated charcoal

3. Precipitate (Sewage + activated charcoal after centrifugation)

The above samples were inoculated on sheep blood agar at 37°C overnight. After growth on blood agar for further procesural treatment,samples were stained with Gram stain.

Standard plate count of the above samples was performed as per the following method:

1. All the glass wares used in bacteriological studies, eg; petri plates, pipettes, conical flasks and test tubes etc. were properly cleaned , washed and sterilized at 160°C for 1 hour.

2. Preparation of Saline solution:

Sodium chloride-2.7 gm

Distilled water-300ml

The salt was properly dissolved in water. It was filled in test tubes in lots of 9 ml each and plugged with cotton. These were then autoclaved at 15 lb pressure for 20 minutes. The sterile blanks were stored in cool and dust free place.

3. Preparation of inoculums: 1 loop ful culture from blood agar plates were inoculated to nutrient broth tubes and incubated at 37°C overnight.

4. Preparation of Dilution:

a. 10 test tubes were arranged in a rack containing 9 ml normal saline each.

b. l ml of inoculated broth was added to test tube no. 1. It was mixed thoroughly and then l ml was transferred from tube no.1 to tube no. 2.

c. Again l ml was transferred from the 2 to the 3 tube and continued mixing and transferring till the last tube. Now the dilutions in tube no.1 to 10 were 10^{-1} , 1 0^{-2}, 1 0^{-3}….. 10^{-10} respectively.

5. A set of 10 Plate Count Agar plates in triplicate were arranged and numbered 1 to 10.

0.l ml of 10 $^{-1}$dilution was then transferred to each plate numbered 1; 0.lml of 10^{-2} dilution in plate numbered 2 and so on till 10^{-10} dilution had been transferred in plate numbered 10. Separate pipettes were used for each dilution.

6. The above procedure was performed separately for each water sample.

Experimental design of phytohormone study -Experiments were performed to study the effect of phytohormone cytokinin & auxin in combination with different molar concentrations of NaCl.The design of experiment was as followed- In yet another study growth of the root of *Glycine max* germinated in distilled water (Control) 50 mM NaCl, 100 mM NaCl and 500 mM NaCl in combination with cytokinin 1 and 2 ppm ,auxin 1ppm and 2 ppm was observed on day 5 and day 8. The hormones were sprayed on day 1 and day 5 and hence the frequency of spray was an important factor.

All sorts of interactions between the treatments, the levels of hormones, durations of germination and the frequency of hormones sprayed was analysed by a 4 way classification of analysis of variance.

∆ **Preparation of samples for enzymological study** -Seeds of *G. max* were germinated in distill water(Control),10mM NaCl & 20 mM NaCl upto 8 days.Data were recorded for length of root, shoot & cotyledon on day 1(no growth),day3,day5& day 8.Samples of all these duration were washed thoroughly with distill water.No attempt was made to fractionate the cotyledon, root & shoot.The whole sample was homogenized at the rate of 20 mg of the tissue in 1ml.of 0.154 ml KCl. Sample was centrifuged & supernatant was used for the analysis of enzyme activity.

Assay of Ca^{++} dependent ATPase-The processed germinated seed sample was used for the assay of Ca^{++} dependent ATPse .An amount of 50 mg of the processed seed was used for the assay .Ca^{++} ATPase activity was assayed according to the method of **Young & Smithwick (1975).**

For the assay of Mg ATPase & Mg dependent Na+-K+ ATPase-The assay of Na+-K+ transport ATPase was measured by the transport ATPase by the use of glycoside ouabain.The assay medium used for each of the enzyme is detailed below-

a) **Mg dependent ATPase**- The assay mixture comprising of 100 mM Tris-HCl buffer, , pH 7.4, 5mM ATP, 5mM tris, 5mM $MgCl_2$

b) **Na+- K+ATPase**-20mM NaCl & 20 mM KCl in a final volume of 1 ml.

c) **Mg++ dependent Na+-K+ ATPase**- The assay mixture comprising 100 mM tris HCl buffer, P^H-7.4, 5 mM tris,5 mM ATP, 5 mM $MgCl_2$,200mM NaCl,20 mM KCl in a final volume of 1 ml.

Δ Additional investigation that approved by SRC of Kota University

d) **Na+- K+ transport ATPase**- Mg+ dependent Na-K activated ATPase was inhibited by digoxin represented the N^+-K^+ transport ATPase.

Aspartate aminotransferase (AST E.C .2.6.1.1) -AST activity was estimated by using colorimetric method of **Reitman and Frankel (1957**) as modified by **King (1965**)

a) **0.1 M phosphate buffer ,pH 7.4** : It was prepared by dissolving 11.928g anhydrous Na_2HPO_4 and 2.175 g KH_2PO_4 in one litre distilled water .

b) **Substrate solution :**2.66 g D-L Aspartic acid and 30 mg of oxoglutaric acid were dissolved in 20.5 ml of 1N NaOH by gently heating .Volume was made to 100 ml with buffer solution .

c) **Aniline Citrate** :It was prepared freshly equal parts of redistilled aniline and a solution of 50g citric acid in 50ml distilled water were mixed .

d) **Color reagent** : It was prepared by dissolving 200 mg ,2.4-dinitrophenyl hydrazine in hot 1N HCl and made upto 1 litre with 1N HCl . It was stored in a brown bottle .

e) **Procedure :**1 ml of substrate solution was taken in a test tube and incubated in water bath maintained at 37^0C for 5 minutes . Then 0.2 ml of supernatant was added and incubated for another 60 minutes ,after one hour 0.05 ml aniline citrate was added and allowed to react for 5 minutes for complete decarboxylation . 1ml of colour reagent was added and incubated for 15 minutes .10ml of 0.4 NaOH was added to each tube and mixed well . Optical density was recorded at 520 nm in Beckman DU-64UV spectrophotometer against blank ,which was prepared by the same procedure except that supernatant was added immediately after addition of aniline citrate .Values were calculated from the standard curve by using sodium pyruvate as

standard .Unit of enzyme activity was expressed in milli micro mole of pyruvate liberated/ mg protein/minute .

Alanine aminotransferase (ALT E.C.2.6.1.2)

ALT activity was estimated by spectrophotometer method of **Reitman and Frankel** (1957) as **modified by King (1965**)

a)0.1 M Phosphate buffer (pH7.4) : It was prepared by dissolving 11.928g anhydrous disodium hydrogen phosphate and 21.175g anhydrous potassium dihydrogen phosphate in one litre of distilled water .

b) Substrate solution : 1.78g D-L-Alanine and 30 mg d-oxoglutaric acid were dissolved in buffer 0.5ml 1N NaOH was added and volume was made to 100 ml with buffer solution .

c) Color reagent :It was prepared by dissolving 200mg 2,4-dinitrophenyl hydrazine in hot 1N HCl and made up to 1 litre with 1N HCl . It was then stored in a brown bottle .

Procedure :

1.0 ml of substrate solution was taken in a test tube and incubated in a water bath maintained at 37 ^{0} C for 5 minutes .Then 0.2 ml of supernatant was added and incubated for another 60 minutes . 1.0 ml of color reagent was added and mixed well .Reation mixture was incubated for 15 minutes .Finally 10 ml of 0.4 N NaOH was added to stop the reaction .Optical density was recorded at 520nm in Beckman DU-64UV Spectrophotometer against blank ,which was prepared by the same procedure except that supernatant was added immediately after addition of color reagent .Values were calculated from the standard curve by using sodium pyruvate as standard .Unit of enzyme activity was expressed in milli micro mole of pyruvate liberated/ mg protein/minute .

Total protein estimation :

Total protein was estimated in the homogenate following the procedure of **Lowry et.al (1951**) .

a) Solution A :2g of reagent grade anhydrous sodium carbonate was dissolved in 0.1 N sodium hydroxide .

b) Solution B : 0.5 % copper sulfate solution was made in 1% sodium potassium tartarate .

c) Alkaline copper reagent : it was prepared by mixing 50 volumes of solution A and 1 volume of solution B just –before use .

d) Folin –Phenol reagent : A prepared solution of sisco research Laboratories Ltd . Bombay was used .

Procedure :

The homogenate was mixed with 5 ml of alkaline copper reagent . It was allowed to stand for 10 minutes . Then 0.5 ml of folin –Phenol reagent 1:1 dilution was added and mixed .Simultaneously 0.5 ml of water as blank and 0.5 ml of standard solution containing various concentration of bovine serum albumin (BSA, 25-200 µg) were similarly treated and color developed was read at 680 nm in Beckman DU-64 UV /Visible spectrophotometer .

Protein concentration was calculated as mg/g of seed homogenate .

Electrophoretic Study :

Polyacrylamide gel electrophoresis was conducted following methods of **Davis (1964**), **Laemli (1970) and Okajima *et al*, (1993**) to separate the constituent proteins according to their surface charge at PH 8.9

Reagents :All the reagents were prepared using de-ionised water and were filtered and stored in amber colour bottles in refrigerator for use .

Reagents	Chemicals	De-ionised water & volume	pH

Solution –A	Acrylamide -14.6g Bisacrylamide -0.4 g	50 ml	8.8
Solution- B	Tris -9.1 g Sodium DoedecylSulphate -0.2 g HCl concentrate -1.0 ml	50 ml	6.8
Solution –C	Tris -3.05 g SDS -0.2 g HCl concentrate -2.1 ml	1 ml	Fresh
Running buffer	Tris -0.9 g Glycine -4.32 g SDS -0.3 gm	300ml	
Sample buffer	SDS -0.25 g Mercaptoethanol -0.5 ml Solution C-2.5 ml	2 ml	

	Glycerol -5 ml Bromophenol Blue - 0.01 g		
Staining solution	Comassie Brilliant Blue -0.25 g Methanol -40 ml Acetic acid 10 ml		
Destaining solution	Methanol -30 ml Acetic Acid -10 ml	60ml	
Storing solution	Acetic acid	100ml	

Table A :- Recipe for the separating gel preparation for SDS-PAGE (for 2 gels)

	5%	**7.5 %**	**10%**	**12.5 %**	**15%**	**20%**
Solution –A	3 ml	4.5 ml	6 ml	7.5 ml	9 ml	12 ml
Solution –A	4.5 ml	4.5 ml	4.5 ml	4.5 ml	4.5 ml	4.5 ml
Solution –A	0.08 ml	0.08 ml	0.08 ml	0.08 ml	0.06 ml	0.06 ml
TEMED	0.01 ml	0.01 ml	0.01 ml	0.01 ml	0.01 ml	0.01 ml
Deionised	10.5 ml	9 ml	7.5 ml	6 ml	45 ml	1.5 ml

Table –B : Recipe for the 4.5 % stacking gel (for 2 gels)

Solution –A	0.9 ml
Solution –C	1.5 ml
Solution –D	0.02 ml
TEMED	0.01 ml
Deionised water	3.6 ml

Casting of gel :

A 15% uniform separating gel was prepared and poured carefully into the gel casting space between the glass plates until about 75% of the space was filled . Water saturated butanol was layered over the gel and the gel was allowed to polymerize at room temperature .After polymerization of the separating gel , the water saturated butanol was drained off by tilting the gel cast assembly and was washed with de-ionised water 3 to 4 times to rinse the butanol if any 4.5 % stacking gel solution was then layered over the separating gel after washing the upper surface by the same gel solution . Slot forming comb was carefully inserted into the upper surface by the same gel solution . Slot forming comb was carefully inserted into the top of the gel casting area until both ends of comb were stopped at top of the side spacer .Water saturated n-butanol was then layered over it . After polymerization of the stacking gel the comb was removed slowly and carefully .

Electrophoretic run :

Samples along with protein marker applied into each slot . The gel cast assemble was connected to power pack ,and electrophoresis was conducted at room temperature at constant current of 20mA and 80 volt initially till tracking dye were stacked and crossed stacking gel . The voltage was increased to 120 volt after the samples reached separating gel . Electrophoresis was continued till the tracking dye reached end of the gel .

Fixing,staining and de-staining of gel :

The gels after electrophoresis were fixed overnight with fixing solution (10% acetic acid and 50% methanol in distilled water) and staining solution i.e 0.125% coomassie brilliant blue R-250 in fixing solution for four hours . The gels were then rinsed with de-ionised water 3-4 times to remove traces of staining solutions . Then gels were destained with several changes of de-staining solutions i.e. 10% acetic acid 30% methanol in de-ionised water .After through de-staining the gels were stored in 7% acetic acid till photographed .

Preparation of protein marker :0.01 ml of protein marker was taken in a 0.25 ml micro centrifuge tube and heated to 95^{o} C temperature for 5 minutes .It was cooled down and used in gel .

Contents of protein marker :Besides the marker protein supplied by the manufacturer ,following proteins were also electrophoresed .

Protein	**Molecular weight in kilo Dalton (KD)**
B-galactosidase	116.0
Bovine serum albumin	66.2
Ovalbumin	45.0
Lactate dehydrogenase	35.0

REase Bsp981	25.0
B-lactoglobulin	18.4
Lysozyme	14.4

The range of marker function molecular weight was from 10 KDs to 170 KDs.

Disppearance of protein bands of MW ~170KDa and ~105KDa is evident in 8 days treatment condition in addition to significant reduction in band intensity of ~94KDa proteins. Molecular weights of protein bands have been marked in the image.

Protein Extraction Protocol:

2.0g of soybean seeds along with the roots were crushed and defatted three times with petroleum ether, b.p. 35-60^{o}C for 30 min each. Proteins were then extracted with 10mLof a solution containing 50mML^{-1} Tris-HCl pH 8.8, 1.5mML^{-1} KCl, 10mML^{-1} dithiothreitol (DTT) 1.0 mML^{-1} phenylmethanesulfonyl fluoride (PMSF) and 0.1% (m/v) sodium dodecyl sulphate (SDS). The samples were mixed for 10 min in an ice bath and insoluble materials were removed by centrifugation at 4^{o}C for 5 min at 5000g.

Determination of total protein concentration:

Protein concentration in all samples were determined according to **Bradford method,**

employing bovine serum albumin as a standard. For this purpose, the samples were appropriately diluted using 1.5molL^{-1} Tris–HCl pH 8.8. The measurements were done at 595nm.

Protein estimation by Bradford method

Bradford reagent was prepared by dissolving 100mg coomassie brilliant Blue G-250 in 50 ml 95% ethanol and adding 100 ml 85% (w/v) phosphoric acid .Solution was made with distilled

water .The solution was filtered 100 ml of glycerol was added and the volume was made up to 1000 ml .

Procedure :

- Various concentration of standard BSA solutions was prepared from the stock solutions (0.2 ,0.4,0.6 ,0.8 and 1.0 ml) into series of test tubes and volume was made up to 1.0 ml of the samples in 1:100 dilution 5.0 ml of coomassie brilliant blue was added and mixed by vortex disill water serve as control after 10-30 minutes absorbance was recorded at 595 nm.
- Absorbance of the standards versus their concentrations . Amount of protein in the sample was calculated from the plot .

Bradford reagent preparation

Dissolve 100 mg Coomassie Brilliant Blue G-250 in 50 ml 95% ethanol, add 100 ml 85% (w/v) phosphoric acid. Make upto 600ml with distilled water. Filter the solution and add 100 ml of glycerol and make up the volume to 1000ml.

Procedure

- Prepare various concentrations of standard BSA solutions from the stock solution (0.2, 0.4, 0.6, 0.8 and 1.0 ml) into series of test tubes and make up the volume to 1.0ml pipette.
- A tube with 1.0 ml water serves as blank. Take 1.0 ml of each of the samples in 1:100 dilution.
- Add 5.0 ml of Coomassie brilliant blue to each tube and mix by vortex.
- Wait for 10-30 minutes and take absorbance at 595nm.
- Plot the absorbance of the standards versus their concentration and calculate amount of protein in the sample.

Molecular weight and Intensity Analysis of SDS-PAGE gels -

Molecular weight Analysis: SDS-PAGE gels were analyzed using *ImageJ* software for calculation of molecular weights of various protein bands. The migration distances was plotted

against the known MW marker standards to generate a standard curve which was used to determine the molecular weights of the protein bands from different lanes of gel, as indicated by arrowheads .

Intensity Analysis: To calculate percentage change in the intensity of protein bands, *ScionJ Image* software was used.

Histological staining-*G. max* seeds were germinated in Distilled water, 500mM Sodium chloride and 1M Sodium chloride, respectively. The sprouted seeds were fixed in 10% neutral buffered formalin for 48 hrs. The formalin fixed seeds were cut in pieces of 2-3 mM thickness in such a position that comprise the cotyledons and portion of germination. The thin seed pieces were washed for 12 hours in running tap water and dehydrated in ascending grade of alcohol (1 hour each in 30%, 50%, 70%, 90%, 95% and absolute alcohol). Clearing of seed was performed in benzene and ultimately the seeds were embedded in paraffin (melting point 58-62 ^{o} C). Seed sections of 4-5 micron thickness were cut by rotary microtome and stained with Haematoxylin and Eosin. The sister seed sections were also stained in 2.5% Toludine blue wherever deemed necessary. **(Luna, 1968).**

Sections were of 10 millimicron ,Photograph where taken at 10 X & 40 X**.**

[∇]**Pot culture study-**Pot culture experiment were carried out during the 2nd week of sept 2011. A pot culture experiment was laid out in CRD, replicated thrice with total twelve treatments at Krishi Vigyan Kendra, Kota during Kharif 2011-12 and 2012-13.

a. Control (irrigated weekly with water)

b. 50 mM NaCl irrigated weekly after two weeks of seed sowing

c. 100 mM NaCl irrigated weekly after two weeks of seed sowing.

Varietal characteristics of crop cultivars used in the experiment

Crop	Variety	Characteristics

[∇] Paper published & presented in National conference (enclosed)

Crop	Variety	Characteristics
Soybean	JS-95-60	High yielding and early maturity; resistant to bacterial pustule, bacterial blight and susceptible to yellow mosaic virus

Date of sowing -12 sept 2011

Date of harvesting – 10Nov 2011

Thoroughly cleaned earthen pots were used for experiments; levelled according to their respective treatment and replicate three times. Twelve healthy and uniform seeds of soybean were sown. There were 12 treatment and 3 replicate, T_1 was kept control, T_2 was 50 mM . NaCl, T3 was 100mM NaCl. Pot culture soil were medium cultured (clay loam) ,basic in reaction with medium water holding capacity (WHC) .The biweekly measurements of total stem length, root length were made. Two soil salinity level (7.8 dsm^{-1} Vs 14.0 dsm^{-1}) are tested resulting in the following treatments –

I) Soil irrigated with distil water during the whole crop cycle as a control (EC_1) ; (T1)

II) Soil irrigated with saline water of electrical conductivity (EC_2) of 7.8 dsm^{-1} (T_2)

III) Soil irrigated with saline water of electrical conductivity (EC_3) of 14.0 dsm^{-1}) i.e.T_3

The experiment comprised of two levels of P_2O_5 i.e. 20 and 40 kg^{-ha} and K_2O i.e. 15 and 30 Kg^{-ha} .Saline water was obtained by addition of NaCl to distil water with the purpose of reaching the desired level of EC. Five plants were raised in each pot. Plant and soil sample were collected from each pot for determination of growth parameters and chemical analysis. Seeds of each pot were separately collected and analyzed for their nutrient content, ash content by drying in furnace. The germination of soy seed grown in pot were observed after 7 days of sowing , as plant growth parameter were recorded at 30, 45 and 60 days after sowing of seeds.

Flame photometric estimation of sodium & potassium-

A simple method for determining sodium and potassium involves the technique of emission flame photometry. This relies on the principle that an alkali metal salt drawn into a non-luminous flame will ionise, absorb energy from the flame and then emit light of a characteristic wavelength as the excited atoms decay to the unexcited ground state. The intensity of emission is proportional to the concentration of the element in the solution. A photocell detects the emitted light and converts it to a voltage, which can be recorded. Since Na^+ and K^+ emit light of different wavelengths (colours), by using appropriate coloured filters the emission due to Na^+ and K^+ (and hence their concentrations) can be specifically measured in the same sample. Freeze-dried hypocotyls, roots and leaves were used for analysis of Na and K contents. The samples were powdered by grinding with pestle and mortar. A portion of the powered samples were digested with concentrated HNO_3 at 110°C for 2 h. Na^+ and K^+ content were estimated by flame photometry (**Horneck and Hanson, 1998**) and K^+/Na^+ ratio was calculated.

Ultraviolet spectral studies

By measuring the absorption spectrum of a substance in UV range, it is possible to identify it in a particular class of compounds. The wavelength at which peak absorption occurs, the **absorption maximum** (λ max), is very useful when trying to identify an unknown. By creating and measuring a series of standards (*e.g.* serial dilutions), it is possible to quantify the amount or concentration of a substance in a sample. In their pure form, the nucleic acids can be quantified by absorption measurements in the UV range at 260 mμ and those of proteins at 280mμ.

Transmittance (T) is the fraction of incident light which is transmitted a sample. Absorbance (A), or optical density (a logarithmic function) of T is expressed as:

$A = \log_{10} (1/T) = \log_{10} (Io/I)$ at 100% transmittance, A = log 1.0 = 0. At 50% transmittance, A = log (1/0.5) = 0.30.

UV spectroscopy precludes the colouring of compounds as it happens in visible range.UV absorbtion is based on double bond present in a compound.

Statistical analysis – For comparison between two variable a pooled standard error method was employed.the validity of pooled SE method was justified as long, there were only two variables,however in most of the investigations here, it was a completely randomized design,therefore it was subjected to analysis of variance (**Snedecor & Cochran, 1967**).The ANOVA was performed using 2 way, 3 way,4 way analysis as deemed necessary.F factor was calculated & significant differences were analysed by Duncan multiple range test**.**The means were also compared by LSD using SPSS (Version 10).

Results- Salinity stress inhibits seed germination and seedling growth, decreases biomass accumulation and yield. There were significant differences in the rate of germination on day 5 and day 8 , root length, shoot length and cotyledon length between different saline concentrations , industrial effluent and sewage . Mean percent germination rate were 64 and 72 on day 5 and day 8 in sewage water.

There were significant differences in the rate of germination on day 5 and day 8 , root length, shoot length and cotyledon length between different saline concentrations , industrial effluent and sewage .Length of root and shoot was shorter in NaCl treatment.

Mean germination rate on day 8 in 1M,2M,500mM,750mM as against control were 32% ,24%,76%,64% respectively .The length of shoot and root of plant treated with 2M NaCl was 58% and 30 % shorter respectively than control plants.Mean percent germination rate were 64 and 72 on day 5 and day 8 .

Effect of salt stress on growth components

Treatment	Quantity (M)	Shoot length (cm)	Root length (cm)	Cotyledon Length(mm)	Germination %

Control	0	11.34	12.48	14	84
NaCl	2M	4.45	4.84	8	24
NaCl	1M	7.61	7.92	9	32
NaCl	750mM	7.93	8.14	10	64
NaCl	500mM	8.12	9.32	12	76
Industrial effluent	---	10.4	12.8	14	82
Sewage water	---	8.01	9.64	13	72

This study shows that salt stress decrease soybean growth and induces changes in root length, shoot length and also change in cotyledon length.Most germination percentage was obtained in control treatment in all cultivars. Increase higher salt stress level, decreased soybean germination percentage. Root and shoot length significantly decreased with the increase of salinity level.After 8 days first and primary leaves had opened in control plants, whereas they remain closed in treatment with 100 mM NaCl and 200 mM NaCl . Seeds of *Glycine max* were germinated in distilled water, 10 mM NaCl and 20 mM NaCl ,root and shoot growth were recorded on day 5 and day 8.

The mean value of the growth of shoots (cm) in different treatments on day 5 and day 8 is presented in table **(I – 1A)**

The analysis of variance for two factors and a completely randomized design is presented in table **(I- 1B).**

Highly significant differences ($p < 0.01$) were observed between the treatments and between the days of germination. A significant difference was observed for the interactions between the

treatment and the days of germination. The coefficient of variation was 26.63%. **Table I-1A** .The mean values of the growth of roots (cm) in distilled water, 10 mM NaCl and 20 mM NaCl on day 5 and day 8 is presented in table **(I – 2A)** . Analysis of variance of roots is presented in table **(I – 2B).**

Highly significant differences ($p < 0.01$) were observed between treatments, between the days of germination and the interactions between treatments and the days of germination .Duncan multiple range test, both in original and ranked order, are presented in table **(I – 2C).** In the ranked order, the mean values differ from each other, the root growth registered the highest growth on day 8 followed by growth on day 5 control. There were no difference in the root growth in 10 mM saline on day 8 and 20 mM saline on day 8.

Table I-I A

Mean shoot length of *G.max* (cm) germinated in distilled water, 10 mM NaCl and 20 mM NaCl

Distilled Water on day 5	3.500
Distilled Water on day 8	6.267
10 mM NaCl on day 5	1.668
10 mM NaCl on day 8	2.267
20 mM day NaCl on day 5	0.900
20 mM NaCl on day 8	1.200

Table I-1B

Analysis of Variance (2 way) for shoot

Source	**Degrees of Freedom**	**Sum of Squared**	**Mean Square**	**F Value**
Treatment	2	48.083	24.042	48.8983 * *
Days	1	6.722	6.722	13.6723 * *
Treatment × Days	2	5.434	2.717	5.5266 *
Error	12	5.900	0.492	
Total	17		66.140	

** P < 0.01 *P < 0.05

Table I-1C

Duncan's Multiple Range Test (For Shoot Length)

LSD Value = 1.248

Original Order				Ranked Order			
Mean	1=	3.500	B	Mean	2=	6.270	A
Mean	2=	6.270	A	Mean	1=	3.500	B
Mean	3=	1.670	CD	Mean	4=	2.270	BC
Mean	4=	2.270	BC	Mean	3=	1.670	CD
Mean	5=	0.9000	D	Mean	6=	1.200	CD
Mean	6=	1.200	CD	Mean	5=	0.900	D

(PLATE 1)

THE EFFECT OF DIFFERENT MOLAR CONC.OF SALINE WATER ON THE GERMINATION OF GLYCIN MAX

(1-A)

100 mM NaCl , 5 Day Growth

(1-B)

50 mM NaCl , 8 Day Growth

(1-C)

1M NaCl , 8 Day Growth

(1-D)

CONTROL 8 DAYS PAPER TOWEL METHOD

Table I-2A

Mean Root Length (cm) of *G.max* germinated in Distilled water, 10 mM NaCl and 20 mM NaCl on day 5 and day 8

Control day 5	3.667
Control day 8	8.200
10 mM day 5	1.800
10 mM NaCl day 8	2.467
20 mM NaCl day 5	1.267
20 mM NaCl day 8	1.267

Table I-2B

Analysis of Variance for Root Length of *G.max* germinated in distilled water 10 mM NaCl and 20 mM NaCl

Source	Degrees of Freedom	Sum of Squared	Mean Square	F Value
Treatment	2	73.938	36.969	131.7703 **
Days	1	13.520	13.520	48.1901 **
Interaction (Treatment × Days)	2	17.973	8.987	32.0317 **
Error	12	3.367	0.281	
Total	17	108.798		

** $P < 0.01$ *$P < 0.05$

Table I-2C

Duncan's Multiple Range Test (For Root Length)

LSD Value = 0.9430

S= 0.3061 at alpha = 0.050

Original Order				Ranked Order			
Mean	1=	3.670	B	Mean	2=	8.200	A
Mean	2=	8.200	A	Mean	1=	3.670	B
Mean	3=	1.800	CD	Mean	4=	2.470	C
Mean	4=	2.470	C	Mean	3=	1.800	CD
Mean	5=	1.270	D	Mean	5=	1.270	D
Mean	6=	1.270	D	Mean	6=	1.270	D

Table I-3A

ANOVA for the root length of *G.max* germinated in distilled water (control), 10 mM NaCl and 20 mM NaCl on day 5, day 8

Source	**Df**	**SS**	**MS**	**F**
TOT	17	108.797778	6.399869	22.6500
Rep	3	0.541111	0.270556	0.9575
Trt	5	105.431111	23.086222	74.6268
Err	10	2.825556	0.282556	1.0000

Table I-3B

ANOVA for the length of roots of specific effects of treatment , duration of germination , and interaction between treatment × durations.

Source	Df	SS	MS	F
s	2	73.937778	36.968889	130.8376
d	1	13.520000	13.520000	47.8490
sd	2	17.973333	8.986667	31.8050
Err	10	2.825556	0.282556	

s-Treatment d-Days sd- Interaction between treatment × days

Table I-3C

Mean tables of root length (1cm)

	d1	d2
s1	3.6667	8.2000
s2	1.8000	2.4667
s3	1.2667	1.2667

s1-Distilled water (control) s2- 500 mM NaCl s3-1 M NaCl

Table I-3D

Standard error of treatments, days and interaction between treatment × days for root length.

	SED	CD (0.05)	CD (0.01)
S	0.30690	0.68381	0.97269
D	0.25058	0.55833	0.79420
SD	0.43402	0.96705	1.37559

SED -Standard error spatial difference

Table I-4A

Mean tables for shoot length (cm)

	d1	d2
S1	3.5000	6.2667
S2	1.6667	2.2667
S3	0.9000	1.2000

s1-Distilled water (control) s2- 500 mM NaCl s3-1 M NaCl

Table I-4B : ANOVA for shoot length of *G.max* grown in distilled water, 10 mM NaCl and 20 mM NaCl

Source	**Df**	**SS**	**MS**	**F**
TOT	17	66.140000	3.890588	19.0094
Rep	2	3.853333	1.926667	9.4137
Trt	5	60.240000	12.048000	58.8664**
Err	10	2.04667	0.204667	1.0000

** $P < 0.01$

Table I-4C : ANOVA for shoot length ,specific effects of treatment ,days and interaction between treatment x days of *G.max* grown in distilled water, 10 mM NaCl and 20 mM NaCl

Source	**Df**	**SS**	**MS**	**F**
s	2	48.083333	24.041667	117.4674**
d	1	6.722222	6.722222	32.8447**
sd	2	5.434444	2.717222	13.2763**
Err	10	2.046667	0.204667	1.0000

** P < 0.01 *P < 0.05

s – Treatment d - Days sd - Interaction

Table I-4D : Standard error of treatments, days and interaction between treatment × days of *G.max* grown in distilled water, 10 mM NaCl and 20 mM NaCl.

	SED	**CD(0.05)**	**CD(0.01)**
s	0.26119	0.58198	0.82784
d	0.21326	0.47518	0.67593
sd	0.36938	0.82304	1.17074

Duncan multiple range test is presented in table **(I – IC)** both in the original order and the ranked order. In the ranked order highest growth was recorded in distilled water on day 8 followed by the growth of the shoot in distilled water on day 5. However the growth in 10 mM saline on day 8 and 20 mM saline on day 5 were statistical non significant.Mean growth in 20 mM saline were statistically non significant and at par . The growth reduce in 20 mM saline on day 5 and was significantly different from others.

Plate 1

1A shows the growth of the roots and shoots on day 5 in 100mM water.

1 B exhibits the growth of shoots and roots on day 8 in 50 mM Water

1 C exhibits the growth of shoots and roots on day 8 in 1M NaCl.

1D depicts the growth of shoots and roots on day 8 in Distilled water(Control).

In an experiment, seeds of *G.max* were germinated in distilled water 500 mM NaCl and 1 M NaCl at an elevated temperature of 30^0C. Measurements of roots and cotyledons were recorded. Six replicates were used for calculations of measurements on day 5 and day 8.

ANOVA for growth of roots (cm) on day 5 and day 8 in different treatments are presented in table **(I – 3A**). There was a significant increase in the size of roots on day 8 as compared to day 5. Mean root length in different treatments on day 5 and day 8 are presented in table (**I -3C.**)

Analysis of variance for the growth of roots in different treatments and different days are presented in table **I-3B**. Highly significant difference ($p < 0.01$) were observed between the treatments and between the intervals. However there was no significant interaction between treatments and between intervals.

Table **I-3D** presents standard error of treatments, days and interaction between treatment × days.

Mean comparison by least square difference showed that performance was best in control as compared to 500mM NaCl and 1 M NaCl while the performance was at par in both the saline concentrations. The coefficient of variation is 6.27 %. Best growth was on day 8.

Mean shoot length in distilled water, 500 mM NaCl and 1.0 M on day 5 and day 8 is presented in table **I-4A**, while there were no significance between treatments.

ANOVA for shoot length of *G.max* grown in distilled water, 10 mM NaCl and 20 mM NaCl are presented **in table I-4B** ($p < 0.01$). Highly significant difference ($p < 0.01$) was observed between treatments .

ANOVA for shoot length of specific effects of treatment ,days and interaction between treatment x days of *G.max* grown in distilled water, 10 mM NaCl and 20 mM NaCl presented in **table I-4C.**

An interactions between treatment and days was not significant .Highly significant difference ($p < 0.01$) was observed between days, mean comparison by LSD showed that growth was best on day 8. The coefficient of variation is 15.46%.

Standard error of the mean and critical difference is presented in table **I-4D**.

Plate 2

(2 -A) depicts the growth of seeds in 500mM NaCl 5 day growth.

(2 -B) shows the growth of seeds in 2 M NaCl 5 day growth

(2 -C) shows the growth of seeds in 1M NaCl 5 day growth

(2 -D) depicts growth of seeds in control 8 day growth

Seeds of *Glycine max* were germinated in distill water (control) , 500 mM of NaCl and 1 M NaCl for a duration of up to 8 days . Seeds were germinated at a higher temperature of 30 ^{0}C .

Table I-5A presents the length of root in different treatment and duration of germination .

Table I-5B presents the ANOVA for the effect of different treatment and replicates . Highly significant difference was observed for the effect of treatments .

Table I-5C presents the ANOVA for the effect of treatment ,days and interaction between treatment x days . Highly significant difference ($p<0.01$) was observed for the effect of between treatment and between days .However the interaction between treatment x days was not significant .

Table I-5D presents the standard error (for roots) of the treatments , duration and interaction between treatment x days and the critical difference at $p<0.05$ and 0.01 level .

Table I-5A : Length of roots in distilled water, 500 mM NaCl and 1 M NaCl on day 5 and day 8

	d1	d2
S1	10.6000	14.3833
S2	9.3667	13.3500
S3	9.5000	13.6500

S1 – distilled water D1 = day 5

S2- 500 mM NaCl D2 = day 8

S3 – 1 M NaCl

Table I-5B : ANOVA for roots germinated in distilled water (control) 500mM NaCl and 1 M NaCl, for replicated and treatments :

Source	**Df**	**SS**	**MS**	**F**
TOT	35	175.787500	5.022500	9.8745
Rep	5	12.175833	2.435167	4.7877
Trt	5	150.895833	30.179167	59.3338 **
Err	25	12.715833	0.508633	1.0000

** P < 0.01 *P < 0.05

Table I-5C : ANOVA for roots germinated in distilled water, 500mM NaCl and 1 M NaCl for specific effects of treatments on days and interaction between treatment and days

Source	**Df**	**SS**	**MS**	**F**
S	2	8.686667	4.343333	8.5392
D	1	142.006944	142.006944	279.1932
Sd	2	0.202222	0.101111	0.1988
Err	25	12.715833	0.508633	

s– Treatment d- days sd – interaction between treatment and days

Table I-5D : Standard error of treatments, days and interaction between treatment × days for roots germinated in distilled water, 500mM NaCl and 1 M NaCl

	SED	**CD(0.05)**	**CD(0.01)**
S	0.29116	0.59965	0.81163
D	0.23773	0.48961	0.66269
Sd	0.41176	0.84803	1.14781

Table I-6A : Mean length of cotyledon (mm) germinated in distilled water 500mM NaCl and 1 M NaCl :

	d1	d2
S1	11.5000	14.0000
S2	9.6667	13.5000
S3	9.5000	13.5000

S1 - distilled water D1 - day 5

S2- 500 mM NaCl D2 - day 8

S3- 1 M NaCl

Table I-6B : ANOVA for cotyledons germinated in distilled water 500mM NaCl and 1 M NaCl. Statistical significance for replicates and treatments:

Source	Df	SS	MS	F
TOT	35	249.888889	7.139683	1.5688
Rep	5	13.555556	2.711111	0.5957
Trt	5	122.555556	24.511111	5.3857
Err	25	113.777778	4.551111	1.0000

Table I-6C : ANOVA for the growth of cotyledons in control, 500mM NaCl and 1M NaCl on day 5 and day 8. Statistical significance for treatment ,days and interaction between treatment x days

Source	Df	SS	MS	F
s	2	11.722222	5.861111	1.2878 NS
d	1	106.777778	106.777778	23.4619 **
sd	2	4.055556	2.027778	0.4456 NS
Err	25	113.777778	4.551111	1.0000

** P < 0.01

S – Treatment d- days sd – interaction between treatment and days

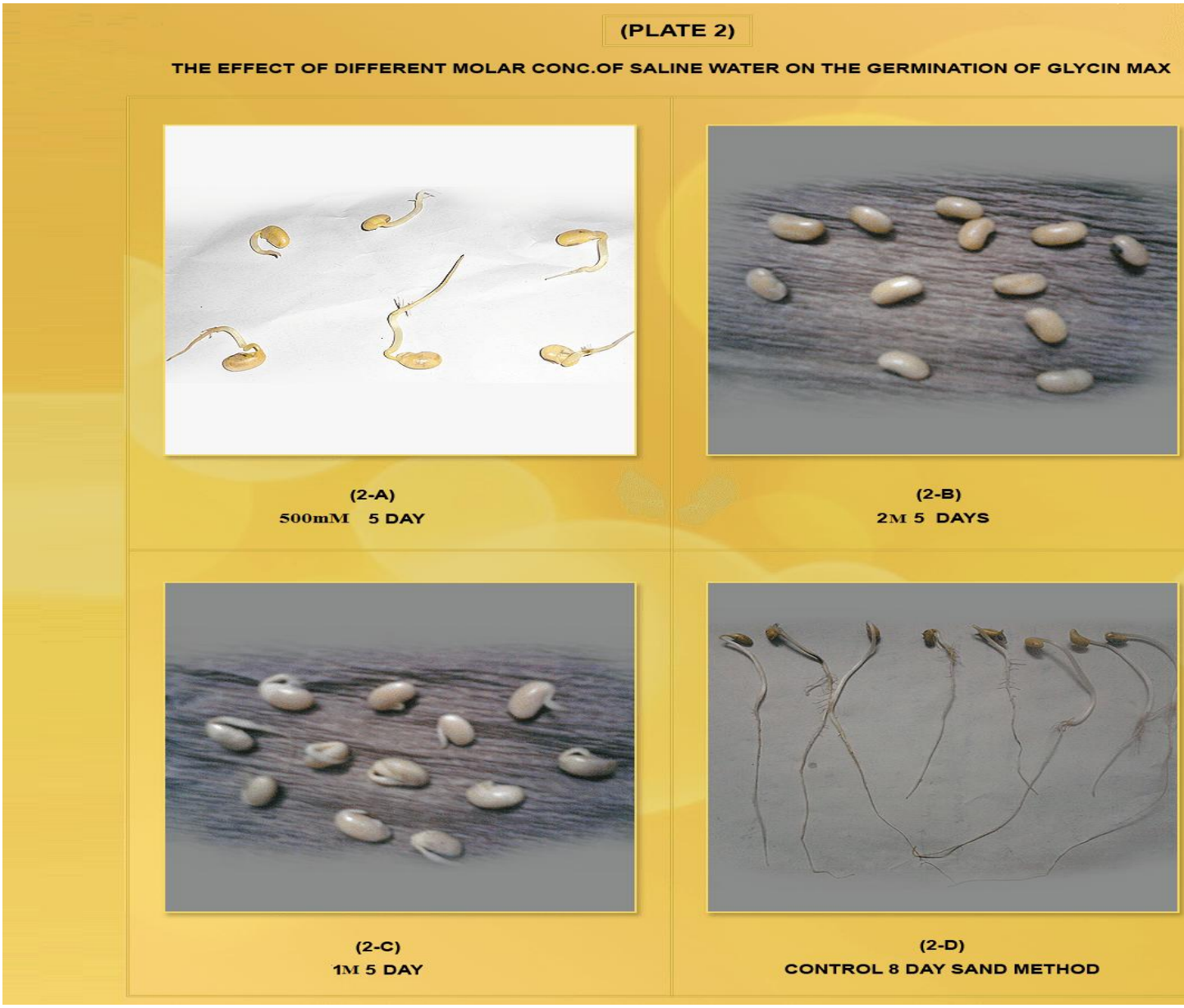


Table I-6D : Standard error of treatments, days and interaction between treatment × days of cotyledons in control, 500mM NaCl and 1M NaCl on day 5 and day 8

	SED	CD(0.05)	CD(0.01)
S	0.87093	1.79371	2.42780
D	0.71111	1.46456	1.98229
sd	1.23168	2.53670	3.43343

Table I-6A presents the mean length of cotyledon in different treatment on day 5 and day 8 .

Table I -6B presents the ANOVA of cotyledon with respect to the effect of treatment and replicate . Treatment effect was highly significant ($p < 0.05$). Replicate was however not significant .

Table I-6C presents the ANOVA for the effect of treatment , days and interaction between treatment x days . The effect of duration of germination was highly significant ($P<0.01$).

Table 1-6D presents the standard error (for cotyledon), critical difference level,treatment & days,treatment xdays.

Table **II -1 A** presents the mean and standard error of the root, shoot and cotyledon length of *G. max* grown in sewage, activated charcoal treated sewage and the industrial effluent. Data were recorded on day 5 and day 8. The mean value of different treatment with respect to root length on day 5 and day 8 are presented in **table II-1B.**

Analysis of variance for the effect of replicate and treatments. Analysis of variance for growth of root of *Glycine max* in sewage, activated charcoal treated sewage and industrial effluent are presented in **table II –1C.**

There was highly significant difference between the treatments. Replicate effect was not significant. The specific effects of the treatments, duration of germination and interaction between the treatment and the days of germination was analyzed by a 2 way classification of analysis of variance.

Table II –1D presents the analysis of variance for growth of root of *Glycine max* in sewage, activated charcoal treated sewage and industrial effluent, The treatment effects and duration of germination were highly significant ($P < 0.01$). The interaction between the treatment and days of germination was not significant.

Mean comparison by least square difference revealed that best root growth was sustained in activated charcoal treated sewage followed by sewage and industrial effluent which were at par. Best growth was sustained on day 8. The standard errors of the mean of treatment, day of germination and the critical difference probability is presented in table **II 1 -E.**The coefficient of variation was 9.67%.

Table II-1A : Effect of different saline (10 mM or 20 mM) concentration on soybean root,shoot and cotyledon length

	Day 5	Day 8
Growth of *Glycin max*(root length) in sewage, activated charcoal treated sewage and industrial effluent (cm)	4.97 ± 0.27	6.97 ± 1.41
Shoot length in sewage (cm)	5.03 ± 0.2	7.6 ± 0.46
Cotyledon length in sewage(mm)	1.5 ± 0	1.8 ± 0
supernatant charcoal centrifuge root length (cm)	6.8 ± 0.23	8.13 ± 0.37
Shoot length (sewage + activated charcoal after centrifugation supernatant)cm	6.77 ± 0.19	8.2 ± 0.31
Cotyledon length(sewage + activated charcoal centrifugation ,supernatant) (mm)	1.6 ± 0	1.7 ± 0
Root length in industrial effluent (cm)	5.37 ± 0.38	7.27 ± 0.29
Shoot length in industrial effluent (cm)	6.17 ±	7.53 ±

	0.35	0.35
Cotyledon length in Indus. Effluent(mm)	1.4 ± 0	1.7 ± 0

Table II-1B

Mean table of root growth of Glycin max (cm)

	d1	d2
s1	4.9667	6.9667
s2	6.8000	8.1333
s3	5.3667	7.2667

S1 – Sewage

S2 – Sewage treated with activated charcoal

S3 – Industrial effluent

D1 – day 5

D2 – day 8

Table II-1C : Analysis of Variance for growth of root of *Glycin max* in sewage, activated charcoal treated sewage and industrial effluent

Source	Df	SS	MS	F
TOT	17	25.465000	1.497941	4.3672
Rep	2	0.563333	0.281667	0.8212
Trt	5	21.471667	4.294333	12.5199**
Err	10	3.430000	0.343000	1.0000

** P < 0.01 *P < 0.05

Table II-1D

Analysis of variance for growth of root for specific effect of treatment , days and interaction between treatment x days of *Glycin max* in sewage, activated charcoal treated sewage and industrial effluent are presented.

Source	Df	SS	MS	F
s	2	7.390000	3.695000	10.7726 **
d	1	13.693889	13.693889	39.9239 **
sd	2	0.387778	0.193889	0.5653 NS
Err	10	3.430000	0.343000	1.0000

** P < 0.01

Table II-1E

Standard error and critical difference of treatments, days and interaction between treatment × days for growth of root.

	SED	CD(0.05)	CD(0.01)
S	0.33813	0.75341	1.07169
D	0.27608	0.61516	0.87503
Sd	0.47819	1.06548	1.51559

The mean values for the length of shoot of *G. max* grown in sewage, activated charcoal treated sewage and industrial effluent on day 5 and day 8 is presented in **table II – 2A.**

Analysis of variance for the effect of replicates and the treatments is presented in **table II – 2B.** There was a highly significant difference ($P < 0.01$) between treatments for the growth of the shoot. Replicate effect was not significant. The analysis of variance for the specific effects of treatments, duration of germination and the interactions between the treatments and the days is presented in **table II- 2C.**

Table II-2D presents the standard error of treatment, duration of germination (days) & interaction between treatment & days.

Table II-2A

Mean length of shoot (cm)

	d1	d2
S1	5.0333	7.6000
S2	6.7667	8.2000
S3	6.1667	7.5333

Table II-2B

Analysis of variance for shoot growth in sewage, activated charcoal treated sewage and industrial effluent

Source	**Df**	**SS**	**MS**	**F**
TOT	17	23.625000	1.389706	4.0359
Rep	2	0.323333	0.161667	0.4695
Trt	5	19.858333	3.971667	11.5344 **
Err	10	3.443333	0.344333	

** $P < 0.01$

Table II-2C

ANOVA for specific treatment effects, days and interaction in sewage, activated charcoal treated sewage and industrial effluent

Source	**Df**	**SS**	**MS**	**F**
s	2	4.093333	2.046667	5.9439 *
d	1	14.400556	14.400556	41.8216 **
sd	2	1.364444	0.682222	1.9813 NS
Err	10	3.443333	0.344333	1.0000

** P < 0.01 *P < 0.05

S – Treatment

d- days

sd – interaction between treatment and days

Table II-2D : Standard error of treatments, durations and interaction between treatment × durations for sewage, activated charcoal treated sewage and industrial effluent

	SED	**CD(0.05)**	**CD(0.01)**
S	0.33879	0.75487	1.07377

D	0.27662	0.61635	0.87673
sd	0.47912	1.06755	1.51854

Seeds of *Glycine max* were grown in sewage and treated sewage. Sewage was treated with activated charcoal, centrifuged and the supernatant was used for germination : Seeds were also grown in industrial effluent.

Table- II-3A Presents the levels of $P^{H,}$ TSS,oil & grease,COD & BOD in industrial effluent.

pH	8.0
TSS	51.0 mg/lit
Oil and Grease	40 mg/lit
COD	80 mg/lit
BOD	18 mg/lit

Composition of Effluent & sewage

Table II–3B Comparison of $_{p}$H , electrical conductivity of pretreated , post treated effluent & sewage-data.

	Effluent		**Sewage**	
	Pre –treated	**Post -treated**	**Pre -treated**	**Post -treated**

pH	**7.8**	**6.5**	**6.99**	**7**
E.C	**1.4ms**	**1.5 ms**	**2.9 ms**	**3.5 ms**

Gram's staining revealed the following results-

1. Sample 1: Gram positive rods, Non haemolytic

2. Sample 2: Gram positive rods, Non haemolytic

3.Sample 3:Gram positive cocci, Non haemolytic

-Plates showing colonies in between 30 and 300 were selected and the colonies were counted using colony counter .The number per ml in the original sample were then calculated.

S. No.	Dilutions										
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	Cfu/ml
1	35	30	12	……..……No Growth…….							$12*10^5$
2	36	30	11	……..……No Growth…….							$11*10^5$
3	……..……No Growth…….										

Dilution factor 10^5

Effect of phytohormone in combination of saline molarity on the growth of *G. max* -The effect of phytohormones cytokinin and auxin in saline treated *G. max* seeds were studied in various concentrations of salinity and 1 part per million (1 ppm) and 2 ppm of cytokinin and auxin .

A combination of salinity and the phytohormones on the growth of roots (mM) is presented in **table III-1A** .Phytohormones were sprayed on day 1. Perusal of the mean ± SE reveals the following salient findings.

There is a decreased growth of roots in 500 mM saline both in auxin and cytokinin sprayed seed. However, there is no significant difference on growth in both the auxins and cytokinins.**Table III-I B** depicts the mean length (cm) of the cotyledon in different combinations of the cytokinins and auxins .

Table III-1A

Effect of saline treatment with phyto hormone (root length) once sprayed on Glycine max.

S.no		Day 5 (cm)	Day 8 (cm)
1	50mM Sal. + Cyt.1	8.45 ± 0.33	11.05 ± 0.56
2	50mM Sal. + Cyt.2	8.83 ± 0.76	12.32 ± 0.34
3	50mM Sal. + Aux.1	8.73 ± 0.55	11.37 ± 0.38
4	50mM Sal. + Aux.2	9.05 ± 0.42	11.63 ± 0.26
5	100mM Sal. + Cyt.1	9.32 ± 0.96	22.65 ± 0.53
6	100mM Sal. + Cyt.2	8.83 ± 0.76	20.92 ± 1.4
7	100mM Sal. + Aux.1	9.42 ± 0.6	17.2 ± 1
8	100mM Sal. + Aux.2	11.06 ± 1.19	21.58 ± 0.6
9	500mM Sal. + Cyt.1	6.6 ± 0.33	7.73 ± 0.29
10	500mM Sal. + Cyt.2	7.52 ± 0.63	13.22 ± 1.08
11	500mM Sal. + Aux.1	7.57 ± 0.42	16.9 ± 0.67
12	500mM Sal. + Aux.2	7.93 ± 0.69	19.57 ± 0.55

Cyt.1 = cytokinin 1 ppm

Cyt.2 = cytokinin 2 ppm

Aux.1 = auxin 1 ppm

Aux.2 = auxin 2 ppm

Table III-1B : Mean length of cotyledons (mm) once sprayed on day 5

	h1	h2	h3	h4
s1	13.0000	13.0667	13.4000	13.4500
s2	10.0333	10.6500	9.5667	9.7667
s3	7.6667	8.9000	6.3833	8.3167
s4	6.3500	8.0500	6.0667	7.2833

s1- Distilled water (control), s2 – 50 mM NaCl,

s3 – 100 mM NaCl, s4 – 500 mM NaCl

h1 – 1 ppm cytokinin h2 – 2 ppm cytokinin

h3 – 1 ppm Auxin h4 – 2 ppm Auxin

A highly significant increase in the root length was observed on day 8, however, the two concentrations of either of the auxin and cytokinin did not have any appreciable difference in changing the growth of the roots.

Growth of roots in 100 mM saline +1 ppm cytokinin on day 8 was highly significant increased while in 500 mM saline + cytokinin 1 ppm was severely retarded .Moreover, there was no significant growth on day 8 as compared to day 5. But for 500 mM saline +1 ppm auxin, all other levels of the hormones, significantly increased the growth of roots in 500 mM saline.

Plate 3

3A depicts the development of roots and cotyledons on day 5 in industrial water **.**

3B presents the development on day 8 in industrial water , **3C** on day 5 in sewage and **3D** germinating *Glycine max* seeds in sewage 8 day growth.

Plate 4

4A shows the development of roots and cotyledons in 100 mM NaCl + 1 ppm auxin, **4B** in 100 mM NaCl + 2 ppm auxin, 8 day growth , **4C** in 100 mM NaCl + 2ppm cytokinin and **4D** exhibits growth in 500 mM NaCl + 1 ppm auxin.

Plate 5

The development of roots and cotyledons in 100 mM saline + 2 ppm cytokinin day 8 is presented in **5A**, in 500 mM NaCl + 1ppm cytokinin in **5B**. Control 8 day growth in **5C** and 100 mM NaCl + 2 ppm auxin in **5D.**

Plate 6

6A shows the developments of roots and cotyledons in 100 mM NaCl + 2 ppm auxin.**6B** in 100 mM NaCl + 1 ppm cytokinin, 5 day growth. **6C** in 500 mM NaCl + 2ppm auxin and **6D** exhibits growth in 500 mM NaCl + 1 ppm auxin 8 day growth.

Analysis of variance for the effect of the 1ppm and 2ppm of cytokinin and auxin respectively in combination with different contractions of saline on the growth of cotyledons on day 5 is presented in table **III-1C.**

A highly significant difference ($p < 0.01$) occurred between phytohormone and treatments. Replicate effect was non significant. Analysis of variance for the treatment, hormones and interaction between hormones & treatment ,highly significant difference is presented in **table-III-1D**

A significant ($p < 0.05$) difference was observed on the growth between the affect of cytokinin and auxin in combination with saline. No significant difference was found between the interactions of saline treatments and two hormones.Based on the least square difference it was observed that control had the best performance followed by 50 mM saline and poorest in 500 mM saline. Cytokinin 2 ppm and auxin 2 ppm had at par performance cytokinin 1 ppm and auxin 2 ppm . The best growth was in cytokinin 2 ppm and auxin 2 ppm followed by cytokinin 1 ppm and auxin 1 ppm. The poorest performance was in cytokinin 1 ppm and auxin 1 ppm.

The standard error of the mean and critical difference at ($p < 0.01$) , ($p < 0.05$)were presented **in table III-1E**. Coefficient of variation for the length of cotyledons was 14.31%.The growth of the cotyledons of *G.max* in control and saline concentrations in different proportions of cytokinins and auxins was studied with two sprays of the hormones. The first spray was done on day 1 and

the second spray was done on day 5. Length of the cotyledons was measured on day 5.Analysis of variance was performed by a 2 way classification.

Table III-1C : ANOVA for the combined effect of saline concentrations and phytohormones and distilled water (control) on the growth of cotyledons on day 5 .Statistical significance of replicate and treatment .

Source	Df	SS	MS	F
TOT	95	826.129063	8.696095	4.0126
Rep	5	57.423438	11.484688	5.2993
Trt	15	606.164063	40.410938	18.6464 **
Err	75	162.541562	2.167221	

** P < 0.01 *P < 0.05

Table III-1D : Analysis of variance for phytohormones in combination with saline concentrations, and control on the growth of cotyledons of *Glycin max* on day 5 for specific effect of treatment ,hormones and interaction between treatment and hormone

S	Df	SS	MS	F

S	3	565.460313	188.486771	86.9716 **
H	3	23.030313	7.676771	3.5422 *
sh	9	17.673437	1.963715	0.9061 NS
Err	75	162.541562	2.167221	1.0000

** P < 0.01 *P < 0.05

S = different levels of saline and control (distilled water)

h = different levels of hormones

sh = interaction between treatments × levels of hormones

(PLATE 3)

EFFECT OF INDUSTRIAL EFFLUENT AND SEWAGE ON GERMINATION OF GLYCIN MAX

(3-A)
Industrial Effluent 5 Day Growth

(3-B)
Industrial Effluent 8 Day Growth

(3-C)
SEWAGE 5DAY

(3-D)
SEWAGE 8 DAY

(PLATE 4)

EFFECT OF PHYTOHORMONE WITH CONJUCTIVE USE OF SALINE WATER ON SEED GERMINAT

(4-A)
100 mM AUXIN -1 ONCE SPRAYED
8 DAY

(4-B)
100 mM AUXIN-2 TWICE SPRAYED
8 DAY

(4-C)
100 mM CYTOKONIN-2 ONCE
SPRAYED 8 DAY

(4-D)
500 mM AUXIN-1 TWICE SPRAYED
8 DAY

Table III-1E : Standard error of treatments, hormone levels and interaction between treatment × hormones for the growth of cotyledons of *Glycin max* on day 5

	SED	CD(0.05)	CD(0.01)
S	0.42497	0.84660	1.12323
H	0.42497	0.84660	1.12323
Sh	0.84995	1.69320	2.24646

SED = standard error of the mean

CD = critical difference

The mean values for the cotyledon length at different levels of salinity and hormones are presented in **table III – 2A**. **Table III – 2B** presents the ANOVA for the effect of replicates and treatments ,a highly significant difference ($p < 0.01$) was observed between the treatments. The replicate effect was not significant.

Analysis of variance for the effect of treatments, levels of hormones and interaction between the treatment and hormones is presented in table **III- 2C**. Highly significant differences ($p < 0.01$) were observed between the treatments and between the levels of hormones. The interaction between the treatments and levels of hormones was not significant.

Best growth of the cotyledons was attained in distilled water (control) and 50 mM NaCl were at par followed by 100 mM NaCl and 500 mM NaCl which were at par based on least square difference, it was observed that cytokinin 2 ppm and auxin 2 ppm attained the best growth were at par followed by cytokinin 1 ppm and auxin 1 ppm which were at par .Worse growth was observed in auxin 1 ppm and cytokinin 1 ppm which were at par. The standard error of the means & critical difference levels are presented in **table III – 2D.** Coefficient of variation is 15.26%.

Table III-2A : Mean length of cotyledons sprayed twice with phytohormones on day 5

	h1	h2	h3	h4
s1	10.4000	10.8500	10.0667	11.2333
s2	10.6167	11.2000	10.2667	10.9000
s3	8.0000	10.5000	8.1667	9.0000
s4	8.3000	9.5000	6.3500	8.0000

s1- distilled water (control), s2 – 50 mM NaCl,

s3 – 100 mM NaCl, s4 – 500 mM NaCl

h1 – 1 ppm cytokinin h2 – 2 ppm cytokinin

h3 – 1 ppm Auxin h4 – 2 ppm Auxin

(PLATE 6)

EFFECT OF PHYTOHORMONE WITH CONJUCTIVE USE OF SALINE WATER ON SEED GERMINATION

(6-A)
100 mM AUXIN-2 ,5 DAY GROWTH

(6-B)
100 mM CYTOKININ-1 5 DAY GROWTH

(6-C)
500 mM AUXIN -2 ONCE SPRAYED
8 DAY

(6-D)
500 mM AUXIN-1 ONCE SPRAYED
8 DAY

Table III-2B : ANOVA for the growth of cotyledons in various combinations of saline and hormones and control twice sprayed on day 5. Replicate and treatment effects are presented.

Source	Df	SS	MS	F
TOT	95	457.266563	4.813332	1.8108
Rep	5	69.388438	13.877688	5.2208
Trt	15	188.514896	12.567660	4.7279
Err	75	199.363229	2.658176	1.0000

Table III-2C : Analysis of variance for phytohormones in combination with saline concentrations, distilled water (control) on the growth of cotyledons of *Glycin max* on day 5

s	df	SS	MSS	F
s	3	127.121146	42.373715	15.9409 **
h	3	41.431146	13.810382	5.1954 **
sh	9	19.962604	2.218067	0.8344 NS
Err	75	199.363229	2.658176	1.0000

** $P < 0.01$

s = Treatment, different levels of saline and control (distilled water)

h = different levels of hormones

sh = Interaction between levels of saline × levels of hormones

Table III-2D : Standard error of treatments, hormone levels and interaction between treatment × hormones for growth of cotyledons in *Glycin max* on day 5

	SED	**CD(0.05)**	**CD(0.01)**
S	0.47065	0.93760	1.24397
H	0.47065	0.93760	1.24397
Sh	0.94131	1.87520	2.48794

SED = standard error of the mean

CD = critical difference

Seeds of *G.max* were germinated in distilled water and different salinity concentrations in combination with 1 and 2 ppm of either of the phytohormones cytokinin or auxin. Hormones were sprayed on day 1 and the length of the cotyledons was measured on day 8.

Table III-3A presents the mean value for the length of cotyledons in twice sprayed on day 8. Cotyledon length was measured on day 8.

Analysis of variance for the length of cotyledon with respect to replicates and treatments on day 8 are presented in **table III – 3B**. Data was analyzed by two way classification. Highly significant difference ($p < 0.01$) was observed between treatments. The replicate effect was not significant. The specific effect of treatments, levels of hormones and the interactions between the treatments and the hormones is presented in **table III – 3C**.Sample were once sprayed.

Observations were recorded on day 8. The standard error of the mean & critical differences are presented in **table III – 3D**. The coefficient of variables was 15.63%.

Analysis of variance was performed for the effect of 50 mM, 100 mM and 500 mM NaCl in combination with either cytokinin 1 , 2 ppm and auxin 1 ,2 ppm and distilled water. The hormones were sprayed on the seeds of *G. max* twice. Length of the cotyledons was measured on

day 8. Analysis of variance was performed by two way classification and it was a completely randomized design.

Table III-3A : Mean length of cotyledons (mm) on day 8 of once phytohormones sprayed

	h1	h2	h3	h4
s1	16.2333	16.4333	16.5500	16.6000
s2	11.3333	11.8000	10.7333	10.9500
s3	11.3333	12.0833	7.9000	10.0667
s4	9.6667	10.6667	7.4333	8.5333

s1- Distilled water (control) s2 – 50 mM NaCl,

s3 – 100 mM NaCl, s4 – 500 mM NaCl

h1 – 1 ppm cytokinin h2 – 2 ppm cytokinin

h3 – 1 ppm Auxin h4 – 2 ppm Auxin

Table III-3B:

ANOVA for the growth of cotyledons in hormones with different combinations of salinity on day 8 .Replicate and treatment effect are presented.

Source	df	SS	MS	F
TOT	95	1202.542396	12.658341	3.5220
Rep	5	75.733021	15.146604	4.2143
Trt	15	857.250729	57.150049	15.9010
Err	75	269.558646	3.594115	1.0000

Table III-3C : ANOVA for growth in control, different concentrations of saline and different levels of hormones once sprayed on day 8 . Specific effect of treatment , hormone and interaction between treatment x hormone are presented.

S	Df	SS	MSS	F
S	3	757.268646	252.422882	70.2323 **
H	3	57.348646	19.116215	5.3188 **
sh	9	42.633437	4.737049	1.3180 NS
Err	75	269.558646	3.594115	1.0000

** P < 0.01

s = salinity levels h = hormones level

sh = interaction between saline concentrations and hormone levels

Table III-3D : Standard error of treatments, hormone levels and interaction between treatment × hormones for growth in control, different concentrations of saline and different levels of hormones once sprayed on day 8

	SED	CD(0.05)	CD(0.01)

S	0.54727	1.09024	1.44648
H	0.54727	1.09024	1.44648
sh	1.09455	2.18048	2.89297

SED = standard error of the mean

CD = critical difference

Highly significant differences ($p < 0.01$) was observed between the treatments and also between the hormones. The interaction between the salinity levels and levels of the phytohormones was not significant ,best growth was in distilled water followed by 50 mM saline and 100 mM NaCl which were at par. The poorest performance was observed in 500 mM NaCl.

Mean comparison by LSD for the effect of hormones revealed that best performance was with cytokinin 1 ppm and 2 ppm. ,which were at par followed by auxin 1 ppm and auxin 2 ppm.

The poorest growth was in auxin 1 ppm and 2 ppm which were at par.Mean length of cotyledons in different hormone concentrations is presented in **table III-4A.**

Table III - 4B presents the analysis of variance for the effect of replicates and the effect of treatments. There was no significant difference between the treatments and the replicates.

None of the treatments and there interactions had any significant effect on growth of the cotyledons. The mean value of the cotyledons with respect to the saline treatments and hormone levels in combination is presented in table **III -4C.**

Standard error of the mean and the probability level of critical differences are presented in table **III -4D.**

The coefficient of variation for the cotyledon length was 18.24%.

Table III-4A

Mean length of cotyledons (mm)

	h1	h2	h3	h4
s1	10.5167	12.2167	9.8667	10.4667
s2	11.9333	12.3000	11.4333	12.2333
s3	12.1667	12.5000	9.5000	11.0000
s4	10.8333	11.0000	10.3333	11.4667

s1- Distilled water (control) s2 – 50 mM NaCl,

s3 – 100 mM NaCl, s4 – 500 mM NaCl

h1 – 1 ppm cytokinin h2 – 2 ppm cytokinin

h3 – 1 ppm Auxin h4 – 2 ppm Auxin

Table III-4B

ANOVA for effect of phytohormones in combination with different concentrations of saline and control on the growth of cotyledons in twice sprayed group on day 8 . Replicate and treatment effect are presented .

Source	**Df**	**SS**	**MS**	**F**
TOT	95	503.139583	5.296206	1.1228
Rep	5	70.049583	14.009917	2.9700
Trt	15	79.302917	5.286861	1.1208
Err	75	353.787083	4.717161	1.0000

Table III-4C : ANOVA for the effect of phytohormone with different concentration of saline and control on the growth of cotyledons on day 8 (twice sprayed).specific effect of treatment, hormone and interaction between treatment × hormone are presented .

S	**df**	**SS**	**MSS**	**F**
S	3	21.044583	7.014861	1.4871
H	3	36.402083	12.134028	2.5723
Sh	9	21.856250	2.428472	0.5148
Err	75	353.787083	4.717161	

S = Concentrations of saline and control

h = levels of hormones

sh = Interaction between saline concentrations and hormone levels

Table III-4D :

Standard error of treatments, hormone levels and interaction between treatment × hormones for the effect of phytohormone with different concentration of salinity on the growth of cotyledons on day 8 (twice sprayed)

	SED	**CD(0.05)**	**CD(0.01)**
S	0.62697	1.24901	1.65713
H	0.62697	1.24901	1.65713
Sh	1.25395	2.49802	3.31427

SED = standard error of the mean CD = critical difference

[B] Root Hormone once Sprayed 5 day

Growth of the root of *Glycine max* in distilled water, 50 mM saline + 1 ppm cytokinin, 100 mM saline + 2 ppm cytokinin, 100 mM saline + 1 ppm cytokinin, 100 mM saline + 2 ppm cytokinin, 500 mM saline and cytokinin , 500 mM saline + 1 ppm auxin and 500 mM saline + 2 ppm auxin was recorded on day 5. **Table III-5A** represents the effect of saline concentration+ phytohormone in cotyledons .Mean±SE are presented on day 5& 8 in once sprayed ,twice sprayed group. The analysis of effect of treatment, the levels of hormones and the interaction between level of salinity and the concentration of hormones is presented in table **III -5B**. Table **III-5C** presents the ANOVA for the effect of different treatments ,hormone was sprayed on day 1.Growth on root was measured on day 5.

A highly significant difference ($p < 0.01$) was observed .The statistical difference for treatment, levels of hormones and the interaction between the treatment and hormones is presented in table **III-5D.**

The growth was best in 50 mM saline followed by distilled water and 100 mM saline which were at par worse growth was 100 mM and 500 mM NaCl which were at par. A highly significant difference ($p < 0.01$) was observed between treatments on the rate of growth. However, neither the effect of different concentrations of the hormones nor the interaction between the treatment x hormones were significant.

Table III-5E presents the standard error of the deviation and critical difference values at $p < 0.05$ and $p < 0.01$ level.The coefficient of variation was 26.26 %.

Table III-5A : Effect of saline concentration phytohormone on cotyledon length (mm) growth on day 5 with phytohormones once or twice sprayed :

		Once sprayed		**Twice sprayed group**	
S. no		**5 Day**	**8 Day**	**5 Day (one spray)**	**8 Day (two spray)**
1	50mM Sal. + Cyt.1	10.03 ± 0.86	11.33 ± 1.15	10.62 ± 0.24	11.93 ± 0.32
2	50mM Sal. + Cyt.2	10.65 ± 0.7	11.8 ± 0.53	11.2 ± 0.28	12.3 ± 0.65
3	50mM Sal. +	9.57 ± 0.55	10.73 ±	10.27 ±	11.43 ±

	Aux.1		0.45	0.34	0.56
4	50mM Sal. + Aux.2	9.77 ± 0.65	10.95 ± 0.38	10.9 ± 0.37	12.23 ± 0.62
5	100mM Sal. + Cyt.1	7.67 ± 0.88	11.33 ± 1.15	8 ± 1.39	12.17 ± 1.45
6	100mM Sal. + Cyt.2	8.9 ± 0.94	12.08 ± 0.34	10.05 ± 1.34	10.5 ± 1.43
7	100mM Sal. + Aux.1	6.38 ± 0.89	7.9 ± 1.03	8.17 ± 1.01	9.5 ± 1.26
8	100mM Sal. + Aux.2	8.32 ± 0.61	10.07 ± 0.56	9 ± 1.06	11 ± 1.15
9	500mM Sal. + Cyt.1	6.35 ± 0.62	9.67 ± 1.93	8.3 ± 0.26	10.83 ± 1.56
10	500mM Sal. + Cyt.2	8.05 ± 0.93	10.67 ± 0.88	9.5 ± 0.76	11 ± 1.39
11	500mM Sal. + Aux.1	6.07 ± 0.58	7.43 ± 1.09	6.35 ± 0.83	10.33 ± 0.88
12	500mM Sal. + Aux.2	7.28 ± 0.61	8.53 ± 0.82	8 ± 1.06	11.47 ± 0.44

Cyt.1 = cytokinin 1 ppm Cyt.2 = cytokinin 2 ppm

Aux.1 = Auxin 1 ppm Aux.2 = Auxin 2 ppm

Table III-5B : Mean table of root length.

	h1	h2	h3	h4
s1	8.5667	8.9333	8.7833	8.8167
s2	10.0333	10.6500	9.5667	9.7667
s3	7.6667	8.9000	6.3833	8.3167
s4	6.3500	8.0500	6.0667	7.2833

s1- distilled water (control), s2 – 50 mM NaCl,

s3 – 100 mM NaCl, s4 – 500 mM NaCl

h1 – 1 ppm cytokinin h2 – 2 ppm cytokinin

h3 – 1 ppm Auxin h4 – 2 ppm Auxin

Table III-5C : ANOVA for growth of *Glycin max* root on day 5 in different levels of saline and distilled water (control) sprayed with different levels of cytokinin and auxin .

Source	**Df**	**SS**	**MS**	**F**
TOT	95	395.913333	4.167509	
Trt	15	164.793333	10.986222	3.8028
Err	80	231.120000	2.889000	

** $P < 0.01$

Table III-5D : ANOVA for the effect of phytohormone with different concentration of saline and control on the growth of cotyledons on day 8 (twice sprayed) .Specific effect of treatment, hormone and interaction between treatment × hormone are presented.

S	**df**	**SS**	**MSS**	**F**
S	3	124.609167	41.536389	14.3774
H	3	26.600833	8.866944	3.0692
sh	9	13.583333	1.509259	0.5224

s= concentrations of saline and control

h = levels of hormones

sh = interaction between saline concentrations and hormone levels

Table III-5E : Standard error of treatments, hormone levels and interaction between treatment × hormones for the effect of phytohormone with different concentration of saline and control on the growth of cotyledons on day 8 (twice sprayed)

	SED	CD(0.05)	CD(0.01)
S	0.49066	0.97647	1.29566
H	0.49066	0.97647	1.29566
sh	0.98133	1.95295	2.59131

SED = standard error of the mean

CD = critical difference

Similar experiments were performed with the only difference that the seeds of *Glycin max* were sprayed with each levels of cytokinin and auxin twice ; first on day 1 and second on day 5.

In another set of experiments, seeds of *Glycine max* grown in distilled water (control) saline levels of 50 mM NaCl, 100 mM NaCl and 500 mM NaCl in conjunction with either of the 1 and 2 ppm cytokinin and auxin were sprayed second time on day 5. The first spray being conducted on day 1, and the growth of the root was measured on day 5. **Table III-6A** presents the pooled mean values of treatments and the hormones , also the mean value of each hormone and each treatment, the level of hormones being 1 ppm and 2 ppm, cytokinin 1 and 2 ppm was in combination with 50 mM NaCl, 100 mM saline and 500 mM saline. Distilled water served as control.

Table III-6B presents the analysis of variance for the overall effect of treatments on the growth of root. Highly significant differences were observed between the treatments.

The effect of treatments, levels of auxin and cytokinin in combination with the saline treatments and the interaction between treatments and the hormones is presented in **table III-6C** .

Highly signficant differences ($p < 0.01$) were observed between the different treatments & the effect of two hormones levels. The interaction between the treatment and the levels of the hormones were non significant.

Table III-6D presents the standard error of pooled mean . Critical difference at ($p<0.05$) and ($p<0.01$) were also significant .

The best root growth was observed in 50 mM saline + hormones followed by control, 100 mM saline + hormones and 500 mM saline which all were at par.

Table III-6A : Mean table of root length on day 5 (twice sprayed)

	h1	h2	h3	h4
s1	8.6000	9.3000	9.1167	9.1667
s2	10.6167	11.2000	10.2667	10.9000
s3	8.0000	10.5000	8.1667	9.0000
s4	8.3000	9.5000	6.3500	8.0000

s1- Distilled water (control), s2 – 50 mM NaCl,
s3 – 100 mM NaCl, s4 – 500 mM NaCl
h1 – 1 ppm cytokinin h2 – 2 ppm cytokinin
h3 – 1 ppm Auxin h4 – 2 ppm Auxin

Table III-6B : ANOVA for effect of distilled water (control), 50 mM NaCl + levels of hormones, 100 mM NaCl hormones and 500 mM NaCl + hormone on the growth of root of Glycin max on day 5.

Source	**Df**	**SS**	**MS**	**F**
TOT	95	437.932396	4.609815	1.2838
Trt	15	150.660729	10.044049	2.7971
Err	80	287.271667	3.590896	1.0000

Table III-6C : ANOVA for the treatment effect, hormone and interaction between hormone x treatment on root length on day 5 (twice sprayed)

s	Df	SS	MSS	F
s	3	92.263646	30.754549	8.5646
h	3	35.709479	11.903160	3.3148
sh	9	22.687604	2.520845	0.7020
Err	80	287.271667	3.590896	

s = concentrations of saline and control

h = levels of hormones

sh = interaction between saline concentrations and hormone levels

Table III-6D : Standard error of treatments, hormone levels and interaction between treatment × hormones growth of root of *Glycin max* on day 5.

	SED	CD(0.05)	CD(0.01)
S	0.54703	1.08865	1.44450
H	0.54703	1.08865	1.44450
Sh	1.09406	2.17730	2.88900

SED = standard error of the mean

CD = critical difference

[B] Root Growth 8th day once sprayed

Table III-7A presents the mean level of growth of roots with respect to treatments ,phytohormones and also the response wih respect to each of the treatment and levels of hormones.

Seeds of *Glycin max* germinated in distilled water (control) , in different level of saline 50 mM NaCl with 1 and 2 ppm of either cytokinin or auxin is presented in **table III-7B.**Highly significant differences ($p < 0.01$) were observed between treatments.

Table III 7-C presents the ANOVA of the specific effects of treatments, the levels of hormones, interaction between the treatment and hormones.

There are highly significant differences ($p < 0.01$) between the treatments and between the level of the phytohormones however, the interaction between the treatment and hormones is non significant.

Mean comparison by least square difference revealed that the growth in distilled water was best, followed by 50 mM NaCl and 100 mM saline which were at par, worse growth was in 500 mM saline combination.

Growth with cytokinin 1 ppm and 2 ppm was best and their performance were at par followed by auxin 1 ppm and 2 ppm,which were at par .The coefficient of variation was 15.97%.

Table III-7D presents the standard error and critical difference levels ($p < 0.01$) and ($p < 0.05$) .

Table III-7A : Mean table of root length on day 8(once sprayed)

	h1	h2	h3	h4
s1	14.4667	14.7833	14.3667	14.4333
s2	11.3333	11.8000	10.7333	10.9500
s3	11.3333	12.0833	9.9000	10.0667
s4	9.6667	10.6667	7.4333	8.5333

s1- Distilled water (control) s2 – 50 mM NaCl,

s3 – 100 mM NaCl, s4 – 500 mM NaCl

h1 – 1 ppm cytokinin h2 – 2 ppm cytokinin

h3 – 1 ppm Auxin h4 – 2 ppm Auxin

Table III-7B : ANOVA for root length of *Glycin max* grown in distilled water, 50mM ,100 mM NaCl and 500 mM NaCl in combination with different levels of cytokinin and auxins .

Source	df	SS	MS	F
TOT	95	832.346563	8.761543	2.0395
Trt	15	488.668229	32.577882	7.5833
Err	80	343.678333	4.295979	

Table III-7C : ANOVA for root length. Specific effects of treatment, hormone and interaction between treatment × hormones on day 8 (once sprayed) are presented .

s	**df**	**SS**	**MSS**	**F**
s	3	388.546146	129.515382	30.1480
h	3	65.745313	21.915104	5.1013
sh	9	34.376771	3.819641	0.8891
Err	80	343.678333	4.295979	

S = concentrations of saline and control

h = levels of hormones

sh = interaction between saline concentrations and hormone levels

Table III-7D : Standard error of treatments, hormone levels and interaction between treatment × hormones for root length .

	SED	**CD(0.05)**	**CD(0.01)**
S	0.59833	1.19074	1.57996
H	0.59833	1.19074	1.57996
Sh	1.19666	2.38149	3.15993

SED = standard error of the mean

CD = critical difference

[B] Growth of root second sprayed 8th day

Analysis of variance was performed for the growth of roots on day 8 germinated in distilled water (control) ,50 mM NaCl, 100 mM NaCl and 500 mM NaCl in combinations with 1 ppm and 2 ppm of cytokinin and auxins. Seeds were sprayed twice with the phytohormones; first sprayed on day 1 and second sprayed was done on day 5.

Table III-8A presents the mean values of the treatments , the levels of hormones and also the each level of combination of treatments , phytohormones.

Table III-8B reveals that there are highly significant differences ($p < 0.01$) between the treatments .The statistical analysis for the specific difference between treatments, the phytohormones and the interaction between the treatments and the phytohormones is presented **in table III-8C.**

Highly significant differences ($p < 0.01$) between the treatments were observed. However there were no significant differences either between the hormone and the interaction between the treatment and the hormone .Mean comparison based on the LSD revealed that the best growth was attained in distilled water (Control) followed by 50 mM NaCl, 100 mM NaCl and 500 mM NaCl in phytohormone combinations and all these levels of saline were at par .The coefficient of variation is 17.38%.

Table III-8D presents standard error, probability values at $p < 0.01$ and $p < 0.05$ level critical difference for the treatments, hormones and there interaction.

Table III-8A : Mean table for length of root on day 8 (twice sprayed).

	h1	h2	h3	h4
s1	14.2167	14.4667	14.3000	14.3167
s2	11.9333	12.3000	11.4333	12.2833
s3	12.8667	10.5000	9.5000	11.0000
s4	10.8500	11.0167	10.4333	11.4667

s1- Distilled water (control) s2 – 50 mM NaCl,

s3 – 100 mM NaCl, s4 – 500 mM NaCl

h1 – 1 ppm cytokinin h2 – 2 ppm cytokinin

h3 – 1 ppm Auxin h4 – 2 ppm Auxin

Table III-8B : ANOVA for root length of *Glycin max* on day 8 (twice sprayed) with cytokinins and auxins in combination with 50 mM NaCl, 100 mM NaCl and 500 mM NaCl.

Source	df	SS	MS	F
TOT	95	658.857396	6.935341	1.2767
Trt	15	224.289063	14.952604	2.7526
Err	80	434.568333	5.432104	

Table III-8C : ANOVA for the specific effect of treatment, hormone and interaction between hormone x treatment on root length on day 5 (twice sprayed)

s	**df**	**SS**	**MSS**	**F**
s	3	181.954479	60.651493	11.1654
h	3	14.927813	4.975938	0.9160
sh	9	27.406771	3.045197	0.5606
Err	80	464.568333	5.432104	

s = concentrations of saline and control

h = levels of hormones

sh = interaction between saline concentrations and hormone levels

Table III-8D : Standard error of treatments, hormone levels and interaction between treatment × hormones for root length on day 5(twice sprayed).

	SED	CD(0.05)	CD(0.01)
S	0.67281	1.33897	1.77664
H	0.67281	1.33897	1.77664
Sh	1.34562	2.67794	3.55329

SED = standard error of the mean

CD = critical difference

ANOVA Root Growth –3 way classification once sprayed Hormone

In order to understand the specific effects of treatments distilled water (control), 50 mM NaCl , 100 mM NaCl and 500 mM NaCl, the levels of cytokinin 1 and 2 ppm, the duration of germination (day 5 and day 8) the interaction between the treatment and phytohormones between hormones and duration, treatment and duration and the interaction between treatment x hormones x days.

Growth of the root of *Glycin max* was analyzed by a 3 way classification. Hormones were sprayed once on day 1.

Table III-9A presents ANOVA (3 day classification) for the effect of treatment (control) distilled water, saline levels in combination of phytohormones on the growth of *G.max*. (once sprayed with hormones) .Highly significant difference ($p < 0.01$) was observed between treatment.

The ANOVA for specific effects of treatments, hormones, duration of germination and different types of interactions between the treatments, hormones and duration of germination is presented in **table III-9B**.

Highly significant difference ($p < 0.01$) were observed between the treatments, the levels of the hormones, the duration of germination and the interaction between the treatment x duration of germination. The interaction between the treatment x hormones, hormone x days and treatment x hormones x days, however were non significant.A mean comparison by least square difference revealed that best growth was attained in distilled water (Control) followed by the order 50 mM NaCl, 100 mM NaCl, and 500 mM .Poorest growth was in 500 mM NaCl as regard the hormones, the best performance was in cytokinin 2 ppm, followed by 1 ppm cytokinin , the poorest growth was in auxin 1 ppm.With regards to the duration of germination, best growth was attained on day 8.

Table III-9A : ANOVA (3 way classification) for the effect of treatment (control) distilled water, saline levels in combination of phytohormones on the root length of *G.max. (*once sprayed).

Source	Df	SS	MS	F
TOT	191	1632.229948	8.545707	2.3788
Trt	31	1057.431615	34.110697	9.4950
Err	160	574.798333	3.592490	

Table III-9B : ANOVA (3 way classification) for specific effects treatments, hormones, duration of germination and different types of interactions on growth of root of *G.max* (once sprayed)

S	Df	SS	MSS	F
S	3	373.231406	124.410469	34.6309
H	3	80.930156	26.976719	7.5092
D	1	403.970052	403.970052	112.4485
sh	9	40.034219	4.448247	1.2382
hd	3	11.415990	3.805330	1.0592
sd	3	139.923906	46.641302	12.9830
shd	9	7.925885	0.880654	0.2451
Err	160	574.798333	3.592490	1.0000

C.V. (Treatment) :: 19.27%

S - Treatment (distilled water control) 50 mM NaCl, 10 mM NaCl, 500 mM NaCl in combination with phytohormome

h - hormone – cytokinin and auxin (1 ppm & 2 ppm)

d - duration of germination on 5 day and 8 day

sh - interaction between treatment × hormone

hd - interaction between hormone and duration of germination

sd - interaction between treatment and days

shd - interaction between treatment × hormone × days

3 way ANOVA – Once Sprayed phytohormone

Growth was best attained in distilled water on day 8, followed by 50 mM NaCl on day 8 and 100 mM NaCl on day 8 which were at par,followed by distilled water on day 5 and 100 mM NaCl on day 5, which were at par. Worse growth occurred in 100 mM saline on day 5 and 500 mM saline on day 5 which were at par.

3 way ANOVA second sprayed day 5.

A three way classification of analysis of variance was performed to analyse the effects of treatments, the effect of duration of germination, the specific levels of the hormones .The interactions between the treatment and hormones, duration of germination and level of hormones x duration of germination , the treatments were distilled water (control) ,50 mM NaCl, 100 mM NaCl and 500 mM NaCl in combination with cytokinin 1 ppm or 2 ppm and auxin 1 ppm and 2 ppm. Seeds of *Glycine max* were germinated and root length was recorded on day 5 and day 8 ,data was pooled.The effect of distilled water(control),50mM,100mM& 500mM NaCl in combination with cytokinin 1ppm , 2ppm & auxin 1 ppm, 2ppm in the growth of roots of *G.max* on day 8, sprayed twice with the hormone on day 1 & day 5 is presented in ANOVA in **table-10A**. Highly significant difference ($P<0.01$) was observed between the treatments.

Table III-10A depicts ANOVA for The effect of distilled water (control), 50 mM NaCl, 100 mM NaCl and 500 mM saline in combination with cytokinin 1 ppm ,2 ppm and auxin 1 ppm ,2 ppm on the growth of root of *G.max*, sprayed twice with the hormone, once on day 1 and the other on day 5.

ANOVA for specific effects of treatments, duration of germination ,levels of specific hormones and interactions between them is presented in the ANOVA table **III-10B.**

Highly significant differences ($p < 0.01$) were observed for the effect of treatments, duration of germination and the interaction between the treatment x duration of germination. The levels of hormones, the interaction between treatment x hormones; levels of hormones x duration and interaction between treatment levels of hormones and the treatments x levels of hormones and duration of germination were, however non significant.

Mean comparison by least square difference, revealed that the best growth was achieved in distilled water and 50 mM NaCl which were at par, followed by 100 mM NaCl and 500 mM NaCl . Maximum growth was attained on Day 8.It was observed that best growth was obtained in distilled water on day 8 followed by 50 mM NaCl on day 8, 100 mM NaCl on day 8 and 500 mM NaCl on day 8 which all were at par . The lowest growth was sustained in distilled water on day 5, 100 mM NaCl on day 5 and 500 mM NaCl on day 5 which all were at par.

Table III-10A : ANOVA for the effect of distilled water (control), 50 mM NaCl, 100 mM NaCl and 500 mM saline in combination with cytokinin 1 ppm & 2 ppm and auxin 1 ppm & 2 ppm on the root length of G.max, sprayed twice with the hormone, once on day 1 and the other on day 5.

Source	Df	SS	MS	F
TOT	191	1491.816667	7.810558	1.7313
Trt	31	769.976667	24.837957	5.5055
Err	160	721.840000	4.511500	1.0000

Table III-10B : ANOVA for specific effects of treatments, duration of germination and levels of specific hormones and interactions between them;

S	Df	SS	MSS	F
S	3	164.668750	54.889583	12.1666
H	3	33.946250	11.315417	2.5081
D	1	395.026875	395.026875	87.5600
sh	9	16.945000	1.882778	0.4173
hd	3	16.691042	5.563681	1.2332
sd	3	109.549375	36.516458	8.0941
shd	9	33.149375	3.683264	0.8164
Err	160	721.840000	4.511500	

C.V. (Treatment) :: 20.00%

s - Treatment (distilled water control) 50 mM NaCl, 10 mM NaCl, 500 mM NaCl in combination with phytohormome

h - hormone – cytokinin and auxin (1 ppm & 2 ppm)

d - duration of germination on 5 day and 8 day

sh - interaction between treatment × hormone

hd - interaction between hormone and duration of germination

sd - interaction between treatment and days

shd - interaction between treatment × hormone × days

4 Way ANOVA of root : Treatment , hormone, frequency, duration and their interactions

ANOVA for the effect of different treatments – distilled water (control), 50 mM NaCl, 100 mM NaCl and 500 mM NaCl in combination with cytokinin & auxin on the growth of root of *G.max* is presented in **table III-11A**. A highly significant difference ($p < 0.01$) was observed between the treatments.

The specific effect of treatments, hormones, duration of germination, frequency of hormone spray and all their interaction are presented in **table III-11-B**. Highly significant differences ($p < 0.01$) were observed between treatments, between the levels of hormones, frequency of hormone spray and the duration of germination on the growth of the root of *Glyine max*. All other interaction that is treatment x levels of hormones, treatment X frequency of hormone spray levels of hormones and frequency of spray, the levels of hormones and the duration of germination, frequency of spray of hormone and duration of germination, interactions between treatment x hormone x frequency, treatment x hormone x duration of germination, treatment x frequency of spray of hormone x duration of germination, level of hormone x frequency of spray of hormones x duration of germination, treatment x hormone level x frequency of hormone spray x duration of germination were all non significant.

Table III-11A : ANOVA for the specific effect of different treatments in different salinity level in combination with cytokinin & Auxin on the growth of root of *G.max* .

Source	Df	SS	MS	F
TOT	383	3183.502891	8.312018	2.0193
Trt	63	1886.864557	29.950231	7.2760
Err	315	1296.638333	4.116312	1.0000

Table III-11B : Specific effects of treatments, duration of germination and levels of hormone spray and all the interactions on growth of root of *Glycin max.*

S	Df	SS	MSS	F
S	3	512.786745	170.928915	41.5248
H	3	107.950078	35.983359	8.7416
F	1	59.456276	59.456276	14.4441
D	1	798.971901	798.971901	194.0990
sh	9	50.333151	5.592572	1.3586
Sf	3	25.113411	8.371137	2.0336
sd	3	243.323203	81.107734	19.7040
hf	3	6.926328	2.308776	0.5609
hd	3	15.162370	5.054123	1.2278
fd	1	0.025026	0.025026	0.0061
shf	9	6.646068	0.738452	0.1794
shd	9	20.101276	2.233475	0.5426
sfd	3	6.150078	2.050026	0.4980
hfd	3	12.944661	4.314887	1.0482
shfd	9	20.973984	2.330443	0.5661
Err	315	1296.638333	4.116312	

C.V. (Treatment) :: 20.00%

S - Treatment (distilled water control) 50 mM NaCl, 1 00mM NaCl, 500 mM NaCl in combination with phytohormome

h - hormone – cytokinin and auxin (1 ppm & 2 ppm)

d - duration of germination 5 day and 8 day

sh - interaction between treatment × hormone

hd - interaction between hormone and duration of germination

sd - interaction between treatment and days

shd - interaction between treatment × hormone × days

f - frequency of hormone spray

Enzyme activity-

Mg^{++} ATPase activity

The Activity of the enzyme with mean values and standard error of the germinated *G. max* on day 1, day 3, day 5 and day 8 in distilled water (control) , 10 mM NaCl and 20 mM saline is presented in **table V-1A.**

Statistic analysis for significant differences in the enzyme activity between the intervals was performed both by 't' test and analysis of variance for a completely randomized design using two way classification .

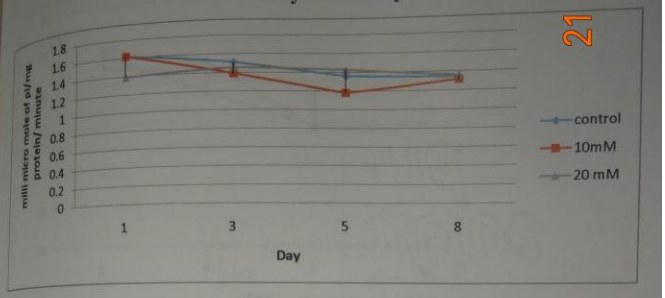
The salient observations were a high variance with in the replicates and so also the coefficient of variation. When t test was performed ,obviously this statistical method did not take in account other variants except the variations with in replicates . **Table V-1B** presents the mean activity of Mg^{+2}ATPase on day 1,3,5, 8 in distilled water (s1) 10 mM (s2) and 20 mM NaCl (s3) **table V-1D** presents the ANOVA for the effect of treatment , days and interaction between treatment x days .

Table V-1C presents the ANOVA for statistical analysis for the differences between treatments, between days , interaction between days and interaction between treatments ,between days. Method of critical difference was used to calculate the significant differences .

Significant differences were observed between days only.The activity was highest and at par on day 1.Activity was lowest and at par on day 3 and day 5.

Figure-11

Enzyme Activity



Mg^{2+}ATPase activity in germinating *Glycine max* seed from Day 1 to day 8

Ca^{2+}ATPase activity in germinating *Glycine max* seed from Day 1 to day 8

$Na^{+}K^{+}$ activity in germinating *Glycine max* seed from Day 1 to day 8

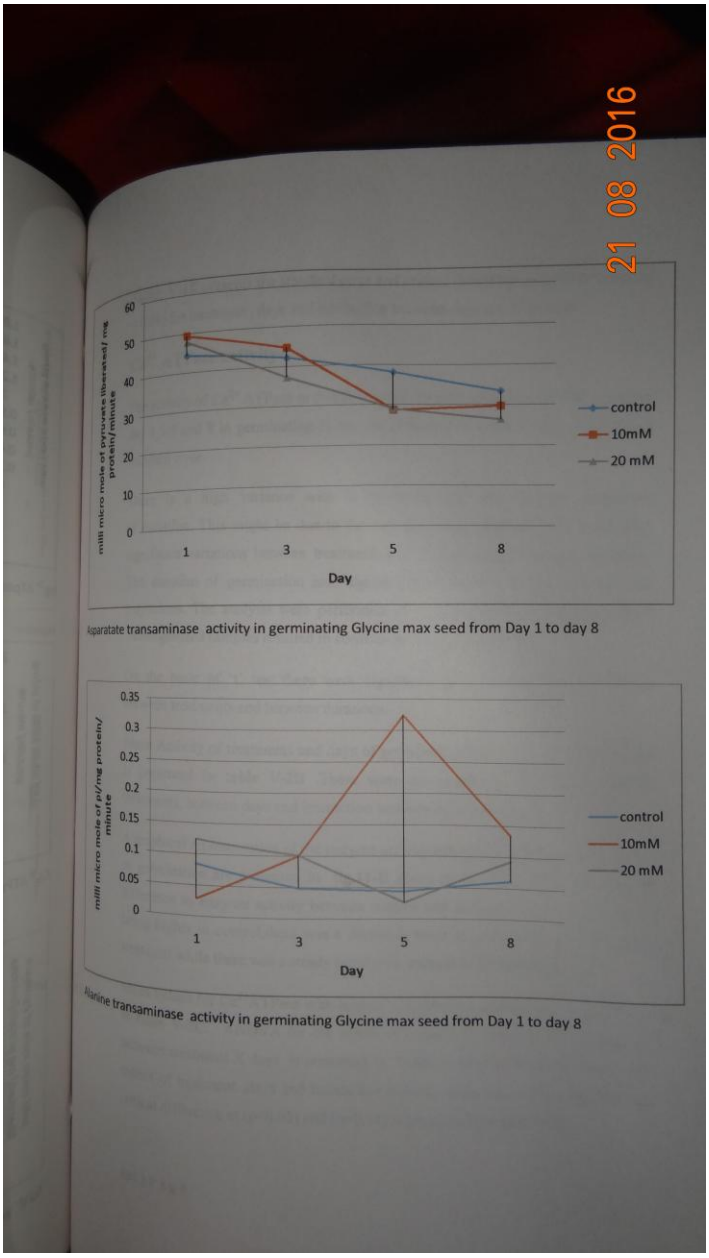


Table V-1E presents the standard error and critical difference at ($p<0.05$) and ($p < 0.01$) for treatment , days and interaction between days x treatment .

Table V-1A

Effect Of Salinity On Mg^{2+}-ATPase				
Salinity	DAY 1	DAY 3	DAY 5	DAY 8
Control	1.62 ± 0.10	1.51 ± 0.06	1.36 ± 0.03	1.34 ± 0.07
10 mM NaCl	1.62 ± 0.08	1.38 ± 0.04	1.18 ± 0.02	1.31 ± 0.09
20 mM NaCl	1.39 ± 0.06	1.45 ± 0.03	1.42 ± 0.05	1.35 ± 0.06

Activity expressed as micromole of pi/mg protein/min

Table V-1B

Mean Activity of treatments and days of germination for Mg^{2+} ATPase in *G.max*

	D1	D2	D3	D4
S1	1.6150	1.5067	1.3567	1.3367
S2	1.6133	1.3850	1.1750	1.3083
S3	1.3867	1.4517	1.4217	1.3550

S1- Distilled water D1 - Day 1
S2-10 mM NaCl D2 - Day 3
S3 -20 mM NaCl D3 - Day 5
D4 - Day 8

Table V-1C

ANOVA for Mg^{2+} ATPase in distilled water ,10 mM and 20 mM saline , between treatment among days

Source	Df	SS	MS	F
TOT	71	2.611865	0.036787	1.4001
Trt	11	1.035349	0.094123	3.5822 0.370 NS
Err	60	1.576517	0.026275	1.0000

Table V-1D

ANOVA for Mg^{2+} ATPase in distilled water ,10 mM and 20 mM saline ,significance of treatment ,days and interaction between treatment x days

Source	Df	SS	MS	F
S	2	0.084444	0.042222	1.6069 0.276 NS
D	3	0.580993	0.193664	7.3706 0.019 *
SD	6	0.369911	0.061652	2.3464 0.162 NS
Err	60	1.576517	0.026275	1.0000

Table V-1E
Standard error ,mean and critical difference at $p < 0.05$ and $p < 0.01$ level

	SED	CD(0.05)	CD(0.01)
S	0.04679	0.09360	0.12449
D	0.05403	0.10808	0.14375
SD	0.09359	0.18720	0.24898

S- Treatment
D- Days

SD – Interaction between treatment & days

The trend of the enzyme activity between treatments and intervals is presented in **fig.11-A.**Control and 10 mM treatments had higher initial activity . There was a trend of decline in activity with intervals specially with 10 mM saline treatment with all levels on day 8 .

Ca^{++} ATPase activity-

The activity of Ca^{2+} ATPase in distilled water, 10 mM saline and 20 mM saline on day 1,3,5 and 8 in germinating *G. max* is presented **in table V -2A** as mean and standard error.

There is a high variance with in replicates and also a high coefficient of variation. This might be due to the fact that the germinated seed had highly significant variations between treatments and among root, shoot ,cotyledon. The duration of germination also significantly varied among root, shoot and cotyledons. The analysis were performed on pooled cotyledons, root and shoot .Homogenized samples resulted in confounded enzyme activities

On the basis of 't' test there were significant to non significant differences between treatments and between durations. Mean Activity of treatments and days of germination for Ca^{2+} ATPase in *G.max* is presented in **table V-2B** .There were no significant variations between treatments, between days and interaction between days and between treatments.

A graphical representation of the enzyme activity between treatments and duration of germination are presented in **fig.11-B** .Even at the initial level there was a difference in enzyme activity between control and 20 mM saline . The activity being higher in control,there was a declining trend in control and 10 mM saline treatment while there was a steady trend with interval in 20 mM treatment .

Mean values for Ca^{2+}ATPase with respect to treatments and intervals is presented in **table V-2C.**

ANOVA for the effect of treatments, intervals and interaction between treatment X days is presented in **Table V-2D**.

The standard error of the means of treatment ,days and interaction between treatment x days and also the critical difference at ($p<0.05$) and ($p<0.01$) is presented in **table V-2E** .

Table V-2A

Effect Of Salinity On Ca^{2+}-ATPase				
Salinity	DAY 1	DAY 3	DAY 5	DAY 8
Control	2.09±.18	1.72 ± .08	1.64 ± 0.09	1.38 ± 0.08
10 mM NaCl	1.78 ± 0.06	1.56 ± 0.04	1.44 ± 0.06	1.37 ± 0.08
20 mM NaCl	1.58 ± 0.06	1.62 ± 0.07	1.55 ± 0.03	1.58 ± 0.06

Activity expressed as micromole of pi/mg protein/min

Table V-2B

Mean Activity of treatments and days of germination for Ca^{2+} ATPase in G.max

	D1	D2	D3	D4
S1	2.0005	1.7400	1.6473	1 .4640
S2	1.6790	1.5825	1.3990	1.4398
S3	1.6363	1.5412	1.5597	1.4152

S1- Distilled water
S2-10 mM NaCl
S3 -20 mM NaCl

D1 - Day 1
D2 - Day 3
D3 - Day 5
D4 - Day 8

Table V-2C

ANOVA for Ca^{2+} ATPase in distilled water ,10 mM and 20 mM saline , between treatment among days;

Source	Df	SS	MS	F
TOT	71	7.722749	0.108771	1.1168
Trt	11	1.878899	0.170809	1.7537 0.431 NS
Err	60	5.843850	0.097397	1.0000

Table V-2D

ANOVA for Ca^{2+} ATPase in distilled water ,10 mM and 20 mM saline ,significance of treatment ,days and interaction between treatment x days

Source	Df	SS	MS	F	
S	2	0.528378	0.264189	2.7125	0.145 NS
D	3	1.073709	0.357903	3.6747	0.082 NS
SD	6	0.276812	0.046135	0.4737	0.807 NS
Err	60	5.843850	0.097397	1.0000	

Table V-2E

Standard error, mean and critical difference at $p < 0.05$ and $p<0.01$ level.

	SED	CD(0.05)	CD(0.01)
S	0.09009	0.18021	0.23968
D	0.10403	0.20809	0.27676
SD	0.18018	0.36042	0.47936

Na^+-K^+ ATPase -

The activity of **Na^+-K^+ ATPase** in control, 10 mM and 20 mM saline on day 1,3,5, and 8 is presented in **table V -3A**.The values are presented as mean and the standard error. The variance with in the replicates is high. The activity of Na^+-K^+transport ATPase was determined by substracting the value of Mg^{2+} dependent ATPase from mg^{2+} Na^+-K^+ ATPase after subjecting the $mg2^+$ Na^+-K^+ to digoxin. The mean value for the activity of Mg^{++} Na^+ K^+ with respect to treatments , intervals & interaction between treatment X intervals is presented in **table V-3B.**

Cardiac glycoside which specifically inhibits Na^+-K^+ transport ATPase, however analysis of variance was performed on pooled value of Mg^+-Na^+-K^+ ATPase. No significant differences were found between treatment, between intervals and interaction between treatments and intervals. ANOVA is presented in **table V-3C.**

ANOVA for the effect of treatments intervals and interactions between treatment intervals is presented in **table V-3D**.

The standard error of the means for treatments, days and interaction between treatment x days and the critical difference for the parameter are presented in **table V-3E .**

Table V-3A

Effect Of Salinity On Na^+ -K^+ ATPase				
Salinity	DAY 1	DAY 3	DAY 5	DAY 8
Control	0.08 ± 0.05	0.04 ± 0.06	0.04 ± 0.09	0.06 ± 0.02
10 mM NaCl	0.02 ± 0.05	0.10 ± 0.07	0.32 ± 0.08	0.13 ± 0.02
20 mM NaCl	0.12 ± 0.05	0.10 ± 0.03	0.02 ± 0.06	0.09 ± 0.03

Activity expressed as micromole of pi/mg protein/min

Table V-3B

Mean Activity of treatments and days of germination for Mg^{2+} dependent Na^+K^+ ATPase in G.max

	D1	D2	D3	D4
S1	1.7052	1.5613	1.4057	1.4025
S2	1.6495	1.4905	1.5052	1.4477
S3	1.5153	1.5585	1.4560	1.4517

S1- Distilled water
S2-10 mM NaCl
S3 -20 mM NaCl

D1 - Day 1
D2 - Day 3
D3 - Day 5
D4 - Day 8

Table V-3C

ANOVA for Mg^{2+} dependent $Na^{+}K^{+}$ ATPase in distilled water ,10 mM and 20 mM saline , between treatment among days

Source	Df	SS	MS	F
TOT	71	2.378670	0.033502	1.1134
Trt	11	0.573273	0.052116	1.7320 0.260 NS
Err	60	1.805396	0.030090	1.0000

Table V-3D

ANOVA for Mg^{2+} dependent $Na^{+}K^{+}$ ATPase in distilled water ,10 mM and 20 mM saline ,significance of treatment ,days and interaction between treatment x days

Source	Df	SS	MS	F
S	2	0.010703	0.005351	0.1778 0.841 NS
D	3	0.401053	0.133684	4.4428 0.057 NS
SD	6	0.161517	0.026920	0.8946 0.552 NS
Err	60	1.805396	0.030090	1.0000

Table V-3E

Standard error ,mean and critical difference at $p < 0.05$ and $p<0.01$ level

	SED	CD(0.05)	CD(0.01)
S	0.05007	0.10017	0.13322
D	0.05782	0.11566	0.15383
SD	0.10015	0.20033	0.26644

S- Treatment
D- Days
SD – Interaction between treatment & days

The trend of changes in the activity of the enzyme Na^+-K^+ATPase in seeds germinated in distill water, 10mM NaCl and 20 mM during different intervals are presented in **fig 11-C** , though, there was a declining trend in the activity in control and 20 mM saline, it is difficult to explain the sudden rise in the activity on day 5 in 10 mM .

Enzyme AST (alanine aminotransferase)-

The mean activity of AST with respect to treatment, days and interactions between treatment x days is presented in **table V-4A**. The activity of the enzyme AST in the seeds of *Glycine max* germinated in distilled water (control), 10 mM NaCl and 20 mM sodium chloride on day 1, 3, 5 and 8 is presented as mean and standard error (SE) in **table V-4B .** Intra and inter variance in the enzyme activity was calculated by 't' test using pooled SE method .Some significant difference were observed in the enzyme activity.

Analysis of variance were performed using 2 way classification, mean comparison was done using least square difference and is presented in **table V-4C**. No significant difference were observed, between treatments , between days, and interaction between treatments and days. Moreover the coefficient of variance is extremely high (36.73%).

ANOVA for the specific effect of treatments, days and interaction between days is presented in **table V-4D** .

The standard error of the mean, critical difference at ($p<0.05$) and ($p<0.01$) is presented in **table V-4E.**

Table V-4A

Effect Of Salinity On Aspartate Transaminase (AST)				
Salinity	DAY 1	DAY 3	DAY 5	DAY 8
Control	39.6 ± 4.71	36.85 ± 0.92	31.96 ± 2.50	29.54 ± 1.56
10 mM NaCl	40.86 ± 1.01	41.23 ± 4.29	26.15 ± 2.21	24.35 ± 0.95
20 mM NaCl	41.47 ± 2.77	31.73 ± 3.08	25.15 ± 1.56	24.06 ± 1.46

Activity expressed as micromole of pyruvate liberated /mg protein/min

Table V-4B: Mean Activity of treatments and days of germination for AST(aspartate aminotransferase) in G.max

	D1	D2	D3	D4
S1	22.8400	22.8483	30.9500	36.4133
S2	35.2500	36.0267	38.0517	35.3017
S3	27.5983	27.4400	33.0517	33.7900

S1- Distilled water D1 - Day 1

S2-10 mM NaCl D2 - Day 3

S3 -20 mM NaCl D3 - Day 5

D4 - Day 8

Table V-4C: ANOVA for AST(aspartate aminotransferase) distilled water ,10 mM and 20 mM saline , between treatment among days

Source	Df	SS	MS	F
TOT	71	8540.951299	120.295089	1.0759
Trt	11	1832.253815	166.568529	1.4897 0.947 NS
Err	60	6708.697483	111.811625	1.0000

Table V-4D: ANOVA for aspartate aminotransferase in distilled water ,10 mM and 20 mM saline ,significance of treatment ,days and interaction between treatment x days

Source	Df	SS	MS	F
S	2	796.346553	398.173276	3.5611 0.096 NS
D	3	644.385726	214.795242	1.9210 0.227 NS
SD	6	391.521536	65.253589	0.5836 0.735 NS
Err	60	6708.697483	111.811625	1.0000

Table V-4E:

Standard error, mean and critical difference at $p < 0.05$ and $p<0.01$ level

	SED	CD(0.05)	CD(0.01)
S	3.05248	6.10589	8.12082
D	3.52470	7.05048	9.37711
SD	6.10496	12.21178	16.24164

S- Treatment

D- Days

SD – Interaction between treatment & days

A graphical representation of the the trend in the changes of AST activity between treatment and between intervals is presented in **fig 11-D**.

There is an overall trend of decline in the enzyme activity during intervals, though there is steadier activity in control at different intervals. The initial activity was almost the same on day 0 in all treatments however, the decline was more with the duration of intervals in 20 mM NaCl**.**

Enzyme ALT (aspartate aminotransferase)

The activity of the enzyme ALT in germinated seeds of *Glycine max* in distilled water ,10 mM NaCl and 20 mM NaCl on days 1, 3, 5 and 8 are presented as mean ± SE in **table V-5A**.The differences between the treatments on individual basis was calculated by t test using pooled standard error method between two groups. There were significant differences between intervals.

Analysis of variance using two way classification was performed to determine the difference between treatments, and between intervals and interaction between treatment and interval is presented in **table V-5C**. There are highly significant differences ($p< 0.01$).

In the interactions between treatment and days however no significant differences was observed between treatments between days. The coefficient of variation was 17.0%. A mean comparison

of the treatments using LSD revealed that day 1 activity was significantly higher as compared to day 3, day 5 and day 8. The activity on day 3 was significantly higher as compared to day 5, day 8. , however the activity on day 5 and day 8 were at par and exhibited poorest activity.

The trend of the activity of enzyme ALT between different treatment and different interval are presented in **fig. 11-E.** Although, the initial activities were the same there was an over all decline in the activity with the duration of germination. The decline in the activity of the control was steadier and steeper in 20 mM NaCl. **Table V-5A** depicts effect of salinity on ALT.

The mean of the ALT with respect to treatments, intervals are presented in **table V-5B.Table V-5C** depicts ANOVA for alanine aminotransferase in distilled water, 10 mM and 20 mM saline, between treatment among days.

ANOVA for the effect of treatment, days and interaction between days x treatment is presented in **table V-5D**. The critical difference at $p<0.05$ and $p<0.01$, SE of the means of treatments, days and interaction between treatment x days is presented in **table V-5E**.

Table V-5A

Effect Of Salinity On Alanine Transaminase (ALT)				
Salinity	DAY 1	DAY 3	DAY 5	DAY 8
Control	44.4 ± 4.25	42.22 ± 1.79	38.17 ± 2.7	33.48 ± 1.81
10 mM NaCl	49.46 ± 2.16	44.74 ± 4.52	28.72 ± 1.83	29.77 ± 2.22
20 mM NaCl	47.89± 2.80	37.30 ± 3.34	29.06 ± 1.52	26.44 ± 0.64

Activity expressed as micromole of pyruvate liberated /mg protein/min

Table V-5B

Mean Activity of treatments and days of germination for ALT(alanine aminotransferase**)** in *G.max*

	D1	D2	D3	D4

S1	44.4000	42.2250	38.1750	33.4850
S2	49.4667	44.7450	28.7250	29.7717
S3	47.8967	37.3017	29.0650	26.4450

S1- Distilled water
S2-10 mM NaCl
S3 -20 mM NaCl

Table V-5C: ANOVA for ALT(alanine aminotransferase**)** in distilled water, 10 mM and 20 mM saline, between treatment among days

Source	Df	SS	MS	F
TOT	71	6940.846665	97.758404	2.2389
Trt	11	4321.087982	392.826180	8.9968 0.557 NS
Err	60	2619.758683	43.662645	1.0000

Table V-5D
ANOVA for alanine aminotransferase in distilled water ,10 mM and 20 mM saline ,significance of treatment ,days and interaction between treatment x days

Source	Df	SS	MS	F
S	2	242.019211	121.009606	2.7715 0.140 NS
D	3	3574.722249	1191.574083	27.2905 0.001 **
SD	6	504.346522	84.057754	1.9252 0.223 NS
Err	60	2619.758683	43.662645	1.0000

Table V-5E

Standard error ,mean and critical difference at $p < 0.05$ and $p<0.01$ level.

	SED	CD(0.05)	CD(0.01)
S	1.90750	3.81558	5.07471
D	2.20259	4.40585	5.85977
SD	3.81500	7.63116	10.14942

S- Treatment D- Days SD – Interaction between treatment & days

Owing to a very high standard of all the enzymes studied, it was a presumption that there could be a significant difference in the levels of all the enzymes with respect to replicate factor. In order to verify whether it could be due to variations in the activity of the enzymes with respect to replicates , two way classification for the analysis was performed , the specific effect of between days, among replicates between days, specific effect due to treatments, replicates and treatment x replicate was performed by ANOVA for the enzymes .No significant differences were observed between days and among replicates .

Table V-6A presents the analysis of variance between replicates .The specific effect of treatment, replicates and interaction between treatment x replicates is presented in **table V-6B** . All the factors were non significant .

ANOVA for Ca^{2+} dependent ATPase with respect to days and among replicates is presented in **table V-7A**. No significant difference were observed due to days and among replicates between days.

The specific effect of treatment, replicates and interaction between treatment x replicates is presented in **table V-7**B

Table V-6A :

ANOVA for the enzyme Mg^{+2} ATPase for the effect of days and replicates

SOURCE	DF	SS	MS	F
Tot	71	2.614638	0.036826	1.1925
Between days	3	0.583570	0.194523	6.2989 ns
Between replicates	17	0.456090	0.026829	0.8688 ns
Err	51	1.574977	0.030882	1.0000

Table V-6B:

ANOVA for the specific effect of treatment, replicates and interaction between treatment x replicates

SOURCE	DF	SS	MS	F
S	2	0.082951	0.041476	1.3430 ns
R	5	0.155855	0.031171	1.0094 ns
SR	10	0.217284	0.021728	0.7036 ns
Err	51	1.574977	0.030882	1.0000

S – Treatment R- Replicates SR – Interaction between treatment x replicates

Table V-7A:

ANOVA for the enzyme Ca^{+2} ATPase for the effect of days and replicates

SOURCE	DF	SS	MS	F
Tot	71	5.158846	0.072660	1.4566
Between days	3	1.345963	0.448654	8.9942
Between replicates	17	1.268875	0.074640	1.4963 ns
Err	51	2.544009	0.049883	1.0000

Table V-7B :

ANOVA for the specific effect of treatment ,replicates and interaction between treatment x replicates

SOURCE	DF	SS	MS	F
S	2	0.359820	0.179910	3.6067*
R	5	0.109574	0.021915	0.4393 ns
SR	10	0.799481	0.079948	1.6027 ns
Err	51	2.544009	0.049883	1.0000

*p < 0.05

S – Treatment R- Replicates SR – Interaction between treatment x replicates

Significant differences ($p<0.01$) were observed between the treatments . A mean comparison by LSD revealed that activity in distilled water and 20 mM NaCl were at par, but significantly

different than 10 mM and 20 mM NaCl, which were at par . The activity in distilled water and 20 mM were higher .

The specific effect of treatment, replicates and interaction between treatment x replicates were not significant. Analysis of variance for the effect of days x among replicates between days for Mg^{2+} dependent Na^{+}-K^{+} ATPase is presented in **table V-8A**. No significant differences were observed either for the days or among replicates, between days.

The specific effect of treatments, replicates and interaction between treatment x days is presented in **table V-8B.** No significant difference was observed for any of the factor studied .Analysis of variance for the factors ,days and among replicates for the enzyme AST is presented in **tabe V-9A .** No significant difference was observed for either of the factor studied .

The analysis of variance for the specific effect of treatment ,replicates and interaction between treatment x replicate are presented in **table V-9B .** None of the factors were significant.

The factors of days and among replicates between days is presented in **table V-10A**.For the enzyme ALT none of the factors were significant .

The ANOVA for the specific effect of treatments, replicates and interaction between treatment x replicates is presented in **table V-10B.** All the factors were non significant .

Table V-8A :

ANOVA for Mg^{2+}+ Na^{+} + K^{+} for the affect of days and replicates.

SOURCE	DF	SS	MS	F
Tot	71	0.984330	0.013864	0.9970
Between replicates	17	0.233446	0.013732	0.9875 ns
Err	54	0.750884	0.013905	1.0000

Table V-8B :

ANOVA for the specific effect of treatment ,replicates and interaction between treatment x replicates

SOURCE	DF	SS	MS	F
S	2	0.074428	0.037214	2.6763 ns
R	5	0.073059	0.014612	1.0508 ns
SR	10	0.085959	0.008596	0.6182 ns
Err	54	0.750884	0.013905	1.0000

Table V-9A :

ANOVA for AST for the affect of days and replicates

SOURCE	DF	SS	MS	F
Tot	71	5587.337388	78.694893	2.1402

Between days	3	2661.680149	887.226716	24.1294
Between replicates	17	1050.414713	61.789101	1.6804 ns
Err	51	1875.242526	36.769461	1.0000

Table V-9B :

ANOVA for the specific effect of treatment ,replicates and interaction between treatment x replicates

SOURCE	DF	SS	MS	F
S	2	186.669700	93.334850	2.5384 ns
R	5	189.740679	37.948136	1.0321 ns
SR	10	674.004333	67.400433	1.8331 ns
Err	51	1875.242526	36.769461	1.0000

Table V-10A :

ANOVA for ALT for the affect of days and replicates

SOURCE	DF	SS	MS	F

Tot	71	6940.846665	97.758404	2.1239
Between days	3	3574.722249	1191.574083	25.8887 ns
Between replicates	17	1018.757490	59.926911	1.3020 ns
Err	51	2347.366926	46.026802	1.0000

Table V-10B :

ANOVA for the specific effect of treatment ,replicates and interaction between treatment x replicates

SOURCE	DF	SS	MS	F
S	2	242.019211	121.009606	2.6291 ns
R	5	180.285324	6.057065	0.7834 ns
SR	10	596.452956	59.645296	1.2959 ns
Err	51	2347.366926	46.026802	1.0000

S – Treatment
R- Replicates
SR – Interaction between treatment x replicates

Ultraviolet spectrophotometry of seeds germinated in distilled water, 10 mM NaCl and 20 mM NaCl on day 5 and day 8 were performed. Absorption maxima was studied from 240 to 300 mμ. Experimental UV absorption data was studied for the cotyledons, root and shoot of *G. max* .

Biochemical transformation occurs during the process of seed germination. The metabolic activities of nucleic acids and proteins are at its zenith. Besides these two main components, other metabolic compounds may have a major role to play. In this investigation, it was postulated that DNA transformation could be roughly approximated from its absorption maxima (λ max) at 260 mμ and the metabolic events of proteins at 280 mμ (λ max of proteins).No attempts were made to purify the DNA or protein of the cotyledons, roots and shoots of seeds germinated on day 5 and day 8. In an ideally purified samples of DNA and Proteins, the λ max of the DNA and protein are sharp. Moreover, the λ max study of the DNA & proteins gives a rough approximation of the pace of the metabolic activities of these compounds. UV spectra also enables to found out the λ max of other unknown compounds . Further, analytical procedures would predict the chemical nature of the compound.

The UV spectral analysis for the cotyledons, root & shoots grown in distilled water (control) and 10 mM NaCl & 20 mM saline was studied from 240 mμ to 340 mμ . Percentage transmission was recorded for the different wave lengths.

Table IV-1 presents the UV spectra of cotyledons grown in control on day 5. **Table IV-2** is the % transmission of shoots in water on day 5.

The spectral percentage of shoot in control,day 8 is presented in **table IV-3** .

T**able IV-4** presents percentage transmission in UV spectral range for day 8 cotyledon in control.

Table IV-5 presented the % transmission of roots on day 8.

Percentage transmission of cotyledons grown in 10 mM NaCl on day 5 is represented in **table** IV-**6** .

The spectral transmission of shoot grown in 10 mM NaCl on day 5 is presented in **table** IV-**7.**

Percentage transmission of roots grown in 10 mM saline on day 8 is presented in **table** IV-**8.**

Spectral transmission for shoot grown in 10 mM saline on day 8 is presented in **table** IV-**9.**

Spectral transmission for cotyledons grown in 10 mM NaCl on day 8 are presented in **table** IV-**10.**

Table IV-**11** presented the spectral transmission of cotyledons grown in 20 mM NaCl on day 5.

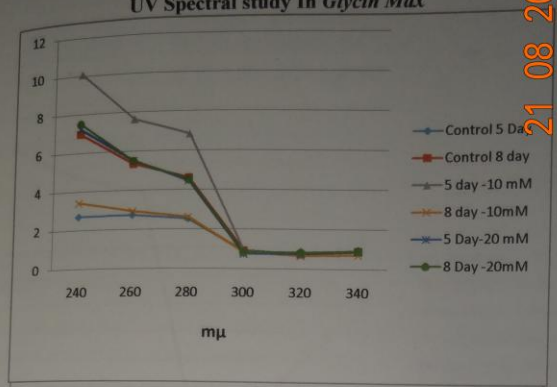
The spectral transmittance of shoot grown in 20 mM saline on day 5 is presented in **table** IV-**12.**

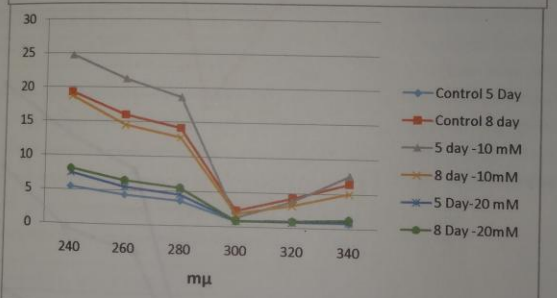
Spectral transmittance of shoot grown in 20 mM NaCl on day 8 is presented in **table** IV-**13.**

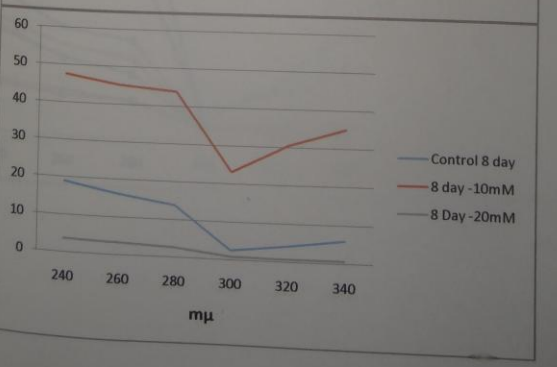
Table IV-14 depicts UV spectra of root 20 mM 8 days root.

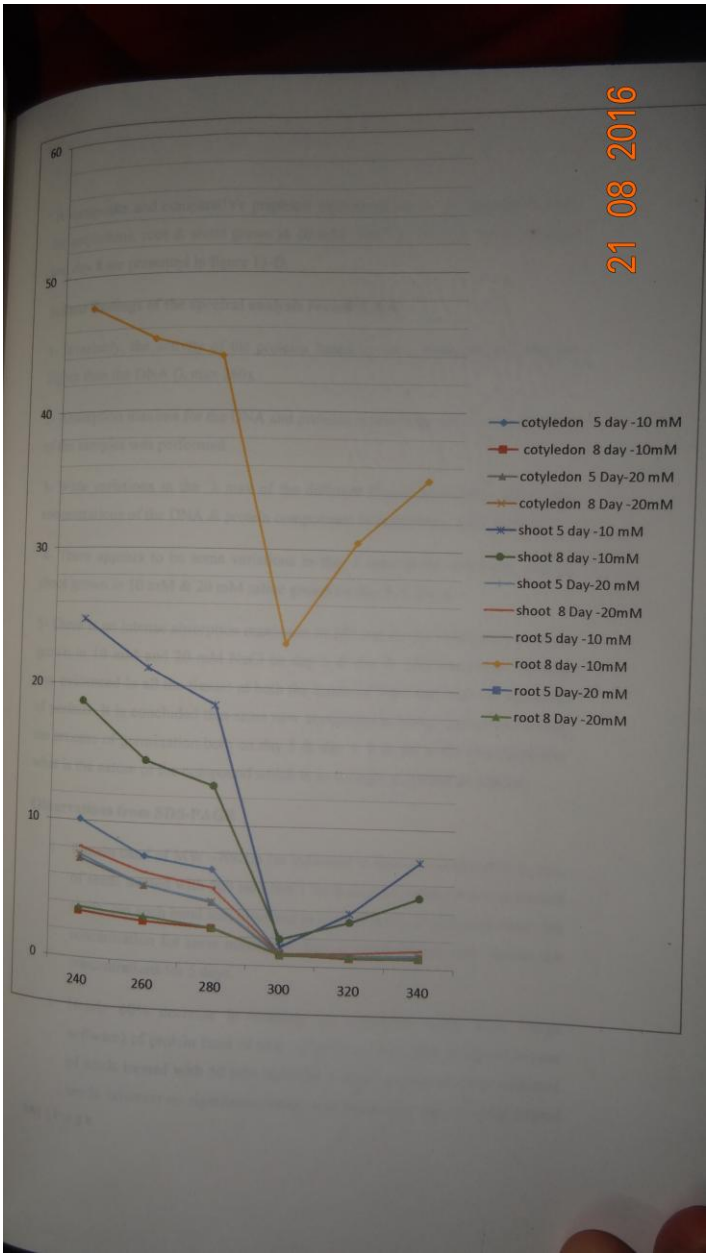
Figure 13

UV Spectral study In *Glycin Max*








21 08 2016
60
50
40
30
20
10
0
240
260
280
300
320
340
cotyledon 5 day -10 mM
cotyledon 8 day -10mM
cotyledon 5 Day-20 mM
cotyledon 8 Day -20mM
shoot 5 day -10 mM
shoot 8 day -10mM
shoot 5 Day-20 mM
shoot 8 Day -20mM
root 5 day -10 mM
root 8 day -10mM
root 5 Day-20 mM
root 8 Day -20mM

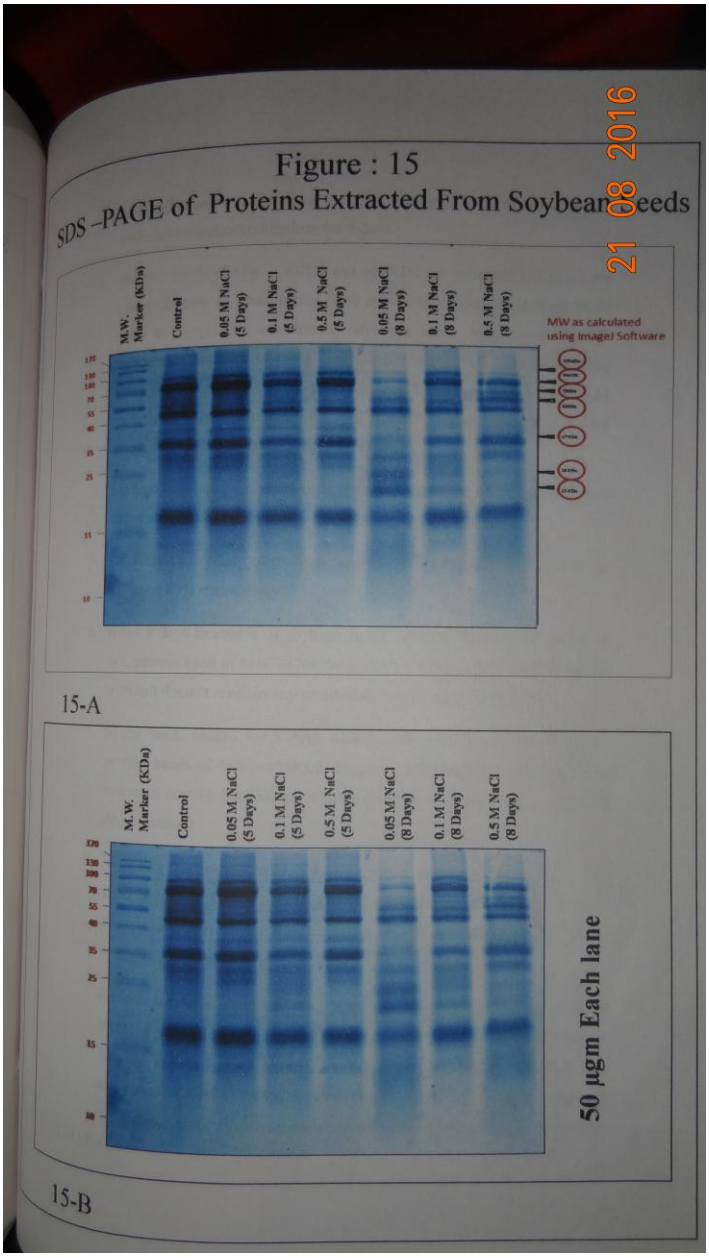


Figure : 15
SDS –PAGE of Proteins Extracted From Soybean Seeds
21 08 2016
M.W. Marker (KDa)
Control
0.05 M NaCl (5 Days)
0.1 M NaCl (5 Days)
0.5 M NaCl (5 Days)
0.05 M NaCl (8 Days)
0.1 M NaCl (8 Days)
0.5 M NaCl (8 Days)
MW as calculated using ImageJ Software
15-A
M.W. Marker (KDa)
Control
0.05 M NaCl (5 Days)
0.1 M NaCl (5 Days)
0.5 M NaCl (5 Days)
0.05 M NaCl (8 Days)
0.1 M NaCl (8 Days)
0.5 M NaCl (8 Days)
50 µgm Each lane
15-B

Table IV-1 CONTROL-5 Day cotyledon

Wavelength	%Transmission
240	2.7
260	2.74
280	2.52
300	0.89
320	0.75
340	0.89

Table IV-2 Control 5 Day Shoot

Wavelength	%Transmission
240	5.47
260	4.39
280	3.64
300	0.82
320	0.85
340	0.79

Table IV-3 Control 8 Days Shoot

Wavelength	%Transmission
240	19.33
260	16.05
280	14.21
300	2.39
320	4.35
340	6.47

Table IV-4 Control 8 Day Cotyledon

Wavelength	%Transmission
240	6.98
260	5.34
280	4.6
300	0.92
320	0.73
340	0.89

Table IV-5 Control 8 Days Root

Wavelength	%Transmission
240	18.97
260	16.18
280	13.83
300	2.52
320	4.15
340	5.96

Table IV-6 10 mM 5 Days Cotyledon

Wavelength	%Transmission
240	10.9
260	7.63
280	6.83
300	0.91
320	0.73
340	0.85

Table IV-7 10 mM 5 DAYS SHOOT

Wavelength	%Transmission
240	24.77
260	21.29

Table IV-8 10 mM 8 DAYS ROOT

Wavelength	%Transmission
240	47.65
260	45.23

280	18.7
300	1.39
320	3.86
340	7.64

280	43.99
300	23.24
320	30.57
340	35.08

Table IV-9 10 mM 8 Days Shoot

Wavelength	%Transmission
240	18.74
260	14.55
280	12.85
300	1.94
320	3.23
340	5.06

Table IV-10 10 mM 8 Days Cotyledon

Wavelength	%Transmission
240	3.41
260	2.92
280	2.6
300	0.84
320	0.66
340	0.71

Table IV-11 20 mM 5 Days Cotyledon

Wavelength	%Transmission
240	7.24
260	5.51
280	4.45
300	0.75
320	0.76
340	0.89

Table IV-12 20 mM 5 Days Shoot

Wavelength	%Transmission
240	7.51
260	5.5
280	4.52
300	0.86
320	0.83
340	0.92

Table IV-13 20 mM 8 Days Shoot

Wavelength	%Transmission
240	8.11
260	6.46

Table IV-14 20 mM 8 Days Root

Wavelenth	%Transmission
240	3.68
260	3.26

280	5.51
300	0.9
320	0.99
340	1.29

280	2.62
300	0.86
320	0.72
340	0.7

A graphical presentation of the absorption spectra of cotyledons grown in 10 mM & 20 mM NaCl on day 5 & day 8 is presented **in figure 13-A.**

The spectral graph for the shoots grown in 10 mM & 20 mM NaCl on day 5 & day 8 is presented in **figure 13-B.**

The spectral analysis of roots grown in 10 mM & 20 mM NaCl on day 5 & day 8 are presented **in figure 13-C.**

A composite and comparative graphical representation of UV spectral analysis for cotyledons, root & shoot grown in 10 mM NaCl & 20 mM NaCl on day 5 and day 8 are presented in **figure 13-D**.

Salient findings of the spectral analysis revealed that

1- Invariably, the activity of the proteins based on the λ max at 280 is slightly higher than the DNA (λ max 260).

2- Absorption maxima for the DNA and proteins is not sharp since no purification of the samples was performed.

3- Wide variations in the λ max of the different tissues is probably due to the concentrations of the DNA & protein components in cotyledons, roots & shoot.

4- There appears to be some variations in the λ max in the cotyledon, root & shoot grown in 10 mM & 20 mM saline grown on day 5 & day 8.

5- There is an intense absorption maximum in 300 mμ for the cotyledons, roots & grown in 10 mM and 20 mM NaCl on day 5 & day 8. This absorption maxima was evidenced in all the tissues at both the lavels of NaCl and both the durations of periods. It is concluded that same new compound is being synthesized during the process of germination both on day 5 & day 8. It is yet to the elucidated that what is the nature of the compound which is so strongly exhibited at 300 mμ

Observations from SDS-PAGE

1. Protein band of MW ~70KDa (as indicated in figure) was observed in case of seeds treated with 500 mM NaCl for 8 days in comparison to untreated seeds. No such band was observed in case of seeds treated with lesser salt concentration for same number of days or seeds treated with various salt concentrations for 5 days.

2. Nearly 60% decrease in intensity (as calculated using Scion Image software) of protein band of MW ~37KDa (as indicated in figure) in case of seeds treated with 50 mM NaCl for 8 days in comparison to untreated seeds, however no significant change was observed in case of seeds treated with higher salt concentrations for same number of days or seeds treated with various salt concentrations for 5 days.

3. Proteins bands of MW ~26KDa and ~21KDa (as indicated in figure) are evident in case of seeds treated for 8 days with various concentrations of NaCl in comparison to untreated soyabean seeds. Approximately 50% decrease in intensity of these bands was observed (as calculated using Scion Image software) with increase in NaCl concentration from 50 mM to 500mM. Also treatment with similar NaCl concentrations for 5 days doesn't result in appearance of these bands.

4. No significant changes in the band intensity of other proteins was observed in the soyabean seeds treated with NaCl for different duration.

5. Decrease in intensity of proteins bands of MW ~26KDa and ~21KDa is 47% and 55% respectively.

6. After 8 days treatment of soybean seeds with various concentrations of salt, protein band of MW ~170KDa disappear. However, 5 days treatment with salt doesn't result in any significant change in band intensity.

7. In the seeds treated for 8 days with 50 mM NaCl and 500mM NaCl, protein bands of MW ~105KDa disappear completely in comparison to untreated control. No change in band intensity was observed in case of 5 days treatment.

8. It appears that protein band of MW ~64KDa increases in intensity in the seeds treated for 8 days with various salt concentrations in comparison to 5 days treatment. However, the change was not found to be significant when image was analyzed using scion image.

Proteins of MW ~94KDa were observed to show reduction in band intensity in case of seeds treated for 8 days with 50mM NaCl and 500mM NaCl in comparison to untreated control. The percentage reduction was calculated to 88% and 44% respectively. However no significant changes were observed in other treatment conditions. For calculation of molecular weight, ImageJ software has been used and protein band intensity has been calculated using Scion Image software. Molecular weight of markers is also indicated. There is effect of NaCl concnetration and duration of NaCl treatment on expression of proteins. The molecular wt markers have also been run in separate lane. There is a observable change in the pattern and intensity of protein bands with molarity and duration of NaCl.

Sr. No.	Sample	Total Protein per gram seed (mg)
1	Control (8 Days)	64
2	0.05N (5 Days)	78
3	0.1N (5 Days)	64
4	0.5N (5 Days)	100
5	0.05N (8 Days)	56
6	0.1N (8 Days)	89
7	0.5N (8 Days)	73

Histology of soybean-

The histological sections of the *Glycine max* seeds in distilled water, 500mM sodium chloride and 100mM sodium chloride were processed & tissue sections were stained with hematoxyline , eosine and 0.25% toluidene blue. .

Plate7

7A shows *Glycine max* seeds (distilled water) germination stained by H&E (4)

7B shows *Glycine max* seeds in distilled water germination ,stained by H&E

7C shows *Glycine max* seeds in distilled water germination, stained by Toludine blue (3)

7D shows *Glycine max* seeds in distilled water germination, stained by Toludine blue (4)

7E shows *Glycine max* seeds in distilled water germination ,stained by Toludine blue (6)

7F shows *Glycine max* seeds in distilled water germination, stained by Toludine blue

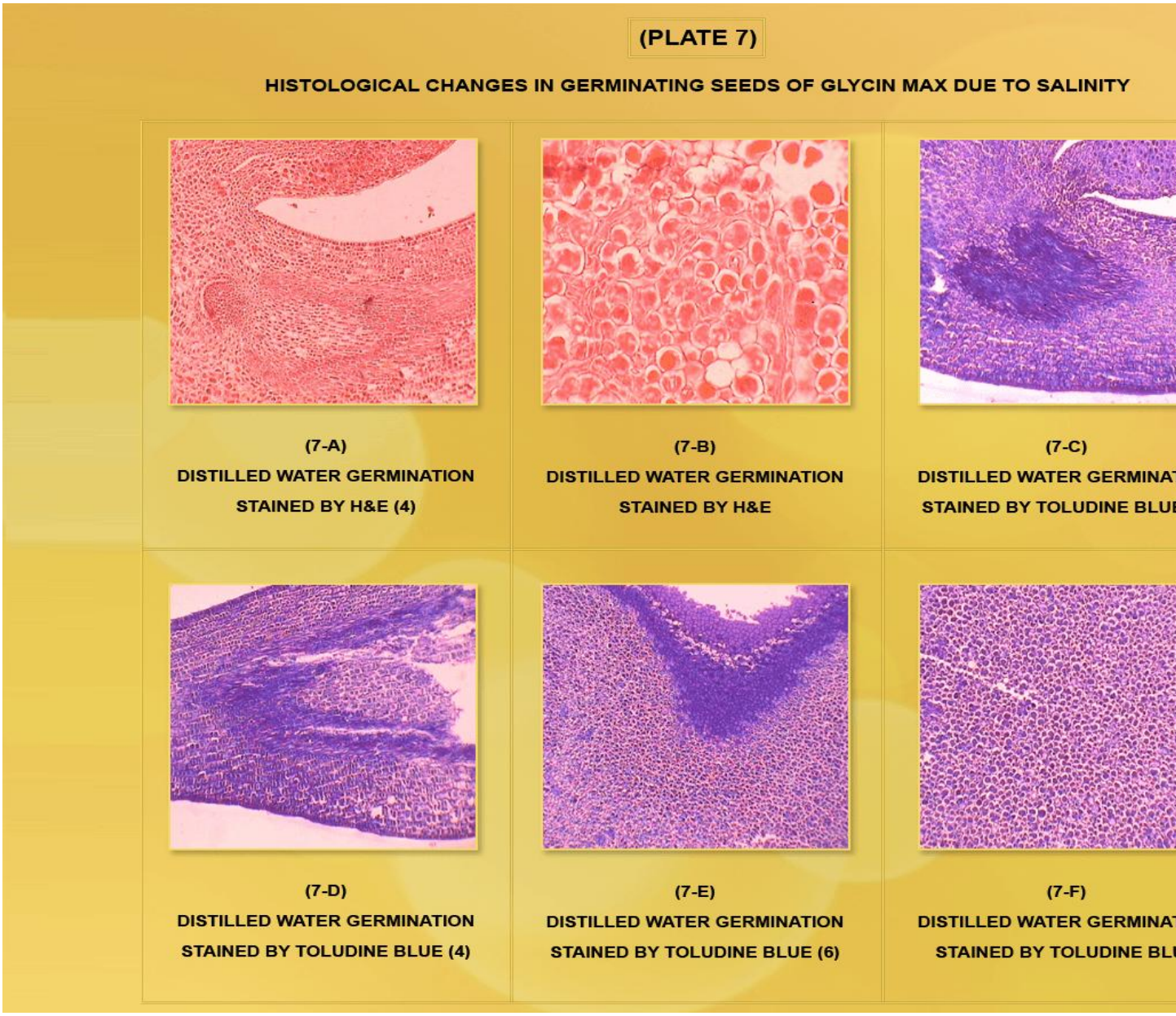


Plate 8

8A shows *Glycine max* seeds in 500 mM sodium chloride, stained by H&E (3)

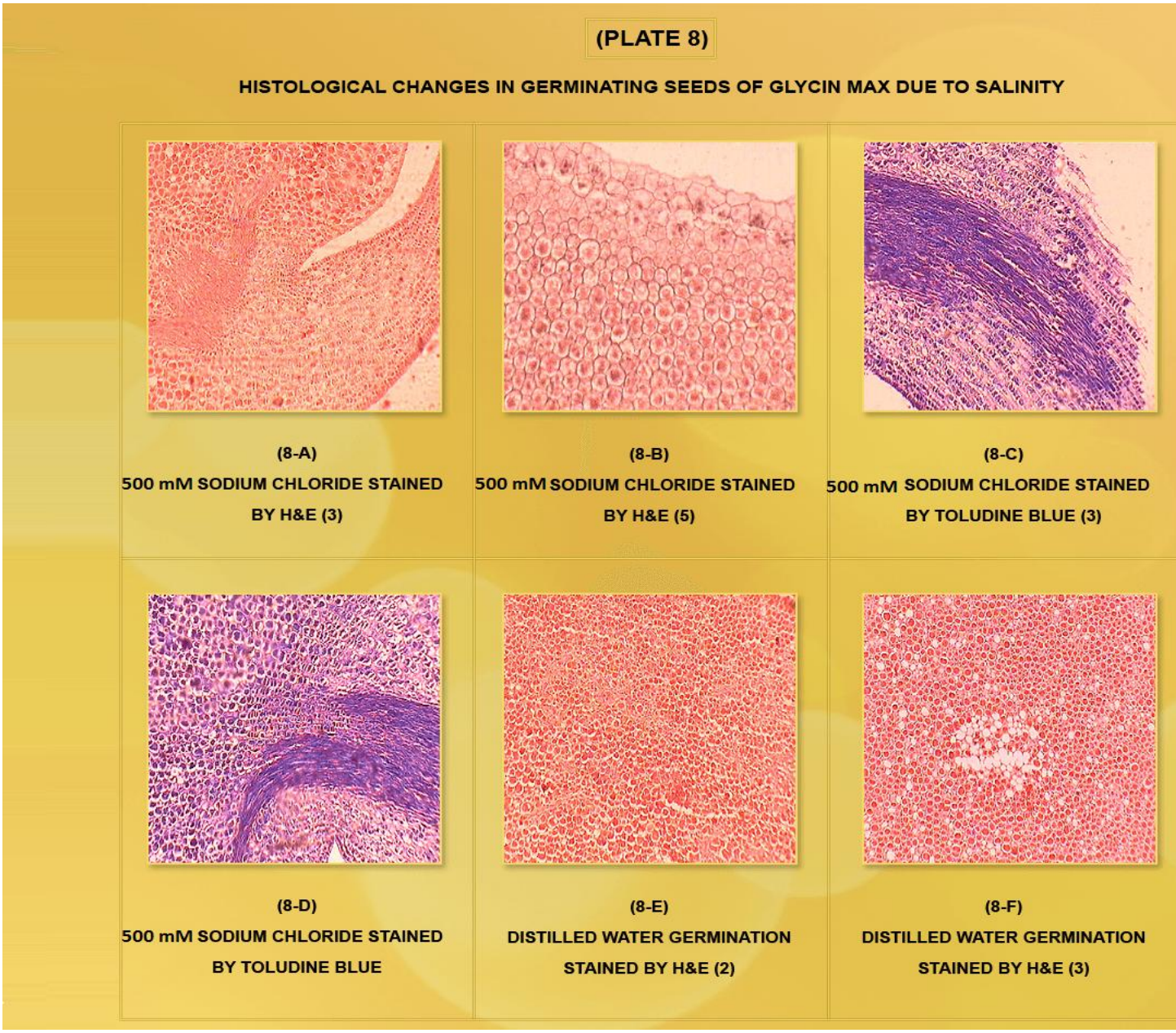
8B shows *Glycine max* seeds in 500 mM sodium chloride ,stained by H&E (5)

8C shows *Glycine max* seeds in 500 mM sodium chloride, stained by Toludine blue (3)

8D shows *Glycine max* seeds 500 mM sodium chloride stained by Toludine blue

8E shows *Glycine max* seeds distilled water germination stained by H&E (2)

8F shows *Glycine max* seeds distilled water germination stained by H&E (3)



Both the methods of staining have their own advantages in deciphering the changes that occur due to saline stress.Sections were observed .The general conclusion is that saline stress resulted in protoplasmic shrinkage & dehydration.

Plate 9

9A shows *Glycine max* seeds germinated in 500mM sodium chloride stained by H&E

9B shows *Glycine max* seeds germinated in 500mM sodium chloride stained by Toludine blue (2)

9C shows *Glycine max* seeds germinated in100mM sodium chloride stained by H&E (2)

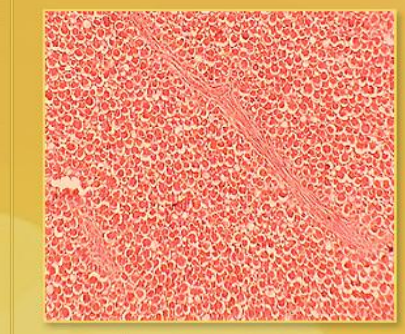
9D shows *Glycine max* seeds germinated in100mM sodium chloride stained by H&E (3)

9E shows *Glycine max* seeds germinated in 100mM sodium chloride stained by H&E (4)

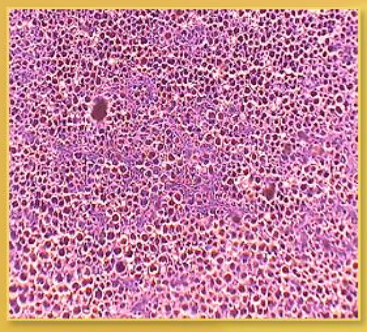
9F shows *Glycine max* seeds germinated in 100mM sodium chloride stained by H&E

(PLATE 9)

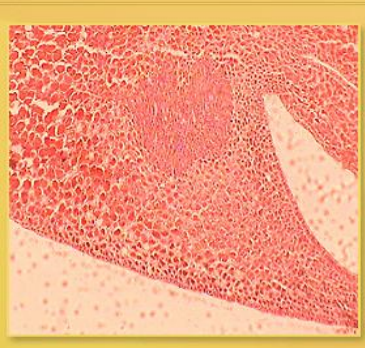
HISTOLOGICAL CHANGES IN GERMINATING SEEDS OF GLYCIN MAX DUE TO SALINITY



(9-A)
500 mM SODIUM CHLORIDE STAINED BY H&E

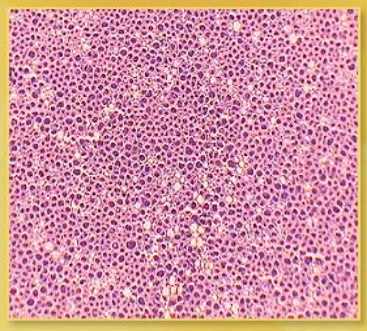


(9-B)
500 mM SODIUM CHLORIDE STAINED BY TOLUDINE BLUE (2)

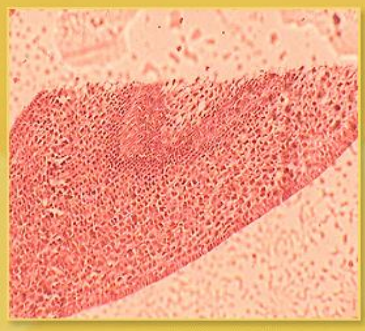


(9-C)
100mM SODIUM CHLORIDE STAINED BY H&E (2)

(9-D)
100 mM SODIUM CHLORIDE STAINED BY H&E (3)



(9-E)
100 mM SODIUM CHLORIDE STAINED BY H&E (4)



(9-F)
100 mM SODIUM CHLORIDE STAINED BY H&E

Field data analysis of G.max and other crops

Soil topography, irrigational facility & climatic factors have profound influence on the production of crops in different Tehsils of Bundi. Maximum area of soybean crop was in Tehsils of K.Patan & Talera. Soybean production varied from year to year. Production was very low in 2003 as compared to 2006. Soybean production was highest in Bundi Tehsil followed by K.Patan & lowest in Nainwa. The average production of Soybean in 2005 was 1306 kg/ha and 1223

kg/ha in 2006 while the target was 1500 to 2000 kg/ha. There is sufficient scope to improve the production of soybean (**Srivastava,2008).**

In Bundi, farmers residing in the upstream of river mej have no facilities for irrigations. Rain fall is scarce. Wells remain depleted. Tube wells are not successful in this area. Government proposes to raise the height of Naya Gaon anicut by 2 meters. This would ensure irrigation facilities from Mej in an area covering Nayagaon, Kheroli, Mal ki jopdhiya, Kharayata, Dhagariya, Pratap garh, Chappanpura, Uttranchal etc.

Table VII-1 represents the agro-climatic zone, agro-ecological situation and blocks in the zone.

Table : VII-1 Agroclimatic zone, Agroecological situation in different blocks and tehsils of Bundi district

S.NO.	Agro climatic zone	Name of AES	Blocks
1	South-East humid plan zone Vth	Rain fed medium rainfall, medium tex. Soil AES-I	Hindoli, Nainwa, Indergarh tehsils
2		Irrigated heavy tex. Soil AES-II	Talera
3		Irrigated heavy calcareous soil AES-III	K.Patan

The agroclimatic zone is mainly south-east humid plain zone in which Hindoli, Nainwa, Indergarh, Talera tehsil are either rain fed or medium rain fall. The soil is medium textured whereas Talera is heavily irrigated. The soil is heavy textured. Keshoraipatan is heavily irrigated but the soil is calcareous.

Table VII-2 presents the area under different types of soil in different blocks of the district.

S.No.	Name of the block	Black		Clay loam soil		Loam Soil		Others
		Area	%	Area	%	Area	%	Area
1	HINDOLI	23537	38	27873	45	3097	5	7433
2	NAINWA	44451	55	16164	20	14548	18	5657
3	TALERA	57959	65	22292	25	2675	3	6242
4	K.PATAN	66102	70	11332	12	14164	15	2833
	TOTAL	192049		77661		34484		22165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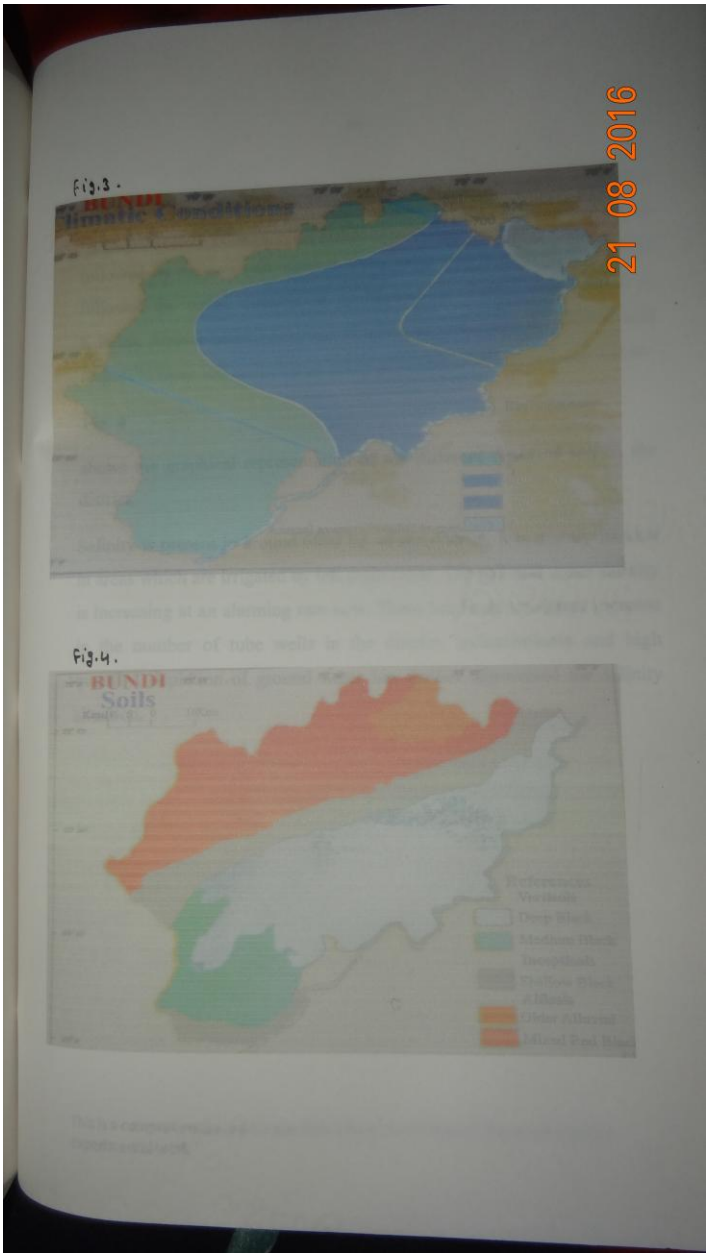
BUNDI
BUNDI
Dams & Canals
Naiwan
Indergarh
Hindoli
Bundi
K.Patan
References



The retention & movement of water in soils and plants is related to free energy which is governed by matrix, osmosis and gravity. Black soils are dark in color and are generally calcerous. They are low in organic matter, high in clay content, high in cation exchange capacity and base saturation. They have high fertility. Black soils contain high proportion of calcium and magnesium carbonate. Based upon phosphorus content soils are classified as low when P is less than 10 kg/ha, medium when 10-25 kg/ha & high when more than 25 kg/ha.

The ECe value of < 1.0 ds/m is normal while values ranging up to 2.0 ds/m are critical for germination of seeds and ECe of 2.0-4.0 ds/m is critical for growth of all sensitive crops while value above 4.0 ds/m causes injury to most of the crops.

The main type of the soil are black, clay loom soil, loam soils and other types. Maximum percentage of black soil are in K.Patan & Talera blocks followed by Nainwa and Hindoli. Clay loam soil is highest in Hindoli followed by equal distribution in Nainwa & Talera and lowest in K.Patan. Almost equal percentage of areas are in K.Patan & Nainwa with respect to loam soil followed by lower percentages in Hindoli and Talera.

Fig. 4 shows the graphical representation of the different types of soil in the district.

Salinity is present in around 6000 ha. of the district. It is mainly located in areas which are irrigated by left main canal. The soil and water salinity is increasing at an alarming rate now. There has been consistent increase in the number of tube wells in the district. Indiscriminate and high quantity, depletion of ground water has further aggravated the salinity situation.

Table VII-3 : Information on problem soils in the district

S.No.	Problem soil	Area in ha.	Extent of severity		
			Very severe	Severe	Mild
1	Saline	6009	-	-	6009
2	Alkaline	9229	-	-	9229
3	Acidic	-	-	-	-
4	Soil erosion	-	-	-	-
5	Iron Toxicity	-	-	-	-
6	Micro-nutrients	35680	-	-	35680

	deficiency				
7	Water logged condition	-	-	-	-
8	Others	-	-	-	-

Sodicity has also increased and there is a consistent increase in the area of sodic soils. There is a large area of soil under micronutrient deficiency which has resulted due to indiscrimate and disproportionate use of fertilizers and the types of the crops grown in different areas.

Figure 4 depicts the severity of salinity in the different blocks of the district. This is based upon the electrical conductivity.

Table V11-4 presents information on the land use pattern in the Bundi district. It presents geographical area, cultivable area, cultivate waste, forest, pasture and area under non agriculture use and barren and uncultivated lands, in the blocks of Hindoli, Nainwa, Talera and K.Patan. The cultivable area as a percentage of the geographical area was higher in K.Patan followed by Nainwa. Barren and uncultivated land was highest in Talera region.

Table VII-5 presents data on the rain fed and irrigated area in the Talera, K.Patan, Nainwa and Hindoli blocks. Highest canal irrigated area was in Talera and K.Patan blocks. The highest area in rain fed cultivation was in Nainwa and Hindoli blocks.

Table VII-6 presents a comparison of the ground water level (m) in different blocks of the district between 1984 and 2005. There has been consistent fall in the level of ground water within last 22 years. If the data is further projected up to 2016, the fall in the ground water level would be more drastic. The fall in the ground water level ranged from -3.48 to -6.69. The highest per year fall was in Nainwa. **Table V11-4** : Information on land use pattern in the BUNDI District 2006-07

S NO	Name of the block	Geographical area	Cultivatable area	Cultivated area	Cultivable waste	Current Fallow	Forest		Pasture	Land put to non agri. Use	Land under misc. plantation	Barren & unculti vable land (waste land)
							Reserved	Open				
1	HINDOLI	134038	61940	44033	15448	2438	10292	37818	5761	8043	21	10421
2	NAINWA	118995	80820	62617	12084	6100	11309	11752	5964	5068	19	3886
3	TALERA	192227	89168	72022	14568	2545	45670	19411	8426	13242	33	16540
4	K.PATAN	136678	94431	75301	13233	5835	3167	12044	4515	13456	62	8794
	TOTAL	581938	326359	253973	55333	16918	70438	81025	24666	39809	135	31506

Table-VII-5

S no	Name of the block	Rainfed area (ha)	%	Irrigated area by canal		Lift		Wells / Bore wells		Tank		Pond		Others	
				A	%	A	%	A	%	A	%	A	%	A	%
1	Talera	27090	23	68258	75	-	-	18946	20	1203	1.5	-	-	3197	3.5
2	K.Patan	30505	29	95906	80	-	-	14334	19	-	-	-	-	1018	1
3	Nainwa	38495	49	1081	2.7	-	-	38549	96.5	234	0.5	-	-	1	0.3
4	Hindoli	27585	40	12068	30	-	-	26241	64	905	2	-	-	1697	4
	Total	123675	33	141313	57	-	-	98070	39.5	2342	1	-	-	5913	2.5

Table-VII-6

S. No	Block	Pre monsoon mean ground water level (m)		Fall in water level (m)	Fall per year (m)
		1984	2005		
1	Hindoli	10.89	14.37	-3.48	-.348
2	K.Patan	9.95	13.61	-4.66	-4.66
3	Nainwa	10.68	17.37	-6.69	-6.69
4	Talera	6.43	10.98	-4.55	-4.55
District Bundi		9.24	14.08	-4.84	-4.84

Table VI1-7 presents the monthly climatic data of the Bundi district. It presents maximum, minimum temperature, cloud cover, dust storms, percent relative humidity mean wind speed and vapour pressure. Higher maximum temperature were observed in May and June and so were the highest minimum temperature. Dust storms were witnessed in may and june. High relative humidity were observed from may to august.

Month	Daily mximum tem. ºC	Daily minimum tem. ºC	Highest tem. in the month ºC	Lowest tem. in the month ºC	Amount cloud cover (tenths of sky)	Dust storms	Relative humidity %	Mean wind speed (km. per hour)	Pressure in mb.	Vapour Pressure
January	24.5	10.6	30.1	6.5	2.1	0.0	49.0	2.2	986.2	9.7
February	28.5	13.1	34.2	8.3	1.6	0.0	38.0	2.3	984.2	8.8
March	34.1	18.5	39.2	13.4	1.4	0.1	26.0	3.9	981.3	8.7
April	39.0	24.4	43.1	19.1	1.3	1.2	18.5	5.2	977.8	8.7
May	42.6	29.7	45.9	24.6	0.8	1.8	22.0	8.2	973.2	12.7
June	40.3	29.5	44.7	24.1	2.8	1.3	41.0	8.2	696.8	21.8
July	33.3	26.4	39.5	23.3	6.0	0.3	61.5	9.1	696.5	28.6
August	31.7	25.4	35.8	23.1	6.1	0.1	74.0	8.2	971.5	27.2

Table –VII-7 : Monthly climatic data of Bundi district

Table VII-8 : Rain fall from 1996 – 2005 in different tehsils of Bundi

Year	Bundi	Talera	K.Patan	Indergarh	Nainwa	Hindoli	Average
1996	1235.6	886.6	742.0	1231.5	491.1	840.0	904.5
1997	899.0	748.4	750.0	919.5	729.7	616.0	776.9
1998	536.0	445.0	513.0	819.5	395.3	541.0	541.4
1999	565.0	622.0	726.0	837.0	631.6	589.0	661.5
2000	606.0	552.0	651.0	459.0	681.6	507.0	576.0
2001	824.0	990.6	786.5	794.0	649.4	810.0	809.15
2002	275.5	269.0	334.5	472.0	259.2	507.0	352.9
2003	754.0	593.0	453.0	808.0	371.0	1176.0	692.5
2004	848.0	544.0	484.0	978.0	503.0	625.0	663.7
2005	545.8	606.0	677.0	691.1	540.1	472.0	588.7
Avg. 10 year	708.8	625.7	611.7	700.9	525.2	668.5	640.1
General Year	773.4	654.9	803.3	765.3	610.1	737.7	724.1

Table VI1-9 presents the trends of cropping pattern in Rabi and Kharif crops in 1974-75 and 2003-2004; a difference of period of almost thirty years. There was an amazing change in the pattern of cropping in both the rabi and kharif crops. While the areas in Kharif crop increased from 33.6 % to 40% between 1974-75 and 2003-2004; It decreased in rabi crop from 66.4% to 60.0% respectively. The area sown in paddy crop declined from 4.7% to 2.2%, jowar from 17.9% to 3.1 %, Soybean was not known to this area in 1974-75 and it increased to 23.7% in 2003-2004. The area under wheat decreased from 31.4%

to 24.8%. Mustard proved to be crop of choice which increased from 0.1% to 22.9% in 2003-2004. Figure 5 presents a change in percent of the area cultivated and the cropping pattern.

Table V1I-9 : Cropping Pattern – Trends from 1974-75 to 2003-04

Scenario for	1974-75	2003-2004
Kharif	**33.6%**	**40.0%**
Paddy	4.7%	2.2%
Maize	2.0%	4.3%
Jawar	17.9%	3.1%
Soyabean	-	23.7%
Urd	0.4%	1.2%
Others	7.4%	5.5%
Rabi	**66.4%**	**60.0%**
Wheat	31.4%	24.8%
Grain	17.1%	1.1%
Mustard	0.1%	22.9%
Coriander	3.4%	7.7%
Others	13.7%	3.5%

Table V1I-10 presents the productivity trends in 1974-75 and 2003-04. There was a significant difference in the trend of productivity (kg/ha) between canal irrigated and rain fed areas. Soyabean productivity was higher in canal irrigated areas. Wheat and Mustard registered a significant increase between 1974-75 and 2003-04. Rain fed areas had significantly lesser productivity in almost all the crops.

Table V1I-10 : Productivity trends from 1974-75 to 2003-04

Items	**1974-75**	**2003-04**	**Non-CAD**
Productivity	Kg/Ha	Kg/Ha	Kg/Ha
Soyabean	Not swon	1303	770
Wheat	2290	3136	2026
Mustard	300	2003	1175
Coriander	500	1330	1110
Fertilizer	37099	129.03	126
Consumption	Kg/Ha	Kg/Ha	Kg/Ha
Cropping intensity	110.84%	138.05%	119%

Table VII-11 presents the production trends in command area prior to the project. A comparative view of the total areas sown and the percentage of total area under each crop in Rabi and Kharif has been projected for the period 1974-75 and 2003-04. Area under paddy decreased from 13.9% to 5.6% while maize registered an increase form 5.9% to 10.0 %. in Kharif crops. There was a decreasing trend in percentage cultivation of wheat, barley, gram and linseed. Mustard was a preferred choice crop which increased from 0.1 to 38.0 % between 1974-75 and 2003-04. Soyabean was not known to the farmers in 1974-75.

Table V1I-11 : Agriculture trends in command area

Kharif Crops – 30% area (Main crops – Sorghum, maize, some pulses)

Rabi crops – 65% area (Main crops –Wheat, linseed, gram & other mix crops)

Cropped area – trends

1. Kharif area (ha.)

Crops	1974-75	% of total area (1974-75)	2003-04	% of total area (2003-04)
Paddy	19500	13.9	12670	5.6
Maize	8200	5.9	24442	10.0
Sesamum	7000	5.0	8489	3.8
Soyabean	Nil	-	133649	60.0
Sugarcane	4000	2.8	78	-
Sorghum	75000	53.4	17486	8.0
Others	26532	19.0	27666	12.6
Total	140232	100	224480	100

* Soyabean becomes major crop

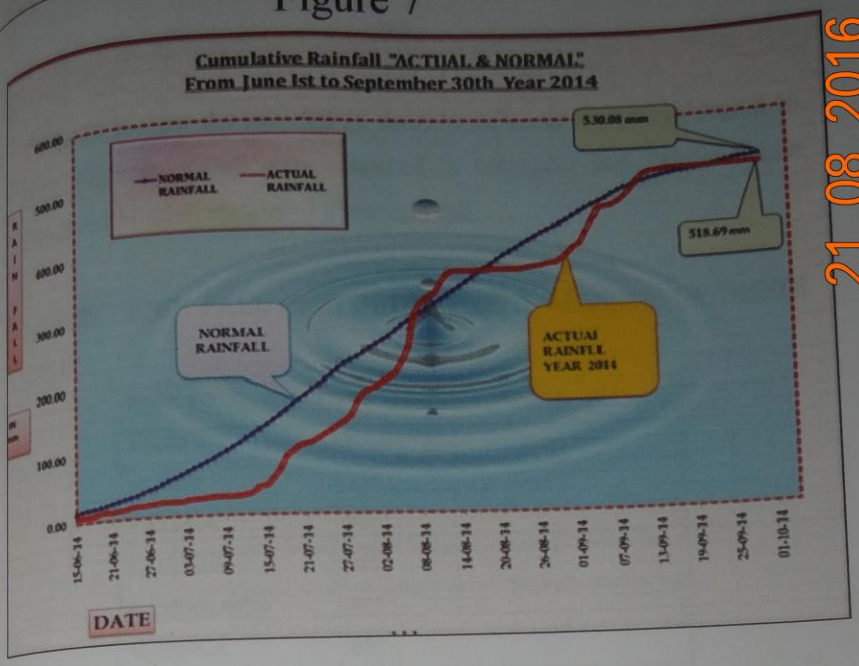
2. Rabi area (ha.)

Crops	1974-75	% of total area (1974-75)	2003-04	% of total area (2003-04)

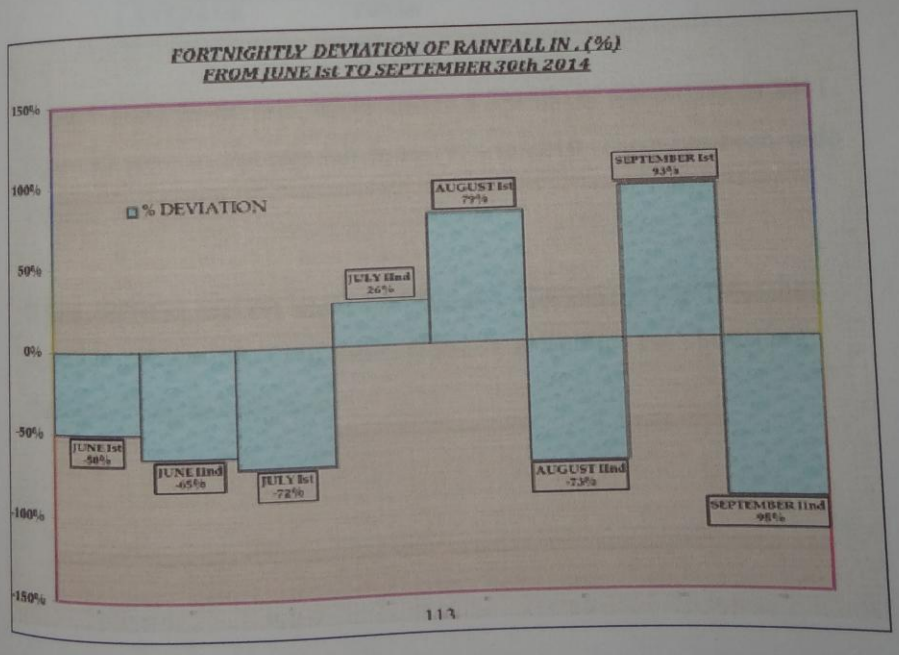
Wheat	130000	46.8	139867	41.0
Barley	18000	6.6	1078	0.3
Gram	72000	25.7	5944	2.0
Mustard	322	0.1	129110	38.0
Linseed	31000	11.1	544	0.2
Coriander	14000	5.1	43196	13.0
Others	12081	4.6	20227	5.5
Total	277403	100	339966	100

Wheat, Mustard & Coriander becomes major crop.

Figure 7

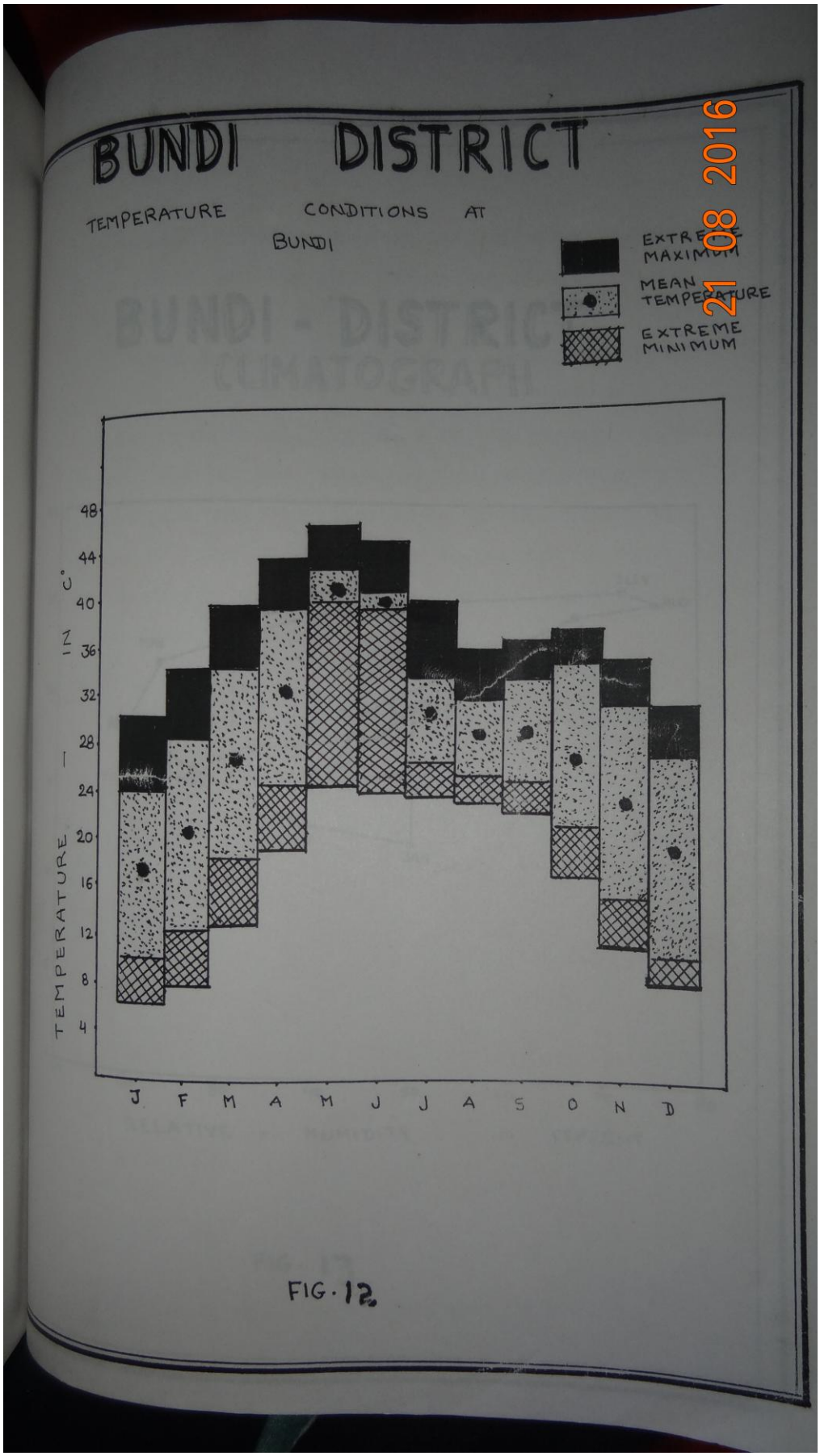


7-A



7 - B

Table VII-12 present the rain fall (6 mm) in different regions of Bundi in 2011. Highest rainfall were observed in Indergarh and Talera. While Bundi and Hindoli registered a low rain fall.


BUNDI DISTRICT
TEMPERATURE CONDITIONS AT BUNDI
EXTREME MAXIMUM
MEAN TEMPERATURE
EXTREME MINIMUM
TEMPERATURE — IN C°
48
44
40
36
32
28
24
20
16
12
8
4
J F M A M J J A S O N D
FIG. 12.

BUNDI - DISTRICT
CLIMATOGRAPH

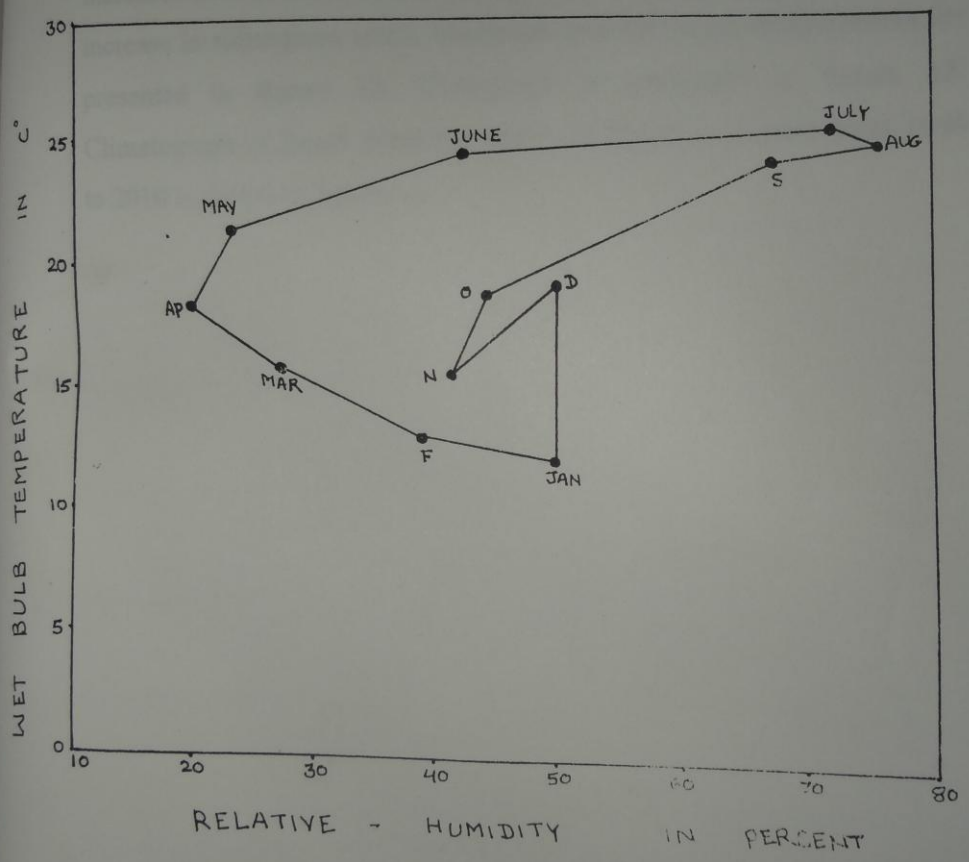


FIG. 13

Table VII-12: Rainfall (mm) in different regions of Bundi (2011)

RAIN FALL (mm) YEAR JAN. 2011 TO DEC. 2011	
TEHSHIL	RAIN FALL
BUNDI	750
TALERA	925
K.PATAN	762
INDERGARH	935
NAINWA	877
HINDOLI	792
AVERAGE	840.16

Figure 9 presents the rain fall in different Tehsils of the district in 2011. Figure 10 presents the rain fall from 1950 to 2010. There has been wide variation in rain fall.

It was reported that the levels of nitrogen, phosphorus and potassium in soil in different tehsils of Bundi is shown in Table VII-13 The salient features of the findings were :

Table : VII-13

Level of nitrogen, phosphorus and potassium in the soil in different tehsils of district Bundi.

Values are expressed as Kg./ha.

Tehsil	Nitrogen	Phosphorus	Potassium
Bundi	259.26	10.32	343.48
K.Patan	278.68	10.76	341.24
Hindoli	246.48	10.49	339.38
Nainwa	263.18	10.3	338.28
Indergarh	252.58	10.79	339.10

Potassium level is high in all Tehsils.

- Phosphorus and nitrogen level is less in most of the areas of Hindoli.
- Phosphorus is less in most of the areas of Hindoli Tehsil.
- Medium level of Nitrogen and Phosphorus are present in some areas of all the Tehsils but only in few projects of Hindoli.
- None of the areas are having higher levels of Nitrogen in any of the Tehsil.
- Higher levels of Potassium have been found in some areas of Tehsils of Bundi, Keshorai patan & Indergarh Tehsils of the district. The level of Nitrogen in these areas were medium.
- Medium levels of Nitrogen , Phosphorus have been observed in some areas of all the Tehsils of the district.

The total and irrigated area under rabi and kharif crops was highest in the Bundi tehsil. Total kharif area was maximum in Bundi & least in Indergarh Tehsil.

The total cultivated and irrigated area in Bundi district from year 2000 to 2006 under Rabi & Kharif crops revealed that the total Kharif area had an increasing trend from 2000 to 2004 but was only marginally increased in 2005 and 2006. The irrigated kharif area did not appreciably

increase in subsequent years. Climograph is presented in **figure 6**. Climatograph of Bundi district is shown in **figure 8** .Rainfall from 1950 to 2010 is shown in **figure 7.**

Pot culture study-

Potassium content was found to be decreasing with increase in salt stress. Results showed minimum Na content in control (EC_1) whereas maximum Na content was found in EC_3. The EC_1 was control, EC2 was 100mM NaCl and EC_3 was 200mM NaCl. Maximum K content were measured in EC_3 whereas minimum K content was found in EC_1 (control).

Where EC_1=0.24 dsm^{-1}

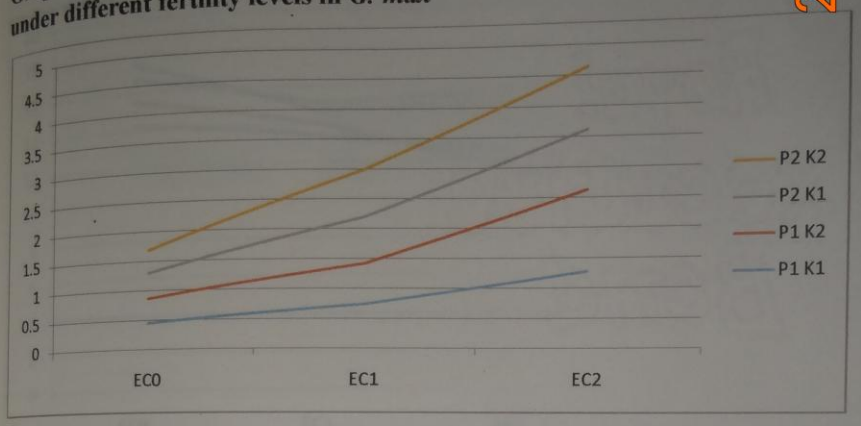
,EC_2=7.8 dsm^{-1},EC_3=14.0 dsm^{-1}

P1= P_2O_5,20 kg^{-ha} ,P2= P_2O_5,40 kg^{-ha}

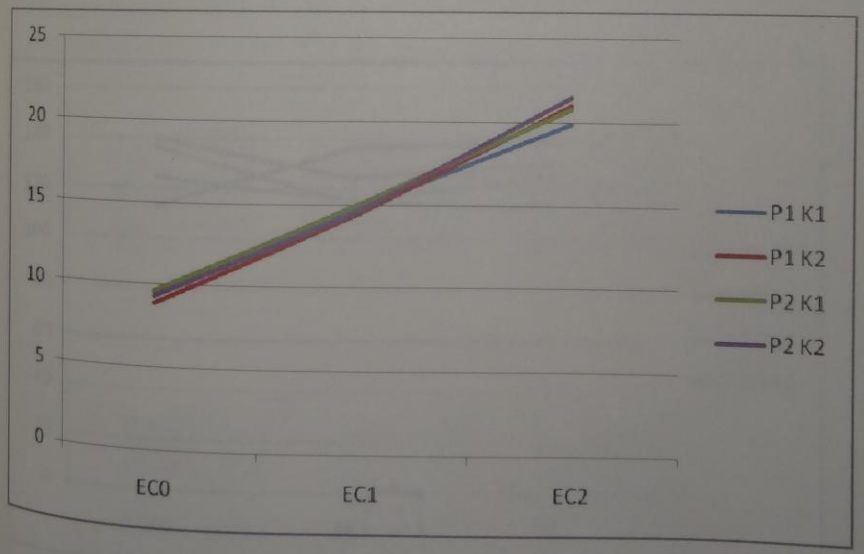
K1= K_2O,15 $Kg^{-ha,}$K2=30 Kg^{-ha}

Figure : 9

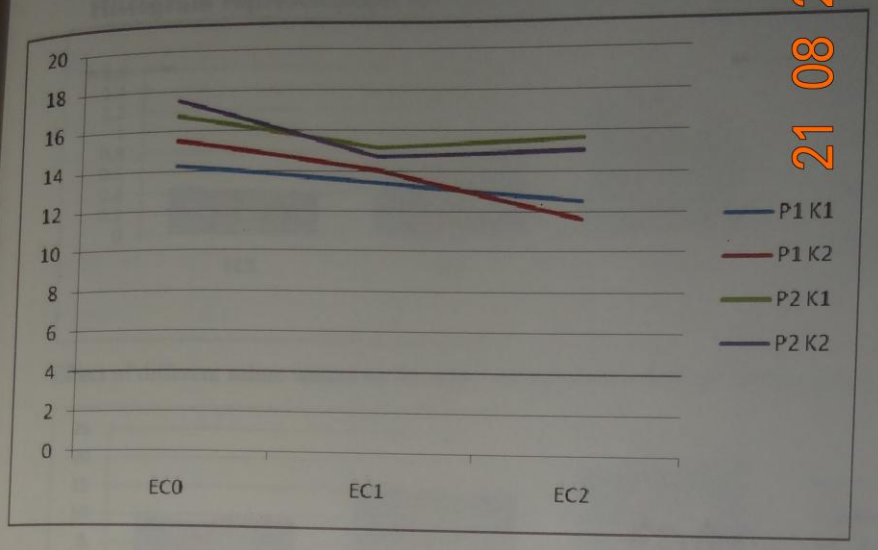
Graphical representation of Effect of different saline concentrations of water under different fertility levels in *G. max*



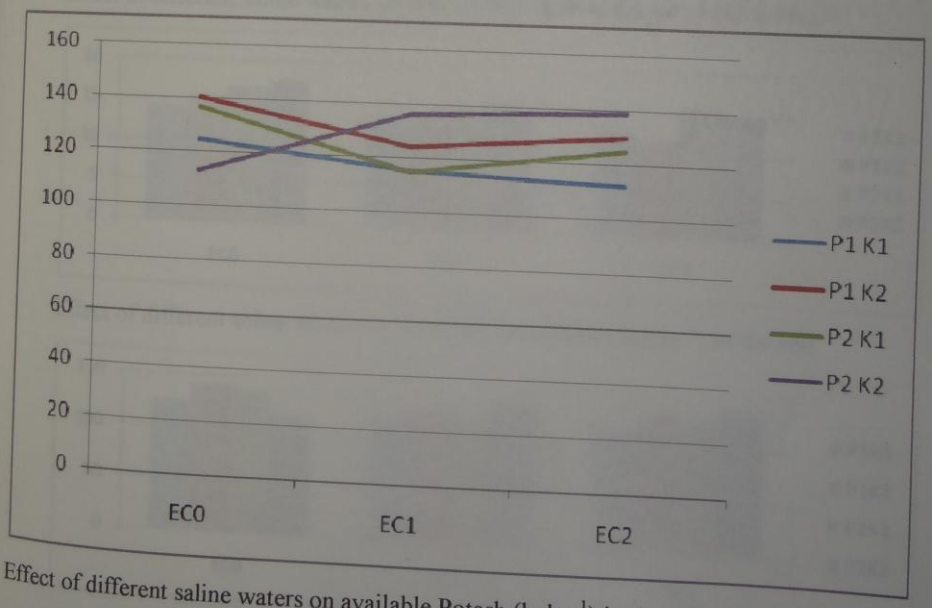
Effect of different saline concentrations of water on EC under different fertility levels.



Effect of different saline waters on ESP of Soil under different fertility levels



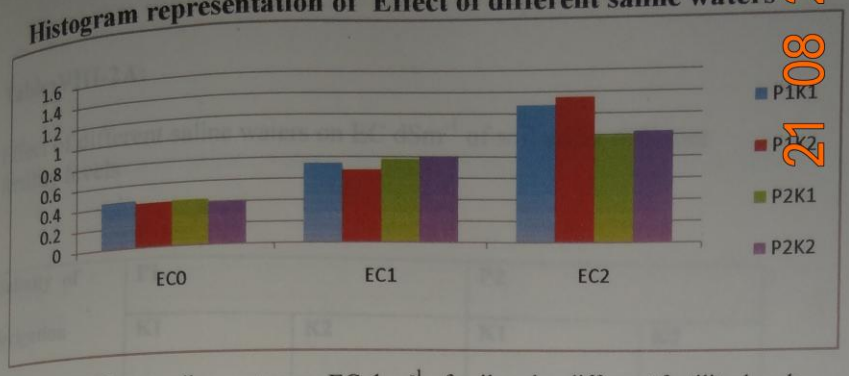
Effect of different saline waters on available phosphorus (kgha-1) in the soil



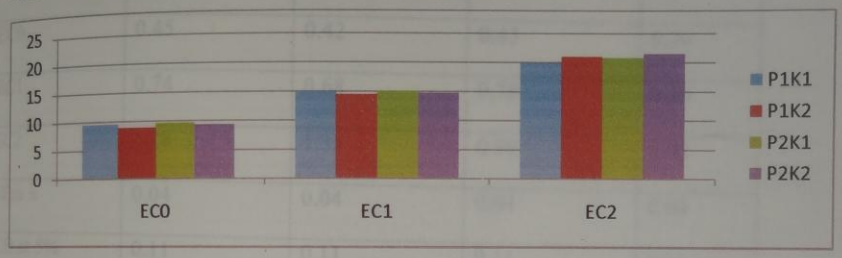
Effect of different saline waters on available Potash (kgha-^{1}) in the soil

Figure :10

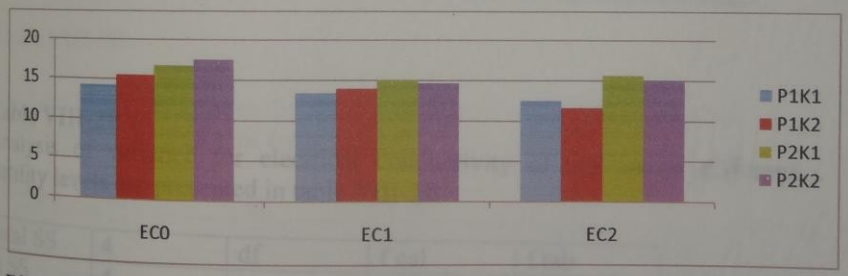
Histogram representation of Effect of different saline waters



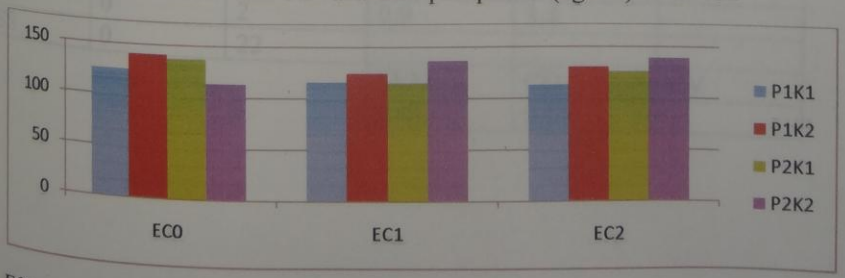
Effect of different saline waters on EC dsm^{-1} of soil under different fertility levels



Effect of different saline waters on ESP of Soil under different fertility levels



Effect of different saline waters on available phosphorus ($kgha^{-1}$) in the soil



Effect of different saline waters on available Potash ($kgha^{-1}$) in the soil

Plate10

10A shows $EC_1P_1K_2$

10B shows $EC_2P_1K_2$

10C shows $EC_3P_1K_1$

10D shows $EC_3P_2K_2$

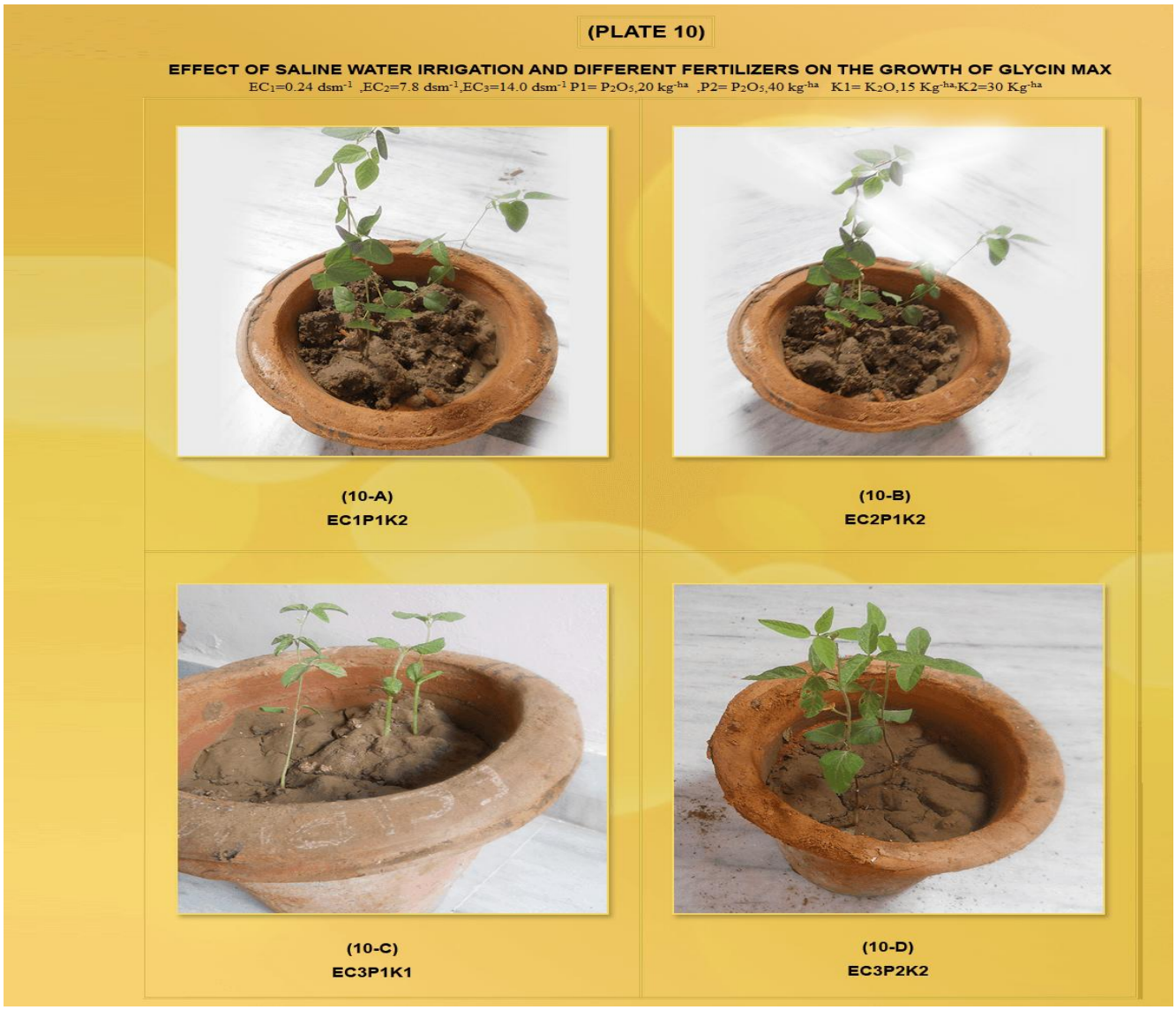


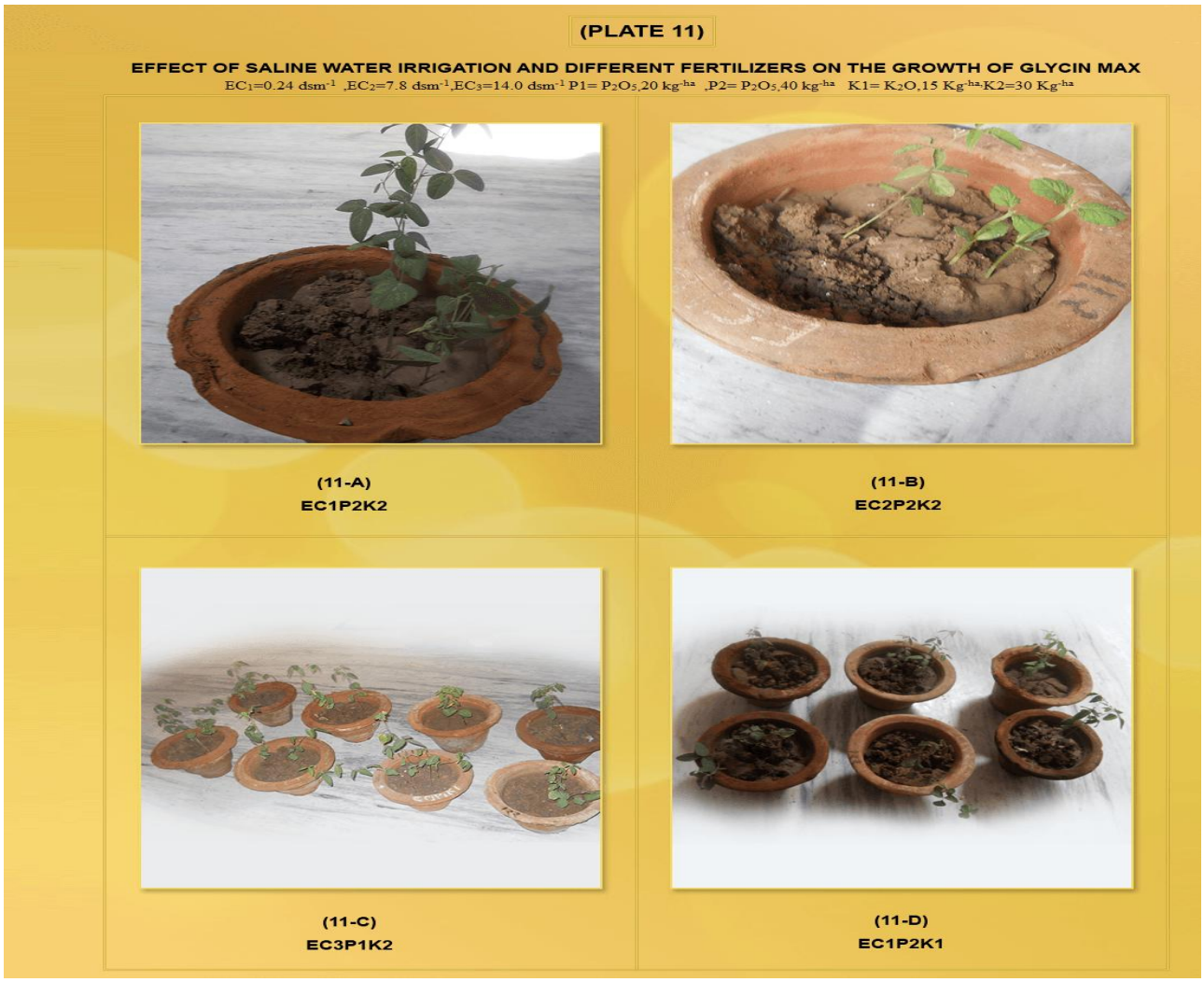
Plate11

11A shows $EC_1P_2K_1$

11B shows $EC_1P_2K_2$

11C shows $EC_2P_2K_2$

11D shows $EC_3P_1K_2$



Sodium content increased significantly as the EC_3 of irrigation water increased whereas change in K content was observed . Increasing levels of salt concentration in irrigation waters from EC_1 to EC_3 (i.e. from 0.24 to 14.0 dsm-1) significantly increased the EC and ESP of the soil with the highest concentration ie. 1.35 dsm-1 under $EC_3P_1K_2$ treatment and 9.50 to

21.50 under $EC_3P_2K_2$ treatment. There is relationship between potassium decreasing and sodium increasing in seedling tissue with sensitivity to salinity.

Table-VIII-1A depicts Effect of salt stress on dry weight of soybean and ash content .

Table:VIII-2A showing effect of different saline waters on EC dSm^{-1} of soil under different fertility levels.

Table VIII-2B showing analysis of variance for electrical conductivity of soil under different fertility levels .

Table: VIII-3A represents effect of different saline waters on ESP of soil under different fertility levels.

Table VIII-3B shows analysis of variance for exchangeable sodium as affected by different saline water under different fertility level.

Table: VIII-4A depicts effect of different saline waters on available P2O5 (kg-ha) in the soil.

Table VIII-4B presents analysis of variance for the effect of different saline water on available phosphorus .

Table: VIII-5A showing effect of different saline waters on available K2O (kg-ha) in the soil.

Table VIII-5B depicts analysis of variance for the effect of different saline water on available potash is presented.

Table-VIII-1A :

Effect of salt stress on dry weight of soybean and ash content ,Variety JS-95-60

Treatment	Quantity	Shoot weight g plant^{-1}		Root weight g plant^{-1}		Ash content		
		FW	DW	FW	DW	Root	Shoot	Leaves
Control	0	2.68	0.52	1.82	0.36	0.043	0.042	0.135
NaCl	0.1 N	1.47	0.32	0.71	0.14	0.023	0.032	0.028
(45 DAS)	0.2 N	0.92	0.21	0.45	0.08	0.012	0.025	0.073
NaCl	0.1 N	1.17	0.28	0.62	0.06	0.011	0.015	0.010
(90 DAS)	0.2 N	0.16	0.09	0.08	0.04	0.008	0.012	0.006
CD at 5%		0.91	0.06	0.04	0.01	0.005	0.008	0.004

FW = Fresh weight, DW = Dry weight , DAS = Days after sowing

Final dry matter (Gram per plant)

Salinity of irrigation water	EC(dSm^{-1})			
	P_1		P2	
	K_1	K_2	K_1	K_2
EC1	0.23 ± 0.004	0.25 ± 0.02	0.26 ± 0.03	0.29 ± 0.03
EC2	0.22 ± 0.004	0.24 ± 0.02	0.22 ± 0.03	0.25 ± 0.03
EC3	0.24 ± 0.004	0.16 ± 0.02	0.11 ± 0.03	0.15 ± 0.03

Number of pod per plant

Salinity of irrigation water	EC(dSm^{-1})			
	P_1		P2	
	K_1	K_2	K_1	K_2
EC1	4 ± 0.41	4 ± 0.85	4 ± 0.85	5 ± 1.03
EC2	3 ± 0.41	3 ± 0.85	3 ± 0.85	2 ± 1.03
EC3	2 ± 0.41	0 ± 0.85	0 ± 0.85	0 ± 1.03

No. of seed per plant

Salinity of irrigation water	EC(dSm^{-1})			
	P_1		P2	
	K_1	K_2	K_1	K_2
EC1	12 ± 1.7	13 ± 2.66	12 ± 2.49	18 ± 3.86
EC2	6 ± 1.7	6 ± 2.66	4 ± 2.49	4 ± 3.86
EC3	4 ± 1.7	0 ± 2.66	0 ± 2.49	0 ± 3.86

Germination days

Salinity of irrigation water	EC(dSm^{-1})			
	P_1		P2	
	K_1	K_2	K_1	K_2
EC1	4 ± 0.41	4 ± 1.08	4 ± 1.08	5 ± 1.31
EC2	5 ± 0.41	5 ± 1.08	5 ± 1.08	6 ± 1.31
EC3	6 ± 0.41	0 ± 1.08	0 ± 1.08	0 ± 1.31

Height of soybean plant (cm) at the harvesting period

Salinity of irrigation water	EC(dSm^{-1})			
	P_1		P2	
	K_1	K_2	K_1	K_2
EC1	31.2 ± 1.72	29 ± 1.19	29.7 ± 1.39	28.1 ± 1.4
EC2	27.4 ± 1.72	25 ± 1.19	26.4 ± 1.39	25.4 ± 1.4
EC3	22.8 ± 1.72	23.3 ± 1.19	22.9 ± 1.39	21.3 ± 1.4

Table:VIII-2A: Effect of different saline waters on EC dSm^{-1} of soil under different fertility levels-

Salinity of irrigation water	**P1**		**P2**	
	K1	**K2**	**K1**	**K2**
EC0	0.45	0.42	0.43	0.39
EC1	0.74	0.68	0.78	0.80
EC2	1.28	1.35	0.99	1.03
SEm ±	0.04	0.04	0.04	0.04
CD at 5%	0.11	0.11	0.11	0.11

Salinity of irrigation water	**P1**		**P2**	
	K1	**K2**	**K1**	**K2**
EC0	0.45	0.42	0.43	0.39
EC1	0.74	0.68	0.78	0.80
EC2	1.28	1.35	0.99	1.03
SEm ±	0.04	0.04	0.04	0.04
CD at 5%	0.11	0.11	0.11	0.11

Table: VIII-3A

Effect of different saline waters on ESP of soil under different fertility levels

Salinity of irrigation water	**P1**		**P2**	
	K1	**K2**	**K1**	**K2**
EC0	9.50	8.90	9.70	9.30
EC1	14.90	14.50	15.10	14.70
EC2	19.90	21.00	20.80	21.50
SEm ±	0.73	0.73	0.73	0.73
CD at 5%	2.15	2.15	2.15	2.15

Table VIII-3B

Analysis of variance for exchangeable sodium as affected by different saline water under different fertility level

Total SS	818	df	f cal	f tab	
trt SS	793	11	67.1	2.3	0
RSS	1	2	0.6	3.4	NS
ESS	24	22			
			SEM	CD	CV
			0.73	2.15	4

Table: VIII-4A

Effect of different saline waters on available P_2O_5 (kg^{-ha}) in the soil

Salinity of irrigation water	P1		P2	
	K1	K2	K1	K2
EC0	14.25	15.50	16.75	17.50
EC1	13.39	14.00	15.10	14.67
EC2	12.50	11.60	15.63	15.03
SEm_±	1.44	1.44	1.44	1.44
CD at 5%	4.23	4.23	4.23	4.23

Table VIII-3B shows analysis of variance for exchangeable sodium as affected by different saline water under different fertility level.

Table: VIII-4A depicts effect of different saline waters on available P2O5 (kg-ha) in the soil.

Table VIII-4B presents analysis of variance for the effect of different saline water on available phosphorus is presented .

Table: VIII-5A showing effect of different saline waters on available K2O (kg-ha) in the soil.

Table VIII-5B depicts analysis of variance for the effect of different saline water on available potash is presented.

Table VIII-4B

Analysis of variance for the effect of different saline water on available phosphorus is presented :

Total SS	188	df	mss	f cal	f tab	
trt SS	92	11	8	2	2.3	S
RSS	4	2	2	0.5	3.4	NS
ESS	92	22	4			
				SEM	CD	CV
				1.44	4.23	8.1

Table: VIII-5A

Effect of different saline water on available K_2O (kg^{-ha}) in the soil

Salinity of irrigation water	**P1**		**P2**	
	K1	**K2**	**K1**	**K2**
EC0	125.00	140.50	137.00	113.20
EC1	115.70	124.30	115.00	136.40
EC2	112.50	130.20	125.50	139.00
SEm ±	3.40	3.40	3.40	3.40
CD at 5%	9.98	9.98	9.98	9.98

Table VIII-5B

Analysis of variance for the effect of different saline water on available potash is presented :

Total SS	4124	df	mss	f cal	f tab	
trt SS	3609	11	328	14.2	2.3	0
RSS	6	2	3	0.1	3.4	NS
ESS	509	22	23			
				SEM	CD	CV
				3.4	9.98	2.2

Morant et. al (2004) found K^+ / Na^+ ratio was higher in salt tolerance of cultivars. Increased Na+ content led to decrease in seed germination level and seedling fresh weight in such plants **(Munns , 2002**).

Discussion

The effect of salinity stress on the germination of *G.max* was investigated in detail. *G. max* seeds were grown in distilled water and sodium chloride concentrations in a range from 10 mM to 1 M. Growth of root, shoot and cotyledon were observed on day 5 & day 8. Though in the milimolar concentration, NaCl had mild effect on the growth of the seeds but the effect was very severe after 100mM onwards. Still higher molarity of NaCl had extremely deleterious effect in terms of growth, percentage of germination & the appearance of germination process. Germination of seeds is governed both environmentally & genetically. This is why, there were variable response depending upon the varieties used in the germination. It not only affected the growth process but also the germination time, percentage of germination & frequent mortality after duration of germination period. Data obtained in all these experiments were

subjected to rigorous statistical treatment in order to derive a valid conclusion which is obvious from the analysis of variance performed on each of the samples. Germination begins within a phasic manner & comprises of three phases: phase 1 is the phase of rapid water uptake which also coinsides with DNA damage repairing process, resumption of the glycolytic cycle & oxidative pentose phosphate pathway. During the second phase active mitochondrial transformation & metabolic activity is at its zenith. About 14000 to 18000 diverse mRNA are present in *glycine max* at different developmental stages (**Goldberg *et al* , 1989**). They are regulated quantitatively at specific developmental stages .

Plate -14 Laser capture microscopy in soybean seed. A smaller number of diverse mRNAs were found to be present in individual soybean globular stage seed region.Approximately 14,000 diverse transcript were detected in the suspenser,including those that encode about 700 transcription factors.

Translation of storage mRNA occurs. During this metabolic phase, reserve mobilization is initiated. In the third phase which is the post germination stage , emergence of radical occur. The sequence of these phases provided an opportunity to approximate the activities of protein and DNA metabolism. In order to study the phenomenon, absorption spectra of the germinated seed component, root, shoot and cotyledons, processed in distilled water (control), 10 mM NaCl and 20 mM NaCl on day 5 and day 8 were studied with special reference to absorption maxima of DNA (260 mμ) and protein (280 mμ). Since neither the proteins nor the DNA were purified, it was observed that the maxima of DNA (260 mμ)& Proteins(280)mμ) were broad, it was expected to be so, since no attempts have been to purify either the protein or the DNA. However, as the phase 1^{st}&2^{nd} of germination are states of massive nuclear & metabolic transformation, a parallelism have been observed in DNA & protein activity. Another λ max was observed at 300mμ which needs further exploration. In subsequent discussion it has been pointed out that a need of additional nuclear and DNA technology is required to answer the question. Morphological variations and transformations either preceed or proceed the biochemical and nucleic acid transformations. This is reflected in the cytological and cellular changes. In the subsequent discussion the role of mRNA translation process, the transcripts and the confirmation of DNA and protein molecules under the stress of salinity has been reported. Many metabolic enzymes are either switched on or switched off, as per requirement of plants to aquire the resistance against abiotic stress. Some

of the RNA ,Tf & proteins are either upgraded or downgraded. Investigation with the use of mutants deficient in acquiring resistance against salinity or confirmity of genes responsible for resisting salinity in different organism have been studied.Even chaperons are subjected to alterations. Therefore, investigation were carried out to study the histological changes in *glycine max* using too different stains, that is hematoxylene & eosine ,toluidine blue at a magnification of 10x and 40x and perusal of the photographs reveal that there is definitive shrinkage of cell cytoplasm under salinity stress. Experiments were also performed to study the effect of salinity on the protein profile of *glycine max* .In further cofirmity type of the protein were assessed on the basis of molecular weight of the different proteins. This study has been discussed elaborately elsewhere. It is well documented that salt stress severely inhibits the growth of *glycine max* which has been distinctly shown in the process of germination. The salt stress effect is mainly due to osmotic or water deficit. Adaptation of the plants against salt toxicity specifically the Na^+ toxicity have already been discussed in detail .

Salinity affects germination in two ways-

a) There may be enough salt in the medium decrease the osmotic potential to such a point which retard the uptake of water necessary for mobilization of nutrient required for germination .

b) The salt constituents or ions may be toxic to the embryo.

Osmotic adjustment in response to salinity results from solute accumulation which occurs through the uptake of solutes, the synthesis of organic compounds, or both. (**Wyn Jones & Gorham ,1983**) . Halophytes typically utilize Na^+ and Cl^- as principal osmotica, while organic solutes may serve an important role in balancing the osmotic presure of the cytoplasm with that of the vacuole; much of the Na^+ and Cl^- is thought to be compartmentalized in the vacuole (**Wyn Jones & Gorham 1983, Yeo 1983**).

During cell division and the initial stages of cell expansion, accumulation of solutes in the cytoplasm would cause its enlargement. The later stages of cell enlargement would involve expansion of the vacuole through accumulation of ions in this compartment. This pattern of accumulation of organic solutes in dividing cells and accumulation of ions in expanding cells is similar to the accumulation of organic solutes in the dividing cells of the shoot meristem

and ions in the expanding cells of leaves of plants under saline condition (**Rhodes *et al*. 1981).**

Soybean cells accumulate several organic solutes along with Na+ and Cl- in response to NaCl stress. Apparently multiple physiological and metabolic changes are induced by external NaCl which result in the accumulation of various solutes, supporting the concept that adaptation of NaCl is genetically complex.

Salinity stress is multigenic involving compatible osmolytes/ solutes, poly amines, reactive oxygen species and antioxidant in defence mechanism , ion transport and compartmentalization of injurious ions. Salinity stress results in leaf senescence & premature leaf abscission .

The product of stress inducible genes are classified in to two groups; one that protects directly the cell against dehydration by synthesizing enzymes that are osmoprotectants, eg: Late embryogenesis abundant protein (LEA) , chaperons & detoxifying enzymes. The second group are gene products such as transcription factor, protein kinases or enzymes involved in phosphoinoside metabolism(**Seki *et al*;2004)** .These gene products regulate gene expression & signal transduction pathway. Salinity stress occurs at cellular, organ & whole plant level. It responds to salinity in two phases, the rapid osmotic phase that inhibits growth of young leaves and an ionic phase that accelerates the senescence of mature cell . Details of counteracting Na^+ toxicity has been discussed else where. Salt injury also leads to electrolyte leakage as a consequence of membrane damage . Vacuolar sorting receptors (VSR) are synthesized denovo during germination. Storage proteins, oil bodies and starch are degraded and used for growth . VSR proteins decrease as germination proceeds from day 1 onwards .

The ubiquitous distribution of auxin , cytokinin & their diverse functions in plants is well documented. Investigations were performed to find out the probable significance of the auxin & cytokinin in alleviating the adverse effect of salinity in *G. max*. In the experiments, seeds were germinated with 1 ppm , 2 ppm of auxin & cytokinin in combination with various molarities of sodium chloride. Distilled water served as control.Seeds were grown upto 8 days & the effects were analyzed on day 5 and day 8. It was investigated whether second spraying on day 5 could be beneficial in alleviating salt toxicity as compared to single spray on day 1. The data were subjected to 2 way, 3 way & 4 way classification of a completely

randomized design of analysis of variance. All sorts of effects such as salinity, hormone, duration of germination & various types of interaction have already been described in the results. The salient findings were that none of the saline hormone condition could be as good as the control (distilled water). The effects of the hormone was attenuated beyond 100 mM concentration. Levels of 500 mM & above level of NaCl were highly injurious & adversely affected the various germination properties. Cytokinin in either of the combination exhibited a superior response as compared to auxin. The pronounced effect of spraying with the hormone was manifested on day 8 of germination . Double spraying was not consequential. Auxin (IAA) supports plant root development in phosphorous stressed proteoids of roots (**Nacry *et al*, 2005).**

Level of IAA is increased in primary roots & young lateral roots. Without IAA only primary roots grow **.**Cytokinin, on the other hand has negative effect on roots and abolishes the auxin effect of increased lateral development. Plants over expressing cytokinin gene (CAXX) reduce the cytokinin level lead to enhanced root growth, lateral and adventitious root formation**.** Phosphorus & nitrogen deficiency decreases cytokinin content accompanied by lateral root formation .Cytokinin represses the expression of phosphorus stress induced genes in arabidopsis. IAA level is elevated in salt stressed *G. max*. IAA overproducing cultivars showed an increased tolerance to NaCl toxicity **(Bianco & Defez 2009) .**

Exogenous application of cytokinin partially restored the germination process (**Gidrol *et al*. 1994)**. Tryptophan, a precursor amino acid for the synthesis of IAA (**Chaiharn & Lumyong ,2011)** , IAA induce nodulation and exogenous application of IAA resulted in increased root and shoot dry weight ,yield of *G. max*. IAA placed a significant role in early infection process of root nodulation **(Spaepan & Vanderleyden 2010**). Rhizobium oxidizes tryptophan secreted by *G.max* into IAA . Under salinity stress plants are unable to secrets tryptophan. The synthesis of IAA is impeded and consequently root nodule formation is inhibited . This could well explain why IAA was not as effective as cytokinin in combating salinity effects (**Spaepen *et al* 2007).**

Exogenous application of tryptophan increased IAA production by rhizobia. IAA is involved in founder cell specification, nodule formation, differentiation, nodule number and nitrogen fixation .

Salinity causes a progressive decline in the level of IAA in the roots (**Sakhabutdinova *et al* ,2003).** Though IAA levels have not been analyzed in the present investigation. It could be one of the causes of the inefficient effect of IAA as compared to cytokinin. A large number of genes which are auxin responsive have indentified and characterized **(Hagen and Gilfoyle,2002**).

The gene families have been classified as auxin GH3 and auxin-up RNA gene formation . Cytokinin regulated cell division, apical dominance chloroplast biogenesis, nutrient mobilization, leaf senescence, vacuolar differentiation, photomorphogenic development, shoot differentiation and anthocynin production (**Davies ,2004**). Cytokinin enhance resistance to salinity and hence its superior effect in the present investigation and resistence against high temperature(**Barciszewski *et al,* 2000**). Cytokinin retards senescence. having effect on membrane permeability to mono and divalent cations, and localized induction of metabolic sinks (**Letham, 1978**). Reduction of cytokinin supply from roots in salt stress alters gene expression in the shoot (**Hare *et al* 1997**). Cytokinins are produced in root tips and developing seeds of plants **(Zahir *et al,* 2001).** They are translocated to shoot by xylem from roots .

The enzymes adenosine triphosphatases are involved in the generation of energy by the hydrolysis of ATP molecules for consumption in various energy requiring reactions. Several types of ATPases ; H^+ pases and P-pases are necessary to maintain the homeostasis specially the regulation of K^+ and Na^+ intra and extra cellularly, osmo-reguation and maintainance of the electrochemical gradient specially when plants are subjected to salinity stress.

In the present investigation, the activity of Mg2+ dependent ATPase, Ca+ dependent ATPase and Mg^{2+}-Na^+-K^+ ATPase have been assayed in seeds of *G.max*. The seeds were germinated in distilled water (control), 10 mM NaCl and 20 mM saline and investigations were carried out on day 1, day 3, day 5 and day 8. Na^+K^+ transport ATPase has a vital role in mammalian system which is obtained by deducting the enzyme activity of Mg+, Na^+K^+ activity inhibited by cardiac glycoside ouabain . Whether such as Na^+K^+ transport also exist in *Glycine max* is controversial , although role of Na dependent and K dependent ATPase in plants have been adequately demonstrated. How these ATPases control the sequestration of Ca^{2+} and Mg^{2+} will

be discussed in subsequent paragraphs, these ATpases are lodged in specific membrane and an exhaustive purification and kinetic studies are necessary to arrive at a definite conclusion.

The present work suffers from the limitation that the seed on day 1 and subsequently germinated seeds were homogenized as a single entity, that is, it comprised of root, shoot and cotyledons. No attempt was made even to partially purify the enzymes. Moreover since 6 replicates were assayed ,therefore possibilities that variations may have crept in depending upon what component of the germinated seed was in majority in the replicate.

Salient findings of ANOVA revealed that there are significant to nonsignificant effects due to treatments and duration. However, graphical representation of the activities reveals higher enzyme activity in control group, secondly, there is a declining phase of activities with duration of germination.

The activity of enzymes was much less than those reported in membrane purified ATPase activity and it was an expected outcome of the present experiments.

The activity of Na^+K^+ ATPase was extreamely low and was not inhibited by ouabain. At present, it can be concluded that Na^+K^+ transport ATPase, as is prevalent in mammalian system, does not exist in *Glycine max.*

However, for precise conclusion, it would need further investigations specially with respect to purification , kinetic studies and gene knockout mutant. The importance of ATPases in conjuction with proton pases and Ppases is discussed in detailed elsewhere .

Protein gradients are crucial for the transport of ions and solute across the different membranes in plant cells. Primary proton transport proteins are:

1) Plasma membrane H^+ATPase

2) The H^+Ppases (type I and type II). Type I Ppases depend on cytosolic K^+ for the activity and are inihibited by Ca^{++}. Type II Ppases are K^+ insensitive and Ca^{++} sensitive .

Type I Ppases acidify plant vacuoles, the resulting H+ and electrochemical gredients are used for the storage of sucrose , organic acid and cytoplasmic detoxification (**Maeshima *et al*; 2001)** .Vacuoles contain H^+ATPase (V –ATPase) and their functions depend on the type of vacuoles where they reside (**Martinoa *et al*; 2007).**

High level of Na+ or high level of Na^+ to K^+ ratio is key requirement for P1B type heavy metal ATPases, they are trans membrane metal transporting proteins and are involved in homeostatis .

Specialized transport proteins in the form of channels, carriers or pumps mediate the movement of heavy metals through membranes (**Williams *et al* . 2000**). P type ATPase translocate diverse sets of ions including H+, Na+/K+, H+/K+ and Ca. Ca^{2+} is the main secondary messenger in *glycine max* seedling .

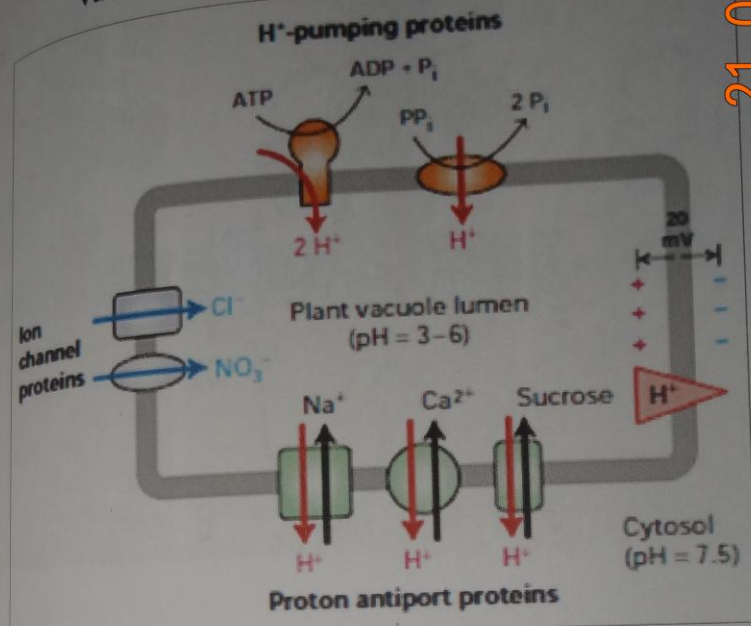
The Ca^{++}ATPase , Mg^{+} $^{+}$ATPase and $Na^{+}K^{+}$ ATPase may not be directly involved in the formation of gradients and channels but does contribute to sequestration and translocation of heavy metals. K+ stimulated ATPase was partially characterized in plasma membrane from meristematic and mature soybean root tissues by others. Activity was greater per unit membrane proteins in the meristematic region.It is reported that ATPases mediate energy transporter to a K+ transporter system, K^+ATPase was associated with plasma membrane of 4 day soybean roots when the Mg^{2+} to ATP ratio was 1:1.

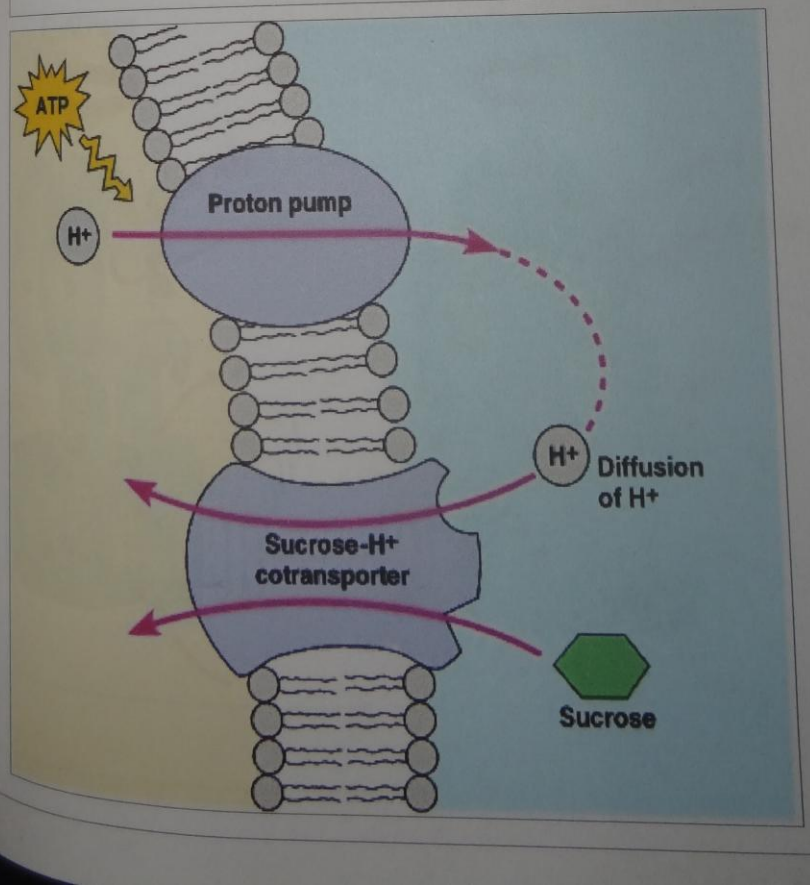
Km value was within the range available in the cells .There is a shift in Mg^2+ and K^+ dependent components during development, the specific activity of K^+ stimulated ATPase of vacuoles isolated from meristematic tissue was 2 to 3 times greater than those for mature tissues (**Scott, 1962).** Mg^{+2} participates in the formation of chlorophyll. Reduced uptake of $Mg+^2$ due to salinity adversely affects growth and development **(Yeo, 1998**).

K+ATPase activity changes with developmental stages. Activity was low in the region of root cap, increased to maximum in the meristematic region, decreased to a minimum as the cell elongation proceeded and increased as the lateral roots developed. K^+ATPase was an integral part of the ion transport system**.**

Figure :

Vacoular H(+) -ATPases (V-AT Pases)





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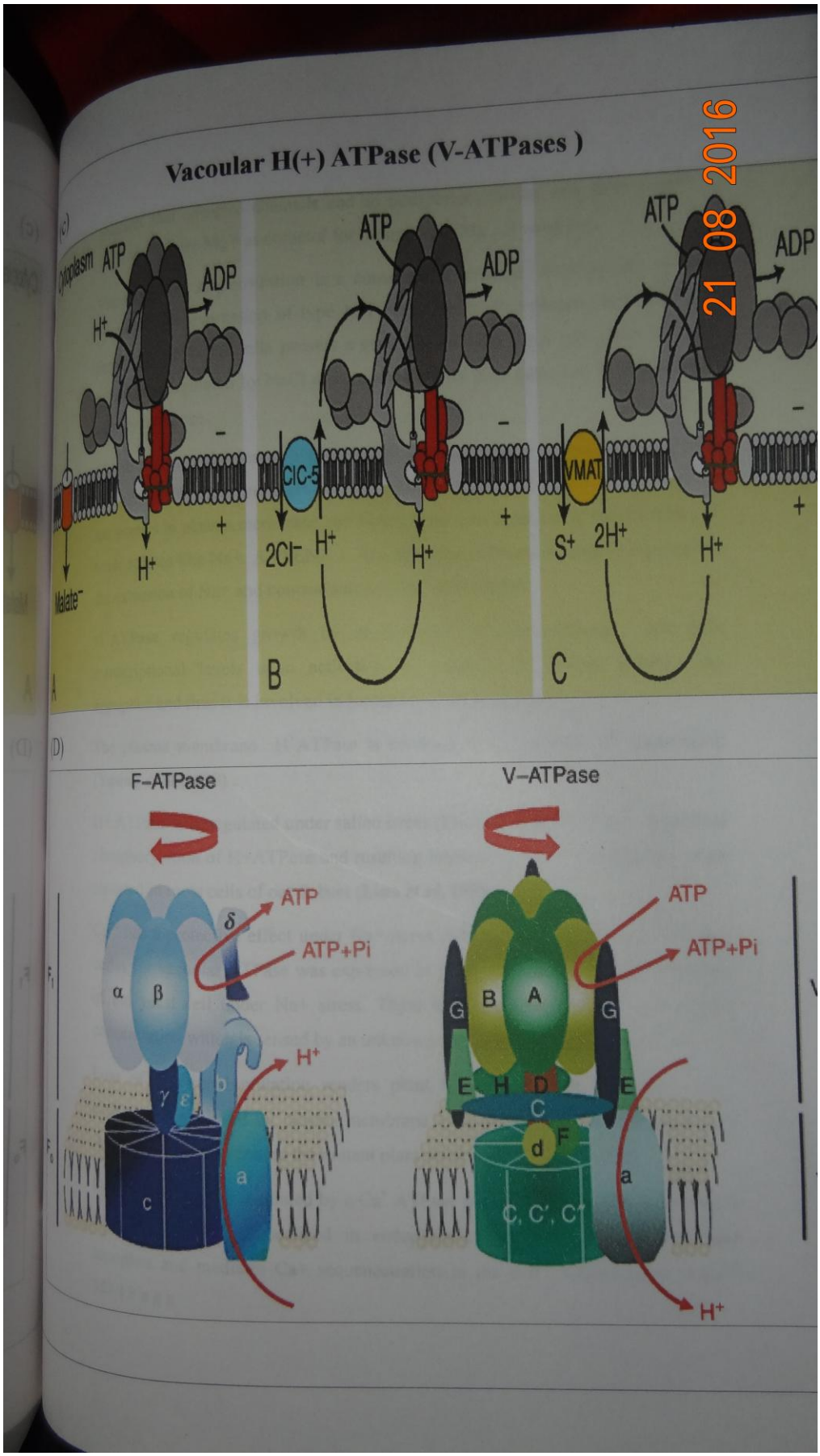

Vacoular H(+) ATPase (V-ATPases)
21 08 2016
Cytoplasm
ATP
ADP
H+
Malate−
A
ClC-5
2Cl−
B
VMAT
S+
2H+
C
D)
F–ATPase
V–ATPase
ATP+Pi
α
β
δ
γ
ε
b
a
c
G
B
A
E
H
D
C
d
F
C, C', C''

P type H+ATPases are active transporters that utilize ATP as energy source to transport H+ across plasma membrane this in turn creates electrochemical gradient that energize channels and co transporters (**Duby and Boutry, 2009**). H+pases require Mg++ as cofactor for formation of Mg ppi complex.

Vacuolar Na+ sequenstration is a conserved mechanism used by salt tolerant species. Over expression of type 1 H+pases results in enhanced salt tolerance.

Bryophyte *physcomitrella* possess a gene that encodes En-A type, Na+ ATPase, which is upregulated by NaCl & is responsible for Na+ extrusion & this helps in salinity tolerance.

Membrane potential across plasma membrane is around - 150mv. This negative potential difference energizes H+ coupled transport for many symporters & antiporters in plasma membrane and hence, allows an uptake of K+ and a efflux of toxic solutes like Na+. A PPENA 1 like Na pump ATPase in fungi is responsible for extrusion of Na+ and concentration of Na+ toxic effect.

H^+ATPase regulates growth by development of transcriptional and post transcriptional levels upon activation by auxins. H^+ATPase acidifies the apoplast and thus it is involved in loosening of the cell wall.

The plasma membrane H^+ATPase is involved in cytoplasmic P^H homeostatis (**Young *et al* ,1998) .**

H+ ATPase is upregulated under saline stress (**Palmgren ,2001**). Ca++ dependent phosphorylation of H+ATPase and resulting inhibition of H+ pumping have been reported in root cells of oat & beet **(Lino *et al*, 1998).**

Ca^{+2} has a protective effect under Na+ stress ,when arabidopsis AcA4 gene that codes for vacuolar ATPase was expressed in yeast, it increased the salt tolerance of the yeast cell under Na+ stress. There is a rise in cytosolic free calcium concentration which is sensed by an unknown receptor binding protein SOS3.

Loss of functional mutation renders plant hypersensitive to salt stress. Na+ exclusion is achieved by plasma membrane localized Na+/K+ anti porter SOS1 .Mutation of SOS3 renders the mutant plant sensitive (**Wu *et al*, 1996).**

Ca^{+2} efflux level is regulated by a Ca^+ ATPase pump. Its expression is increased under salinity. It is localized in endoplasmic reticulum, plasmalemma and tonoplast, mediates Ca+2

sequentration in the cell. Calereticulin, a Ca^{+2} binding protein with a chaperone function plays a critical role in Ca^{+2} homeostasis and protein folding in endo plasmic reticulum in plants.

Another mechanism to reduce accumulation of cytosolic Na+ is achieved by the action of Na+/H+ antiporters on the tonoplast. The proton gradient that drive the antiporter is generated by tonoplast H+ ATPase.

Vacuolar HKT type transporters that are involved in homeostasis, show selectivity for K+ over other mono valent cation & specifically modulate K+ uptake & transport in plants **(Gamble& Ozumi;2006).**

K+ channel & transport system play multiple role such as opening & closure of stomatal pores, leaf movement & ion uptake. AKT1 is an onward rectifying channel for K+ uptake in arabidopsis roots. Elevated cytoplasmic Na+ impaired the K+ permeability modulated by AKT1 **(Qi & Spalding; 2004**).

OSaKT1 is a dominant salt sensitive K+ uptake channel in rice root (**Fuchs *et al*; 2005).** Inhibition of K+ uptake modulated by these channels is a possible cause of Na+ toxicity. Na+/K+ ratio rather than the absolute intra concentration of Na+ determines salt tolerance in plants. Down regulation of 50 S ribosomal proteins mediates the inhibitor effect of Na+ on protein biosynthesis.

Na+ extrusion from the cytosol & partitioning within the vacuole are two major ways to reduce excess of Na+ in the cytoplasm . In vacuolar membrane, another H+ ATPase creates the potential required for the uptake of K^+ or Na^+ in the vacuoles by Na+/H+ antiporters which regulate Na+ sequestrationing into the vacuole**,** firstly by increasing the activity of vacuolar Na^+/K^+ ($Na^{+/}H^+$) antiporter that mediates Na+ for vacuolar H+ , thus solute concentration is increased & secondly by an increased H+ pump activity by vacuolar membrane inducing movement of H+ into the vacuolar & generation of a high proton electrochemical gradient. Both approaches enhance Na+ accumulation in the vacuoles & reduced Na+ toxicity in the cytoplasm leading to higher salt tolerance. Sequestering of Na+ in the vacuoles confers two advantages: reduced toxic levels of Na+ in cytosol, and increased osmotic potential of the vacuole and therefore, more negative water potential that aids water uptake by the cells and water retention under high salt concentrations (**Lubbers *et al*., 2007)**. Over expression of two Na+/H+ antiporters NHX1 & SOS1 for salt overly sensitive has

conferred salt tolerance in transgenic plants by sequestering Na+ in the vacuole or transporting the Na+ across the plasma membrane out of the cell .The capacity of the plants to maintain a high K+ to Na+ ratio is upregulated by dehydrin like protein essential for the regulation in phycomitrella under salt stress . Na+K+ ATPase & a SOS1 homolog in red algae porphyra, living in the sea at high salinity regulates against salt toxicity. In arabidopsis, a mutant disrupted in H+ATPase . AH4 gene has increased sensitivity to salt stress **(Vitart *et al*;2001).** Enhanced membrane conductance for K+ depolarizes the membrane thereby reducing the driving force for Na+ influx. Substitution of Na+K+ gene & its transcripts , complements in *G.max*. as is found in red algae may provide an additional mechanism against salt toxicity. ATP production falls sharply under anoxia due to decreased oxidative phosphorylation by switching from anerobic to aerobic respiration. This reveals cytosolic acidification and inhibition of ATP dependent proton pump activity .

The dynamic nature of the plasma membrane and the role of proteins in membrane activity have been documented (**Guroorn;1972,Mows;1975).** It seemed reasonable, that if the requirement for K^+ changes during development, then the change might be accompanied by changes in the level or activity of the K^+-stimulated ATPase.The enzyme is apparently a dynamic, component of the plasma membrane. One major implication is that the developmental status of the plasma membrane of a given root tissue might then be deduced from its level of K^+stimulated ATPase activity.

There is greater uptake of K^+, on a per cell basis, by mature cells. It is hypothesized that as membrane development progresses, there may be an increase in total ATPase protein coupled with a decrease in specific activity.Consequently there should be an increase in ATPase protein by severalfold to account for the observed differences in K^+ uptake.

Stomata do not only open and close in order to exchange gases, they also form a gateway for pathogens to enter the interior of the leaf. When receptors at the cell surface recognize pathogens, one defense response to close the stomatal pore to prevent bacteria from entering the leaf interior (**Melotto *et al.*2006**). Pathogenic bacteria have evolved strategies to suppress the closure of stomata. One example is the RIN4 protein known to negatively regulate plant response to pathogens (PAMP-triggered immunity, PTI) (**Kim *et al.* 2005).**

In resistant plant genotypes the interaction between RIN4 and the H -ATPase is prevented, presumbly by post-translational modification of the RIN4 protein.

Protein kinase Pk5S is a regulator of H-ATPase . PKS5 belongs to a family of calcium regulated Serine/Threonine protein kinases (PKS/CIPK11) containing 25 members in *Arabidopsis* (**Guo *et al*. 2001; Kolukisaoglu *et al*. 2004; Kudla *et al*. 2010).**

The protein kinase family SOS2/CIPK24 phosphorylates the Na^+/H^+antiporter (SOS1) upon salt stress (**Qiu *et al*. 2002**), while CIPK23 phosphorylates the K channel AKT1 (**Laloi *et al*. 2007).**

Since environmental stresses often cause protein denaturation, therefore chaperones are key components helping to maintain proteins in their functional conformation during stress conditions.Plasma membrane H-ATPase is revealed as a response to limited amounts of phosphate. To mobilize sparingly soluble P forms in soils, cluster roots release substantial amounts of carboxylates and concomitantly acidify the rhizosphere.Fusicoccin stimulated citrate exudation, whereas vanadate, an inhibitor of the H-ATPase, reduced citrate exudation. The increase in protein secretion may be due to both an increased transcription level of a H-ATPase gene as well as activating post-translational modifications of H-ATPase protein involving binding of activating 14-3-3 protein (**Tomasi *et al*.2009).**

Growing tissues and exponentially growing cells generate large amounts of pyrophosphate. It is tempting to speculate, that the H-PPase could be serving two purposes: the generation of the proton gradient required for vacuolar transport/expansion and the scavenging of PPi to alleviate its well documented inhibitory feedback effect.The expression of type II H-PPase has been documented in young seedlings, cotyledons, rosette trichomes,sepals and stamen filaments. It has been suggested that the type II H-PPase may be required during cell expansion (**Mitsuda *et al*. 2001a**).Transcriptional regulatory networks that drive organ specific and cell-specific patterns of gene expression and mediate interactions with the environment represent a fundamental aspect of plant cell signaling.

The expression levels of the H-PPase are precisely controlled at the transcriptional level in response to various environmental conditions or developmental stages (**Maeshima 2000**). It has been shown that cis-acting regions regulate the expression of promoter AVP1 in pollen. AtCAMTA5 and AtCAMTA 1 (calmoduline binding TFs) were shown to bind to the pollen-specific cis-acting region of AVP1 promoter . Promoter AVP1 expression in pollen might be regulated via Ca2þ signaling (**Mitsuda *et al*.2003**). Reversible phosphorylation of tyrosine residues in proteins has been envisaged to play a critical role in the regulation of a variety of cellular processes, including the signal transduction and development in higher plants. The

protein phosphorylation is closely regulated by both protein kinases and specific protein phosphatases. Plants contain a few proteins, which dephosphorylate tyrosine residues in plants proteins, and their roles are poorly understood. The *thaliana* genome contains several genes that correspond to protein tyrosine phosphatases. These proteins have characteristic features that of classical protein tyrosine phsphatases, like conserved active site motif, (1/V)HCXAGXXR(S/T)G within the catalytic domain, and strongly inhibited by oxy anion (vanadate) and inactivated by SH-blocking agents. The role of protein tyrosine phosphatases in relation to mitogen-activated protein kinases (MAPKs) and with respect to abiotic and biotic stresses in plants is well documented.

The protein kinases and protein phosphatases that catalyze these processes and modulate the phosphorylation status of the target proteins are classified into two major groups, depending on their substrate specificities. These are: (i) protein serine/threonine kinase/phosphatases , and (ii) protein tyrosine kinases / phosphatases. , serine / threonine protein kinases and phosphatases are well characterized , have been shown to play a prominent role in various processes of plant growth and development much less is known about the functions of tyrosine kinases and more so of protein tyrosine phosphatases (PTPases) in plants, however, several members of the PTPase family have been characterized from *thaliana* **(Xu *et al*;1998**) and a significant protein of the genome encodes protein kinases and protein phosphatases that catalyze the reversible phosphorylation of protein**,** suggesting that the PTPases may also perform key functions in plants. . Plant PTPases play their role in the regulation of mitogen-activated protein kinases (MAPKs), which under stressed condition are activated by phosphorylation of tyrosine residues of protein**.** It is quite interesting to note that there are about 20 genes that encode about 20 putative PTPases , but only one encode tyrosine – specific PTPase and remaining 19 or so genes encode dual-specificity PTPase **.**All PTPases are characterized by their sensitivity to vanadate and insensitivity to okadaic acid, a specific inhibitor of protein serine/threonine phosphatase **.** In addition, PTPases are very sensitive to thiol – specific alkylation reagents such as iodoacetate, N-ethyl maleimide, and 5,5'-dithio-2-nitrobenzoic acid. There are other stress responsive enzymes that included Lipoxygenase (LOX), Phenylalanine(PA), Ammonia-lyase catalase (ALC) , Peroxidase (POD) and Superoxide dismutase (SOD) were reported in bacteria and control-treated soybean plants.

For a precise analysis of the region of the legume seeds, advance techniques are necessary to obtain the precise region.In the present investigation,crude method of isolation has been used.Various compounds and microorganism can be used to increase the N- utilization. To be able to isolate different regions from any legume seed and embryo is to make use of Laser Capture Microdissection (LCM) technology **(Day *et al*., 2005; Nelson *et al*., 2006).** LCM technology makes it possible to study gene activity in the entire seed because any seed compartment region, or tissue can be isolated easily throughout development.

Genes, including those encoding transcription factors, that are active specifically in the embryo proper and suspensor of SRB and soybean globular-stage embryos genes that are active specifically in other compartments of the seed (e.g. endosperm ,integuments, hilum) have been indentified.Some model calculations and field investigations that demonstrate the effect of root water uptake on the salinity of the root surrounding soil fraction (rhizospheric soil).Root hair length and rhizospheric soil volumes are factors most relevant for understanding crop salt tolerance, when growing in soils. It is postulated that short root hairs contribute to a lower salt tolerance ,whereas long root hairs enhance water uptake from saline soils and crop salt tolerance. An interactions between roots and soil contribute to the salt tolerance of crops under field conditions.In the past decades the focus of research to elevate salt tolerance of plants mainly referred to biochemical and physiological aspects (**Koyro and Huchzermeyer 1999**). Genes responsible for salt tolerance of some crops (e.g. soybean, tomatoes, grasses, rice) have been identified **(Jaradat 1999**). However, in spite of the large efforts put into the understanding of biochemical and physiological processes in plants grown under saline conditions, results are disappointing with respect to their relevance for crop yields under brackish or saline agriculture .

Proteome analysis of leaf, hypocotyl and root of 7-day-old seedlings to determine the importance of salt-responsive proteins in vegetative stage have already been reported.Proteins common among leaves, hypocotyls and roots show organ and developmental stage specificity at the mRNA level:The relative amount of glyceraldehyde-3-phosphate dehydrogenase mRNA was decreased in the leaves, hypocotyls and roots relative to controls by NaCl treatment. The decrease was significant for hypocotyls and roots. The expression of this protein was down-regulated in leaves and hypocotyls. These results indicated that the down-regulation of glyceraldehyde-3-phosphate dehydrogenase was caused at both the mRNA and protein level by NaCl treatment (**Jeong *et al*;2001).**

Calcium is a main secondary messenger for soybean seedlings under salt stress. Down-regulation of the 50S ribosomal protein indicates the inhibitory effect of NaCl on soybean protein biosynthesis and presumably leads to the consequent reduction in plant growth.
The accumulation of starch granules is a common phenomenon observed in seed during germination. The conversion of different reserves into starch seems to be a routine process in different crops during seed germination. It is still an open question why plants sacrifice some ATPs to re-synthesize starches rather than directly utilize the reserves .Improved salt tolerance of potato was obtained by transfer of the G3Pd gene **(Holmberg & Bulow ;1998**). Under salt stress, which inhibits photosynthesis, the substrates for glycolysis decrease and there is a resulting decrease in the rate of the glycolytic reactions.

Cell membrane thermostability was measured as the procedures outlined by Blum and Ebercon **(Blum and Ebercon ; 1981**) . Electrical conductivity (EC) of the samples were measured with the help of EC meter . The relative injury (RI) was determined by

Relative injury (RI) = $(1-(1-(s_1/s_2))$ x $100)/ [1-(c_1/c_2)]$

where s is the treatment, c is the control, and 1 and 2 are the **f**irst and second readings of conductance, i.e., before and after the autoclaving.

Experiment with various crops and soils have clearly demonstrated that the additivity of the water potentials on the plant water supply and plant growth cannot be considered as a general rule, but there are specific combinations of soils and plants, where the matric and osmotic water potential may affect plant growth additive.

Molecular breeding has been considered the most efficient way to improve salinity tolerance in, cotton (**Gorham *et al.*, 2009).** Cotton plants over-expressing TsVP [an H^+-PPase gene cloned from *Thellungiella holophila*) **(Lv *et al.*, 2008)],** the AVPI gene encoding a vacuolar pyrophosphatase as a proton pump on the vacuolar membrane has been utilized for this purpose(**Pasapula *et al.*, 2011**)]. A CMO gene cloned from *Atriplex bortensis* (**Zhang *et al.*, 2009**) have been achieved which significantly increased tolerance of salinity stress. Molecular breeding approaches, including transgenic modification and quantitative trait mapping with marker-assisted selection, have shown some success however will continue to increase our understanding of the complexity of plant physiological pathways (**Lubbers *et al.*, 2007**),

current transgenic cotton with improved salt-tolerance ability is still far behind the requirements for commercial production (**Zhang *et al*., 2009**).

The mechanism of action of phytohormone and mode of application have variable response.Foliar spray of **G. barbadense** cultivars with GA_3 alone or in combination with boron, increased growth and monovalent cation contents under salinity, but decreased chloride content . The GA3 also alleviated the detrimental effects of salt on photosynthetic pigments in seedlings .Analog can potentiate phytohormone action treatment (of imbibing seeds and/or as a foliar spray) with the cytokinin analog, MCBuTTB. Improved the germination growth and yield of cotton plants subjected to salinity (**Stark. 1991**). Another cytokinin analogue, polystimuline K, alleviated the effects of salt on photosynthetic activity in two week old cotton plants (**Ganieva *et al*., 1998**).

A complex transcription regulation system is involved in phosphorus starvation (**Francis-Zorril *et al* 2004**). A rise in Tf (PTF1) is involved in response to phosphate starvation. This Tf is expressed in phloem cells of primary root, leaves and lateral roots. Over expression of this transcript enhances tolerance of rise to phosphorus starvation and enhanced expression of H+ pump (**Yi *et al* ,2005).**

As dicussed earlier, massive metabolic transform and DNA damage repair changes occur during the process of germination. Several of the enzymes are either over expressed or remain quiescent. DNA transformation proceeds simultaneously. Under the circumtances, a host of transcripts mRNA translational factor, chaperons synthesize new protein are all involved in the process. Literature is replete with the changes which occur in the domain of protein .It was therefore, decided to study the protein changes in *G. max* germinating seeds. Seeds were germinated in distilled water (control) and different molar concentrations of NaCl upto 8 days. It is already reported that radical changes start from day 1 and various transformations occur with the emergence of radicles. The role of salinity stress on *G. max* has already been discussed .

It has also been discussed that how the tolerant strains combat the salinity effect by transforming the protein, upregulated enzyme, downgrading others and an array of other compound like chaperonin help to maintain the configuration & conformation of proteins to prevent misfolding.

Germinated seeds of *G.max* in distilled water and various molarities were isolated for its cotyledon and roots. Cotyledon and roots of day 5 and day 8 grown in distilled water and different molarities of NaCl were subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) for protein separation . Molecular weight of the protein were obtained by electrophoresis of known molecular weight of proteins .The mobility of the proteins were compared with the mobility of known molecular weight standards and a plot of mobility vs molecular weight enabled to calculate the molecular weight of seed proteins.

The role of salinity stress on the germination, growth & development of *G. max* is well documented. It has already been discussed that how the plants combat the adverse effect of salinity by modifying the quantum of enzyme activities, changing the configuration of proteins and genetically regulating the changes through their transcripts. Though the SDS PAGE technique has reasonably resolved a number of protein, resolution of protein could further be accenuated by using the modern technology. Seed proteins were resolved into different protein fractions having high to low molecular weights. During the process of germination from day 5 to day 8. Several proteins either disappeared or some new protein bands appeared. Salinity strikingly resulted in a change in the protein bands. Some proteins disappeared or new protein appeared. It appears that control gene, structural gene & operator gene of the operon is activated in the salinity stress. It has not been possible to identify the nature of the protein bands. The possibilities of protein transformation due to salinity & period of germination are being discussed in context with the work of others.

Changes in the gene expression occur in the salinity stress, genes have been isolated, whose expressions are influenced by salinity. cDNA that encodes tolerance have been selected using salt sensitive mutant during seed development .Newly synthesized storage protein are transported and stored in protein storage vacuoles such as δ –tonoplast intrinsic protein (**Jiang *et al*, 2000**). A cys protease sulphahydryl-endopeptidase (SH-EP) is synthesized denovo in cotyledon. Soluble protein are transported to lytic vacuoles in the plant cells which are receptor mediated involving vacuolar sorting receptor proteins (VSR) . These proteins are decreased from day 0 to day 4 and new band proteins have been detected. Aleurain is also synthesized during germination. VSRs are recycled from multi vascular body to golgi for a further round of protein binding **(Oliviusson *et al*; 2006**). To demonstrate DNA binding of legume TFs one has to resort to electrophoretic mobility shift assays (EMSAS), hybridize labeled DNA to TFs on filters and DNAase-1 foot printing. This technology has helped a

cDNA encoding ALFIN1-protein with putative Zn binding (**Bastola *et al*; 1998).** Tf activity can be documented by transactivation in vivo in cell culture by particle bombardment of cotyledons . Soybean seed protein identification have been complimented with density gradient centrifugation protein which have resolved into 2.2 S, 7.5 S and 11.8 S.

On saline treatments, metabolic related proteins were mainly down regulated in leaves, hypocotyls and roots. A 31 kDa glycoprotein precursor was upregulated glyceraldehydes -3 -phosphate dehydrogenase was specifically down regulated. A late embryogenesis abundant protein analyzed by proteomic technique was involved in process of adaptation to salt stress at early seedling stage. Leaf proteins were identified by N terminal sequencing and MALDI-time of the flight mass spectrometric technique. These proteins were ATP synthase, G-6-P dehydrogenase, 20 KDa chepronine to name a few, were identified. Proteins involved in metabolism, defence, storage were up regulated while proteins involved in photo synthesis and metabolism were down regulated by NaCl treatment (**German *et al* 2004).**

Transcription factor are DNA binding proteins that react with transcriptional regulators. They are involved in chromatin modeling or modification of proteins to recruit or block access of RNA polymerase to DNA template. There are thousands of legume genes that were differentially expressed during development and differentiation in saline stress (**Buitink *et al* 2006**). Further course of analytical procedure would require the technique of gene silencing and transgenic complementation, determination of quantitative trait loci (QTL) to identify gene confirming resistance. A QTL has already been mapped for salt tolerance. Transcriptome atpases have already been developed for transcriptional informations.

In spite of enactments, sewage and industrial effluents are not being treated for further purification. No systematic sewage disposal plants are being used. Similarly, no attempt is being made to treat the industrial effluent. This has resulted in wide spread pollution. The toxic elements and heavy injurious metals are being disposed in the fields resulting in a wide spread health hazard. Farmers are frequently using sewage for agricultural production. In case of industrial effluents, growth of agricultural productions are severely hampered. Also, some highly toxic material accumulate in food grains which prove extremely toxic and lead to even cancer problems. The index of the processed industrial effluent is gauged by the chemical composition of the processed effluent. The criteria for purity is assessed from the composition

such as pH, electrical conductivity, total dissolved solids, biological oxygen demand, chemical oxygen demand and bacterial loads.

An experiment was therefore devised to study the germination of *Glycine max* in sewage and industrial effluent. Industrial effluent & sewage were analyzed for pH, electrical conductivity, total dissolved solid, biological oxygen demand, chemical oxygen demand and bacterial load. In the case of sewage seeds were germinated in the raw sewage as such and sewage treated with activated charcoal and supernatant used for the germination of *G. max*. Total bacterial load and types of bacteria on the gram positive and gram negative basis was counted in the raw sewage and sewage treated with activated charcoal and the resultant supernatant which was used for germination & the centrifugal residue. ANOVA was performed for the treatment effects .It was found that best growth was sustained in activated charcoal treated supernatant followed by raw sewage. Poorest growth was observed in industrial effluent. BOD and COD were more than the permissive limit. There was no change in total bacterial load and the gram positive and gram negative bacteria in raw and treated sewage . This was not unexpected since activated charcoal adsorbs heavy metals and is not a bacterial filter. Improved germination in activated charcoal treated sewage may be due to elimination of heavy and toxic metals. Microbial processes which are often dependent on environmental factors such as temperature, moisture, and nutrient availability, are likely to be affected by climate change. Enhancement of soil respiration rates and plant responses attributed to an interactive effect between warming, decline in soil moisture, limitations in soil nutrient supply for plants, decline in the availability of labile C sources for soil microbes. As soil biodiversity regulates nutrient dynamics and many disease risks, nutrient availability to crops change as could the exposure to soil-borne diseases.

Two factors of climate change can mostly initiate the soil degradation process. They are increase in temperature and decrease in precipitation which form a close-linked chain of soil erosion, desertification and salinization. The associated effects may be the reduction in SOC concentration and structural aggregation, and also disturbances in hydrologic cycle leading to more evaporation, evapotranspiration, surface run-off, and frequency of drought.

Key indicators affected by climate change include aggregate stability, SOM, carbon and nitrogen cycling, microbial biomass and activity. The overall major impacts on soil health resulting due to climate change would be in the organic matter supply, declining SOC level,

temperature regimes, hydrology and salinity. Increase in soil temperature will decline soil available-N through processes such as mineralization, volatilization and denitrification.

Sustenance of soil organic carbon (SOC) in soil is of utmost importance for soil health and sustainable agriculture. Soil carbon storage with turnover rates of biomass under different IPNS modules in a vertisol under the predominant soybean-wheat cropping system of central India revealed improvements in carbon sequestration through balanced and integrated nutrient management. The highest C addition and storage was recorded under 16 and 8 t ha^{-1} FYM addition to soybean and wheat, respectively. Carbon addition and storage relationship revealed that to maintain the initial SOC 554 kg C ha^{-1} is required. The vertisol of Central India have high C storage potential and the soybean-wheat cropping sequence hold promise to maintain its SOC even in the event of no fertilizer and no manure addition .The decay rate constant of native soil carbon was 0.0061 $year^{-1}$. Rising temperature can hasten the rate of organic matter decomposition, release of organic carbon from the soil into the atmosphere and thereby accelerate the already declining status of organic matter or under cooler conditions there can be an accumulation of soil organic carbon (SOC). The following table presents the rating of levels of TDS.

Level of TDS (milligrams per litre)	Rating g
Less than 300	Excellent
300 - 600	Good
600 - 900	Fair
900 - 1,200	Poor
Above 1,200	Unacceptable

It should be ensured that waste water should not be allowed to discharge in agricultural fields as such. A suitable efficient irrigation system could identify the useful resources in effluents such as plant nutrients and organic matter and not make the quality of land deteriorate through soil structure degradation and salinisation. The agro biological effects of heavy

metals are distinctly visible when the plants absorb toxic metal such as cadmium, lead, nickel and mercury. The heavy metal accumulation can be rectified by bioremediation Microbes are generally useful for assistance in reclamation of sites with heavy metal problems. Several fungi are also good in accumulation of heavy metals. There has been considerable interest in the remediation of contamination by various physiochemical and biological techniques. Commonly used methods are precipitation (**Mullen et at 1989),** electro chemical (**Casey, 1997**), ion exchange (**Mullen *et al* 1989**), biological methods **(Pfister ; 1999**) and reverse osmosis. All these methods have their sets of drawbacks and hence a synergy has to be stuck between all of them to achieve optimum remediation of the metal contaminants. Activated carbon has been recognized as a highly effective adsorbent for the removal of heavy metals. Charcoal adsorption helped to change the pH and a reduction of suspended solids and dissolved substance. Evidently there is an over all reduction in suspended and dissolved solids, dissolved oxygen & biological oxygen demand (BOD) (**Verma *et al,* 2007).**For the purification of industrial effluent & sewage, activated charcoal bed was used.Subsequently ,the filtrate was centrifused and used for germination studies and change in bacterial population.The basic principle of activated charcoal adsorbtion based on the equation given below-

Adsorbents follows the equation of $t_b = \frac{N_O}{1000EvC_o} D - \frac{1}{KC_O} \ l_n\left(\frac{C_O}{C_b} - 1\right)$

Where t_b = time inter break through (min.)

CO = intial concentration of solution (mg/l)

V = fluid velocity or loading rate (m / min)

E = porosity of the filter

K = quasi – chemical rate constant (l/ mg-sec)

NO = capacity of the media for each pollutant in a multi component solution (mg pollutant per cubic meter of filter volume)

D = depth of the filter bed.

Adsorption on activated charcoal depends on Langmuir adsorption isotherm, Freundlich isotherm & Gibbs phenomenon. Generally graphs are plotted for the rate of adsorption against time according to equation of **Cooney (1999**). The adsorption capacity is dependent upon the equilibrium time and optimum pH.

Absorption on activated charcoal is represented by an equilibrium isotherm, which is the plot of the quantity of sorbate, retained on adosorbent as a function of the equilibrium concentration of the sorbate in liquid phase. According to Langmuir adsorption isotherm, biosorbents have high monolayer saturation capacity and can accumulate dye more than its weight.The Freundhich isotherm model encompasses the heterogeneity of the surface and the exponential distribution of sites and their energies. Langmuir equation integrates solid phase sorbate concentration, sorption capacity and intensity of sorption.The parameters of Langmuir adsorption isotherm and Freundlich absorption isotherm fits well with charcoal adsorption bed. Besides these adsorption isotherm, molecular size and molecular weight of the dyes also influence the extent of biosorption. **Haglund** et al(**1996**) has established a relationship between molecular weight & molecular size by the expression:

Molecular size = (molecular weight) / 3. Therefore, it is sufficient to use molecular weight as a measure of molecular size.

Electrical conductivity of water is directly related to the concentration of dissolved ionized solids in the water. Ions from the dissolved solids in water create the ability for that water to conduct an electrical current, with the increase in concentration of soluble salts ,the electrical conductivity of the soil treated with undiluted effluent would be maximum .

TDS is used as a general indicator of water quality. Higher concentrations of suspended solids can serve as carriers of toxics, which readily cling to suspended particles. TDS is made up of inorganic salts, as well as a small amount of organic matter. Common inorganic salts that can be found in water include calcium, magnesium, potassium and sodium, which are all cations, and carbonates, nitrates, bicarbonates, chlorides and sulphates, which are all anions.TDS is a measure of all dissolved substances in water, including organic and suspended particles that can pass through a very small filter. TDS is reported as mg/l.

DISSOLVED SALTS

Dissolved salts in irrigation water form ions. The most common salts in irrigation water are sodium chloride, (NaCl). calcium sulphate, ($CaSO_4$),magnesium sulphate, ($MgSO_4$) and sodium bicarbonate ($NaHCO_3$), Salts dissolve in water and form positive ions (cations) and negative ions (anions). The most common cations are calcium (Ca^{2+}), magnesium (Mg^{2+}), and sodium (Na^{+}) while the most common anions are chloride (Cl-), sulphate ($SO_4$2-) and bicarbonate (HCO_3-). The ratios of these ions,however vary from one water supply to another. Furthermore, high concentrations of sodium would cause deficiency of other elements such as potassium and calcium in plants; whereas, high amounts of calcium can reduce the negative effects of sodium chloride (**Cramer & Läuchli, 1986).**

The salinity of irrigation water is sometimes reported as the total salt concentration or total dissolved solids (TDS). Electrical conductivity (EC), is a much more useful measurement than TDS. The salt content in the water is directly related to the EC. The EC can be reported based on the irrigation water source (ECw) or on the saturated soil extract (ECe).

Transform infrared spectroscopic analysis have highlighted amino acid-metal interaction in single metals ion solution and found responsible for sorption phenomenon **.**

Higher terrestrial plants can also be used for environmental restoration by the use of phyto-stabilization and phyto decontamination. Certain plants are hyper accumulator which generally uses either the extraction, stabilization, immobilization, volatilization or degradation as the mode for the remediation of contaminated sites. However, such plants have very slow growth rate and there are also limits to plant growth due to additional factors like temperatures, soil type and water availability. Certain biotechnological approaches such as ecological improvement of rhizosphere and use of genetic engineering for improvement of plants for bio remediation have been successful. Genetic engineering is being employed for metal sequestering, transporting and modifying biomolecules for metal sequestering proteins and peptides and enzymes to enhance phytovolatilization.

Fungi secrete a variety of extra cellular enzymes that facilitate the decomposition of some pollutants. *Phanerochaete chrysosporium, P. sordia* and *Trametes hirsuta* degrades pentachlorophenyl in soil and a number of toxic genobiotics. The basidiomycetous fungus, *Plesuratos streatus* produces fungal laccace which degrades atrazine and orgonophosphorous. *Aspergilus terrus, A. flavus, A. niger* and *A. fumigatus* have the ability to decolourise dye.

Decolorization of dyes is mediated through fungal enzymes (**Devi & Kaushik, 2005).** Copper containing phthalocyanin was completely decolorized with in 7 days by *Phanerchaete chrysosporum*. The possible mechanism of decolouriztion could be dependent upon functional dye molecules, fungal biomass and enzyme mediated fungal metabolism (**Fu & Viraraghavan, 2002).**

Bacteria are also good degraders of toxic pesticides such as halocarbons, polychlorinated triphenyl trichloro ethylene. *Pseudomonas putida* degrades aromatic hydrocarbons. Methanogenic bacteria degrades perchloro ethane. Mono & dichloro benzene can be degraded aerobically by-*Pseudomonas* and *alcaligenes* strains.The ability of anoxygenic phototrophic bacteria in organic and inorganic compound degradation and waste recycling is well known, especially under anaerobic conditions (**Merugu *et al*, 2007**). The species widely used are *Rb. sphaecoides, Rb. capsulatus, Rc, gelatinosus* & *Rps. palustris*. They can remove H_2S, NO_3, sulphate and nitrate. The ability of *Rb. sphaeroides* to produce antiviral substances has found application in treating waste water. *Rhodobactur spheroides* can tolerate heavy metals like CO^{2+}, Fe^{2+} and MnO_4. RpS acidophilia is efficient in remediation of sewage. Its efficiency was evident by a decrease in BOD, COD and organic matter in treated water.

Interaction of heavy metals occurs in microbes. Bacteria transform and convert metallic ions by reduction in different polluted environments. The metal resistant micro organisms from polluted environment can serve as indicators of potential toxicity to other forms of life mechanisms. Heavy metal tolerance of marine bacteria was studied by **Das *et al* (2007).** Generally Gram negative were more tolerant than Gram positive. Micro-organism undergo selection pressure in the presence of toxic compounds and develop resistance. In metal polluted habitats, the frequency of tolerant bacteria increases. The development of tolerance, however, is associated with the concentration of both total and soluble metals. Soil having higher clay and humus, absorb larger amount of heavy metals due to its better cation exchange capacity. The status of $CaCO_3$ and soil pH are also crucial factors in term of availability of heavy metal in sewage irrigated soils. The sewage irrigated soils have been found relatively low in Ca content but variable amount of metals like Cd, Cr, Cu, Pb and Zn. There is a negative correlation between pH & $CaCO_3$ with heavy metals. Long term addition of sewage-sludge & pathogenic processes can result in elevated heavy metals concentration in the soils (**Mani & Kumar, 2006).** Higher retention of heavy metals was correlated with the

organic matter. Recycling through plant uptake and sewage-sludge deposition is a dominant process in the long term retention of metals in the soil. Treated sewage effluent should have a suspended solids not exceeding 30 mg/L and a BOD not exceeding 20 mg/L. This standard is often referred to as the 30:20 standard. Effluent complying with the 30:20 standard is generally safe.

Generally a water treatment system consist of rapid mixed unit flocculation and filtration and performance of each unit is interdependent. A design of such an order is possible through non-linear programming & dynamic programming (**Gupta & Shrivastava, 1997).** Recently, genetic algorithm has been used which is based on mechanism of natural selection and natural genetics. It is a stochastic procedure.

The transaminases AST & ALT have been studied in *G. max* seeds germinated in distilled water, 10mM NaCl & 20mM NaCl. Samples were obtained on day 1, day 3, day5 & day 8 of germination. Enzyme activity was studied with respect to the treatments, durations of germination & replicates. It was thought necessary to analyse the replicate effect because of the two limitations of this experiment. Firstly, no attempt has been made to purify the enzymes of the germinated *G. max*. Secondly analysis was performed on the entire germinated plant, that is, no attempt has been made to separate the root, shoot & cotyledon components & analyse the activity separately. Thus, there are greater chances of sampling error.The statistical design, therefore, required an analysis of variance to analyse the effect of replicates & was determined as "Between replicates".

The replicate effect was not significant but considered on the basis of coefficient of variation, much of the experimental error in the ANOVA may have been incorporated due to replicate effect.

Statistical analysis showed that, but for few analysis the differences are not significant, however, the trend of decline in the activity with the duration of germination is obvious. The graphical representation clearly shows that there is definite trend of progressive decline in the activity of the enzyme with the duration of germination. It is also evident that the activities are higher in the control group. Though transaminases in the plants are ubiquitous but the specific functioning of the AST & ALT are not much defined. The transaminases are stimulated by cofactors. Pyridoxal phosphate (PLP) stimulated AST by 50% while it did not have any stimulatory effect on ALT . The effect was only with the purified samples.

Pyridoxamine phosphate activated the enzymes much more slowly. Pyridoxal phosphate has no effect on crude enzyme . AST from phosphate deficient plants exihibited PLP stimulation in crude extract also. Whether metabolic inhibition by inhibitors have any role is questionable. Since, transamination steps have rarely been identified as rate limiting in the metabolic pathway of plants. Divalent metal ions did not improve the rates of transamination, nor did chelating compounds inhibit the activity . **Splittstoesser 1970**, however, found significant effect but not complete inhibition by EDTA of AST. This inhibition could be counteracted by Mg2+. In an unpurified enzyme, as in the present investigation it is difficult to be certain whether the reactions are all being catalyzed by the same enzyme or by different enzymes present in the preparation.

Cruickshank and Isherwood;1958 established that wheat germ AST& ALT activities, which they did not separate, differed in their dependence on supplied PLP and their response to inhibitors. There is a general tendency for the affinity of the keto substrates to be greater than the affinity of the amino acid substrate. However, it is difficult to predict the physiological direction of the reaction solely on the basis of kinetic data. AST will probably consume glutamate during period of growth and active nitrogen assimilation. In senescent tissue, or in the other cases where protein and amino acid degradation are going on, these enzymes may operate in the other direction to generate glutamate which can be oxidatively deaminated. 2-oxoglutarate via a transamination may permit assimilation to continue in the absence of net 2-oxoglutarate synthesis. Amino transferases are also of undoubted importance in the degradation of amino acids, where they catalyze conversion of amino acid to oxidizable oxo acids or aldehydes. The mechanism may provide one means of metabolizing aspargine aspartate shuttle depends on amino transferases activity both inside and outside the chloroplast .

The enzymatic transfer of amino group plays an important part in many metabolic processes where the inter-conversion of nitrogen containing molecules is involved. Nitrogen following its initial assimilation in glutamate and glutamine can be distributed to many other compounds by the action of amino transferases. The final step in the synthesis of several amino acids is a transamination of the 2 keto analogue of the amino acid .For example, alanine formed from pyruvate and aspartate from oxaloacetate.

Glutamate is often the amino donor substrate in bio-synthetic transamilation reactions, with the result the 2-oxoglutarate will be a product. 2-oxoglutarate is a substrate for nitrogen assimilation by both the glutamate dehydrogenase and glutamate synthetase routes. Glutamates-utilizing transamiliations therefore, regenerate the carbon precursor for ammonia assimilation.

Further part of the discussion is an overview, constraints in designs and experiments of such investigations, technological advances in instrumentation, application of artificial neural network and genetic algorithm, microbial genetics, mutant production and screening for salinity tolerance and future scope of the work to improve the productivity of soya bean using a molecular breeding approach. It is also a brief presentation of the inter-disciplinary approach which would help to derive a definite conclusion from experimental work.

In most of the experiments in pot culture,hormonal spray,& even the levels of saline solution treatments,the quantity of the ingredients is arbitratily set.At certain limit, optimization can not be obtained by analytical procedure.

An optimization beyond the limits of analytical procedure can be deduced from the derivation of artificial neural network (ANN) & genetic algoritham. A neural network is a massively parallel distributed processor made up of simple processing units which has a natural propensity for storing experimental knowledge and making it available for use. A neuron is an information processing unit that is fundamental to the operation of a neural network. A two-layer ANN with sigmoid axon transfer function was used for input and output layers.

ANN utilizes interconnected mathematical neurons to form a network that can model complex functional relationship (**Saha & Edwards;2007).** A two-layer ANN model using a back propagation (BP) algorithm may be used to predict the reduction of nutrients like Na^+, K^+ in various parts of the plant **(Kardam *et al,* 2010).**This can also be used to deduce the reduction of phytohormones in various organelles of the plant.

Aftificial Neural Network model based on the combination of back propagation and principle component analysis can predict the reduction in nutrient elements/hormones uptake with a sigmoid axon transfer function. The Levenberg- Marquardt algorithm provides minimum mean squared error for training and cross validation(**Kardam *et al,* 2010).**Optimization of the ANN structure was utilized to predict the reduction of Cd uptake by zea mays in the range of metal concentration with which experiments was not conducted. Varying the number of

hidden layers and the number of nodes within, based on the cross validation technique, the optimal neural network configuration was ascertained. The performance and prediction of neural network simulation was optimized to obtain minimum mean squared error and was examined for its ability to predict the response of experimental data not forming part of the training program.A simple back propagation of the recurrent network using the momentum training algorithm is effective to generalize and predict the degree of reduction .It is proved as a meaningful supplement for the complicated mathematical models in the prediction of bioprocess (**Kardam *et al*, 2010).**

It is well known that diffusion concerning water uptake in germinating seed and plant is of fundamental importance for the growth, development and crop production. Earlier models on water absortion were either based on the relative concept of water potential of water content and mostly assumed the seed to be of spherical geometry. Controversy has arisen on the use of the water potential concept .The accuracy obtained in the determination of water diffusivity of germination of seed was confined to the assumption of seed to be a sphere and therefore the data obtained were in errors .Most of the experimental values were determined under non-steady state conditions and were therefore not comparable.The seed germination experiments determined only the average time for germination and provided no information about the critical moisture attained during this time .

A proper co-relation needs to be established between the critical time for germination, critical moisture content, percent germination and crop production. Different genotypes of a crop differ in composition, structure and physiology. All experiments of diffusion of water, electrolytes & hormone etc must conform to the laws of diffusion.They must conform to the laws of Langmuir Adsorbtion isotheorm,Gibbs adsorbtion,Isotherm & Ficks law of diffusion.It is a wrongful presumption that seed subjected to water, saline or hormonal spray will absolutely penetrate the seed coat.Diffusion will depend on the shape, size & nature of the seed .This is proved by the experimental & mathematical derivation of diffusibility. A new theory of water diffusivity was formulated **(Sood;2007)** which was based on particle density gradient concepts rather than concentration gradient. The validity of the theory was established by carrying out experiments using genotypes of soybean.The concepts of critical time for germination and critical moisture content were developed and established on the basis of steady-state conditions. of germination of seed was confined to the assumption of seed to be a sphere and therefore the data obtained were in errors .

The theory of water diffusivity in which **fick's law** has been restated in terms of particle density gradient in place of concentration gradient is mathematically related to the flux and **diffusion constant (**D) as

$$J\ (t) = -D\ \frac{\partial N}{Na}\ I\ \partial X$$

Where N is number of water molecules per unit volume and N_A is the Avogadro 's constant for diffusion in germinating seed the theory developed

$$\overline{D} = \frac{I}{\beta seed\ t}\ In\ \frac{\Delta N\ o}{\Delta N\ f}$$

Where β_{seed} is the seed constant defined by

$$B_{seed} = \frac{A}{I}\ \frac{1}{V1} + \frac{1}{V2}$$

In which A and I are the effective area and pore length of the seed membrane/coat and V_2 and V_2 are the volumes of the water and that of the seed respectively. $\Delta N^0 = N_1 - N_2$ and $\Delta N = N^3 - N^4$ are the initial and final number of water molecules before and after the diffusion process and t is the time of diffusion in seconds. To determine D , β_{seed}, t, ΔN_o and ΔN_f must be known.

The diffusion concerning electrolyte/non-electrolytes of germinating seed involved called seed constant as β_{seed} and was a specific characteristic of seed coat/membrane of a genotype.

A new term critical time for germination t_{ctg} was obtained in water diffusion experiment and could easily be extended to any solution(**Sood ,2007**). Accordingly this critical time for germination is very specific for a particular seed genotypes since it is at this time the seed is assumed to attain a certain fixed moisture levels or content and called 'CM_c'. It is at this stage that the seed is at the peak of its physiological and biochemical activities necessary for

germination. The seed if tested for germination should germinate provided minimum moisture levels exists so that the seed after attaining the CM_e should not dehydrate otherwise there is a possibility of germination getting effected. The seed constant obtained for two genotype also clearly showed that these two genotypes were genetically different and they absorbed water at different rates **.**

It is possible to quantify the critical moisture content (CM_c) from the critical time for germination (t_{ctg}) which could be obtained from diffusion experiments for any seed of a genotype and of any crop. Water-relations in developing seeds, plants tissues, seed germination in soil and nutrients/water uptake in plant roots growing in soil etc. are also based either on water potential or water content(**Grange, & Finch;1992)**

A theory of diffusion concerning electrolytes/nonelectrolytes for germinating seed and pollen was developed . Diffusion in germination of seed on the basis of absolute particle density gradient produced by the actual number of water molecules rather than water potential or concentration gradients and water diffusivities in wheat and soybean varieties at 30^0C was developed .A correlation between the critical time for germination with the time of steady-state conditions obtained during diffusion experiments was observed (**Sood;2007)**

The characteristic of the seed (D) which depended upon the effective area available for diffusion , the average effective pore length (l) and the volumes of the seed (V_2) and of the water (V_2). The validity of the theory of water diffusivity was derived on the basis of particle density gradient rather than the water potential gradient for soybean at room temperature. The water diffusivity values obtained ranged(4-30) 10^{-4} cm^2/h i.e (0.0111-0.083$\bar{3}$) 10^{-5} cm^2/s (**Sood;2007**) .These were found to be $(0.0339)10^{-5}$ cm^2/s and $(0.0365)10^{-5}$ cm^2/s for the two genotypes viz. PK-416 and SL-295 at 30^0C showing clearly that the orders of magnitude was the same and the values was within the above range. From the results of seed constants, (β seed) and D values it was also clear that absorption of water by two soybean and wheat genotypes were not only governed by physiological factors but also due to seed coat/membrane characteristics . Seed coat/membrane to water penetration and subsequent germination has been demonstrated

.The time during which steady-state conditions was achieved for each genotypes of soybean and wheat seeds was the critical time for

germination since the seed at this stage is at the peak of its biochemical and physiological activities and must have attained the critical moisture content conducive for maximum germination.

Present investigation suffers from the limitation that the varieties and genotypes of the seeds were unknown.This may have led to logical variation in the results.It also needed to use known genotypes and further subjected to molecular technology. It,therefore required to analyze the relatedness among the genotypes,such a relatedness has been derived from a dendrogram which was constructed on the basis of similarity matrix representing Jaccard's coefficient using the UPGMA algorithm. Experiments have shown a weak correlation between morphological and molecular marker (**Li *et al*;2008 , Vetelainen *et al* ;2005,. Mwase *et al* ;2010**) The lack of correlation between morphological and molecular markers showed that most of the growth and morphological traits were influenced by several genes (**Vetelainen *et al* ;2005**).Molecular markers are neutral and thus do not reflect the diversity in functional characters **(Li & Jin ;2007)**. Morphological traits are under natural selection and their expression is partially under the influence of environmental factors . The use of DNA markers offers potential applications in plant pathology(**Hallerman & Beckmann ;1988**)Additionally, these markers can be used to detect and characterize quantitative trait loci (**Anderson *et al*;1994**), thereby enabling marker-assisted selection to be applied as an additional component to the selective breeding programme.

To generate molecular species-specific DNA marker, random amplified polymorphic DNA RAPD-PCR has been utilized, which involves DNA amplification using arbitrary short primer sequences (**Williams *et al* ;1990**). This also proved to be useful in genetic diversity and species identification studies. Nucleotide sequence and genomic organization is often species-specific as well as chromosome specific (**Willard & Waye ;1987**).Nucleic acid probe technology has given a new era in research and diagnostics. Non-radioactive Digoxigenin (DlG) labeled probes are more sensitive and effiecient compared to P^{32} or S^{35} lebeled probes DIG labeled probes , have limited half-life. These non-radioactive DIG labeled probes are useful in diagnosis and hybridization experiments using filter/ nylon membranes blotted with nucleic acid either by Southern transfer/Colony Transfer/Northern Transfer or by dot-blot. PCR based markers such as RAPD and ISSR are considered powerful tools for assessing the existing genetic variation at the species, genus and population level. These

markers are neutral to the environmental intlucnces, high in providing number of variations and do not require DNA sequence information of a species (**Agarwal *et al* ;2008**). Application of restriction fragment length polymorphism (RFLP)using chloroplast and mitochondrial DNA (**Pradhan *et al* ;1992)** and inter simple sequence repeat (ISSR) markers **(Warwick *et al* ;1992**) have been used. In recent years, several **genetic** loci in legumes that affect rhizobial root nodule **and** AM symbioses have been characterized through positional cloning.

Although these loci were defined initially as root nodulation mutants, they also limit AM infection .**Genetic** loci identified to date impairing AM symbiosis **and** nodulation include: a Leu-rich, receptor-like kinase (*dmi2*, *LjSYM2*, PsSYM19); a lig**and** gated ion channel (*dmi1*); **and** a calcium-calmodulin dependent kinase (*dmi3*,*PsSYM9/30*). Down regulation of the 50S ribosomal protein indicates the inhibitory effect of NaCl on soybean protein biosynthesis and presumably leads to the consequent reduction in plant growth.
Kinesin proteins are a large family of plus- or minus-end-directed microtubule motors important in intracellular transport, mitosis, meiosis, and development , control and maintenance of the cell cycle and cell integrity are critically important for cell-to-cell communication and signaling, especially under stress **(Oppenheimer ;1997**). To understand a basic or crucial role of a given gene product in gene regulation or signal transduction, protein-protein interaction study is fundamental. The widely used systems are to use yeast two hybrid (Y2H), biomolecular fluorescence complementation (BiFC), affinity pull-down coupled with mass spectrometry (AP-MS), and structural analysis of protein crystals.

Phylogenetic relationships within the family Brassicaceae have been studied using genomic sequence tagged microsatellite sites (STMS) .Crossspecies transferability of genomic STMS is reported in many crop genera and it has been observed that its frequency is highly variable among plants. However, transferability of rye markers to wheat and triticale has been observed to be 25 and 39%, respectively **(Kuleung *et al* ;2004**).Transferability of STMS markers with variable frequency has also been reported in Brassicaceae (**Saal *et al* ;2001, Yadava *et al* ;2009, Marquez-*et al*;2010**) Proteomic analyses performed of soybean seed proteins in mature seeds (**Mooney and Thelen, 2004**), seed filling and seed germination (**Xu *et al*., 2006**) revealed seeds and young seedlings were frequently confronted with much higher salinities than vigorously growing plants, because germination usually occurs in

surface soils, which accumulate soluble salts as a result of evaporation and the capillary rise of water. The adaptive physiological and biochemical responses of a plant to salinity are controlled by genes that encode salt tolerance mechanisms (**Casas** *et al*., **1992**). Since salinity tolerance is a complex trait, it is most likely controlled by interactions of hundreds of salt responsive genes (**Sahi *et al*., 2006, Winicov,1998).** Plants recognise a salinity stress and condition adaptive response mechanisms (**Hasegawa & Bressan, 2000).** Reported responses involve many molecular processes such as ion homeostasis (membrane proteins involved in ionic transport), osmotic adjustment and water regime regulation (osmolytes), as well as scavenging of toxic compounds (enzymes; **Benke *et al*., 2010, Blumwald *et al*., 2004)** signal transduction pathways that regulate plant ion homeostasis during salt stress (**Zhu;2002.).**

Soybean transcriptome atlases have been developed for deposit, download, or further study of transcriptional information (**Libault *et al*.,2010**) also, the database of SoyDB (http://casp.rnet.missouri.edu/soydb/) is specifically curated for soybean transcription factors.

The Arabidopsis COMATOSE (CTS) gene was originally identified in a genetic screen for loci that promote germination (**Russell *et al*., 2000**). CTS is a single-copy gene encoding a full-length ATP-binding cassette transporter that is required for the import of several biologically important molecules into the peroxisome, including not only very-long-chain fatty acids associated with breakdown of seed-storage lipids, but also precursors of auxin and jasmonic acid biosynthesis **(Hayashi *et al*., 2002; Theodoulou *et al*.,2005**). Several mutant alleles of CTS demonstrated an inability to complete germination, suggesting a role for CTS in the dormancy to radicle protrusion transition (**Russell *et al*., 2000; Footitt *et al*., 2002)**. The influence of CTS on gene expression in. the imbibed seed and to define the developmental status of cts mutants at the transcriptome level have been reported. Transcriptome analysis has previously been used to identify not only the stored (S) mRNA population and the effect of imbibition, but also the changes in gene expression in relation to dormancy status (**Nakabayashi *et al*., 2005; Cadman *et al*., 2006).**

Many of the problems that impede the productivity of *G.max* varieties have defied solution through conventional breeding approach. These include widespread moisture stress (> 65% of the area particularly under rainfed and dryland conditions), expanding salinity, new pests and biotypes of higher virulence and poor shelf-life. There is thus a distinct need for

innovative technologies to find solution to existing and emerging problems and thereby increase the overall productivity and stability of our major crop (**Gadwal;2003) .**The conventional breeding methods are based on heritability estimates & crossing between high yielding varieties is a long drawn procedure,moreover macro & micro environment largely decide the heritability of the characters as is evidenced by the following formula of-

Heritability estimate (h^2)

h^2 of a character =($\sigma^2_A + \sigma^2_D + \sigma^2_i$)/ ($\sigma^2_A + \sigma^2_D + \sigma^2_i + \sigma^2_{E1} + \sigma^2_{E2} + \sigma^2_{HE}$ + 2 cov HE)

Where

σ^2_A = Additive genetic variance

σ^2_D = Variance due to dominance

σ^2_i = Variance due To epistasis

σ^2_{E1} = Variance due to macroenvironment

σ^2_{E2} = Variance due to microenvironment

2 cov HE =Covariance due to heredity x environment

σ^2_{HE}= Variance due to interaction between heredity x environment

Relevance and need for biotechnology

The high-yielding technology that heralded the Green Revolution has, no doubt, rescued the country from chronic food deficiency and starvation but it has had its adverse effects too. The high input cultivation of rice and wheat has led to excessive water use and eroded soil quality. Limited variability for yield-related traits is showing down the progress in yield enhancement. Hybrid technology, through a potential technological option, has not yet become a reality in several crop plant for want of stable cytoplasmic male sterility-fertility restoration system. **UV Spectra –** Fluorescence spectroscopy could compliment UV spectroscopy.

337 and 410 nm-excitation of static fluorescence of seeds of the bitter gourd at immature (or raw) and mature (or ripe) stages of growth revealed developmental changes. In the 337 nm-excitation, emission bands appear approximately at wavelengths 410 and 450 nm, along with ones at 500 (in mature stage only) and 630 nm. The intensity of the 410 nm band became lower, while those of the 500 and 630 ones became higher at the mature stage of the fruit. For the excitation of 410 nm, bands appear approximately at 630 and 670 nm, along with the one at 500 nm. Intensities of 500 and 630 nm bands increased whereas that of the 670 nm one decreases. Fluorescence intensity ratios F 630/F 670, determined for the two stages show marked variations—a clear indication of the process of ripening the fruit.

Fluorescence is a phenomenon by which light absorbed by a system at specific wavelenth is emitted at a different and higher wave length. When a molecule absorbs energy (visible or UV) it accordingly goes to a specific vibrational level in the excited state, and before it reverts back to the ground level, it looses the excess vibrational energy by collision with solvent molecules or by nonradiative loss , dropping down to the lowermost vibrational level of the excited state. Due to this loss of energy it emits light at a higher wave length . The ratio of absorptions at 260 nm vs 280 nm is commonly used to assess DNA contamination of protein solutions, since proteins (in particular, the aromatic amino acids) absorb light at 280 nm. 260:280 ratio has high sensitivity for nucleic acid contamination in protein. **(Warburg, & Christian ;1942)**

Fluorescence being sensitive to environment of the chromophore has been known as a biophysical tool for getting structural, stereochemical information regarding molecular dynamics, ligand binding, solvent interaction, localization of ultramicroscopic amounts of rare substances in vivo and differentiation of living and dead cell. Fluorescence resonance energy transfer (FRET) between donor and acceptor reporter groups, being distance dependent is a technique used for measuring structural changes of biomolecules, relative motion and interaction between two different molecules. Single molecule FRET (sm FRET) can probe structural changes of biological molecules during biological events in real time. Single molecules can be detected by attaching a fluorescent dye at a well defined position in the molecule and cutting down the background light. During folding of RNA or proteins the two-state fluctuations (high & low) in FRET values can be seen as a function of time. Using three spectrally different fluorophores (three color sm FRET) it is possible to study changes involving more than one distance in complex biological system i.e. both, a conformational

change within the RNA and the binding of the protein to the RNA can be followed simultanously.

Based on the study of the intensity of the fluorescent radiation, pyrimidine and it's ribo counterpart are highly fluorescent.

A large number of fluorescent molecules such as xyribofuranosy 1-2.7- dioxopyridol are used as probes or reporter groups. If to a living cell excess of acridine orange is added, the value of the two ƛmax obtained are significantly different i.e. one for nuclear double stranded DNA (green fluorescence) and the other for cytoplasmic single stranded RNA (red fluorescence). Living cells take up SITS which is restricted to vesicles and fluorescences.

Synthetic sequences of DNA/RNA are becoming extremely important because of their multipurpose application. A large number of reporter groups are available as probes for nucleic acids as well as proteins like spin labels and triplet labels but fluorophores are mostly used because of their ease of application, stability, direct detection and discriminable emission spectra. Fluorescence provides sufficient sensitivity for real time optical detection of the small amount of DNA present in DNA sequencing gels ($\sim 10^{-15}$ mole per band. Single molecule FRET (smFRE1) enables to probe of structural changes of biological molecules during biological events in real time be used in future to characterize proteins , metabolic pathways, cell signaling or any biological phenomenon. FRET has nanometer sensitivity since it measures the molecular motion in its center of mass frame.

FRET has already been used to detect the Mg^{2+} induced conformational changes on a single molecule using Cy3 (donor) and Cy5 (acceptor) **(Kim *et al* ,2002 , Agalarov *et al* ;2000 ,Tsutsui.*et al* ;2008 , Benedix *et al* ;2009)).**

Biophysical and physiological mechanisms of damage by high temperature appears at all levels of structural organization in plants changes in transpirational kinetics involves hastened senescence and is manifested at whole plant level .Water loss from chloroplasts damage primary photosynthetic process membrane disorganization may lead to changes in the molecular mobility and others biophysical properties of tissues water . A rapid and nondestructive method for studying cell membrane thermostability and permeabitlity for heat stress might be through low resolution (^1H) .The changes in cell membrane permeability are manifested in changes in the molecular mobility and biophysical state of water which are in turn reflected in longitudinal and transverse relaxation characteristics of tissue water .

The membrane thermostability of leaf decreases with temperature rise . The relative injury (RI) of the cultivars increased with increase in temperature with maximum injury at 48^{o} C **.** Cellular membranes are composed primarily of proteins and lipids . High temperature not only denaturates membrane proteins but also cause lipids phase transitions and changes in these two components are likely to affect membrane structure and integrity. These are in turn expected to be reflected in the longitudinal and transverse relaxation times (T1 & T2) of leaf tissue relaxation characteristics which indicate the molecular mobility and biophysical state of water .Thus relaxation times of tissues water are influenced by a delicate balance between the total water content , macroscopic and microscopic distribution of water in different sites /phases . macromolecular-water interactions and exchange between different water phases are important consideration .

Field data analysis of Gmax and other crops

There are two water quality parameters to consider when assessing irrigation water quality for potential water infiltration problems. These are the ECw and the sodium adsorption ratio (SAR). The SAR is an indicator of the amount of sodium in the water relative to calcium and magnesium. Both a low salt content (low ECw) and high SAR can predict a high potential for permeability or water infiltration problems. A low ECw or high SAR can predict separately or collectively to disperse soil aggregates, which in turn reduces the number of large pores in the soil. These large pores are responsible for aeration and drainage. A negative effect from the breakdown of soil aggregates is soil sealing and crust formation. Soil and water salinity is recognized as the most important problem involved in crop plant establishment and growth. Transpiration and evaporation from the soil surface, low quality of irrigation water and lack of proper drainage are major causes of area land salinity leading to crop loss. A waste water agrocycling packages offers a low cost alternative but it has the potential toxicants and repeated application of such waters in agricultural soil may cause heavy metal toxicity & build up of salinity.

Kinematic analysis has provided important insights into the biology of growth by revealing the distribution of expansion within growing organs. Modern methods of kinematic analysis have made use of new image-tracking algorithms and computer-assisted evaluation, A new image-analysis program (Kine Root), has been developed to study spatio-temporal patterns of growth and curvature of roots. Detailed analysis of plant growth requires measurements that

capture the large spatial and temporal heterogeneity of the expansion and differentiation of plant organs. While measurement of the aggregate growth of a plant organ provides important information, such as overall growth rate and velocity, the spatial distribution of growth is not described by these measurements. Kinematic analysis-has been used to study the dynamics of physical motion (e.g. acceleration, velocity etc.) without reference to the forces resulting in the movement. Kinematic analysis has been used to study the influence of environmental factors on spatial and temporal growth patterns, e.g. effect of water stress(**Liang *et al*., 1997; Sacks *et al*., 1997)**, shoot irradiance, and temperature on maize (*Zea mays*) primary root elongation, and influence of nitrogen supply and water stress on leaf growth. Kinematic analysis has also been employed to describe the influence of biotic stress, such as aphid infestation, on elongation rate of shoot . Kinematic analysis has also been used to analyze the effect of phosphorus deficiency on the elongation rate of the primary root of *Arabidopsis thaliana* . Recently,**Van der Weele *et al*. (2003**) introduced a new computer-assisted technique that involved the combination of two methods, the structure-tensor (**Jahne, 1997**) and robust matching algorithms (**Black and Anandan, 1996**), to measure the expansion profile of a growing root at high spatio-temporal resolution.

To estimate the yield potential, **Maas and Grattan (1999**) have provided an extensive list of salinity coefficients for a number of horticultural and agronomic crops. These coefficients consist of a threshold and slope. The salinity threshold (a) is the maximum average soil salinity (ECe) the crop can tolerate in the rootzone without a decline in yield (salinity threshold value). The slope coefficient (b) is the percent loss in relative yield the crop will experience for every unit increase in ECe above the threshold. Using these coefficients, the yield potential (% yield) can be estimated from the following expression :

(Mass and Hoffmann,1977) **% Yield** = $100 - b\,(ECe - a)$

Where 'b' is the yield loss in % per unit increases of soil salinity ECe (1 ds m^{-1}) ranging from <3 % (ds $^{m-1}$) for salt tolerant to > 30 %/ (ds m^{-1}) for sensitive crops and 'a' is the soil salinity threshold value, ECe, where yield decrease starts (ranging from 1.5 ds m^{-1} for sensitive to 10 ds m^{-1} for salt-tolerant crops).

Pot culture study showed that salt stress decreases soybean growth and induces changes in growth attributes such as shoot length, root length, total weight (seed + plant) and Increasing

salt concentration of irrigation water restrict the growth of soybean grown on swell shrink soils due to increased salt concentration in soil solute as well as exchangeable sodium percentage .Increased quantity of available P and K was analyzed under P_2 and K_2 doses are due to applied higher doses of these nutrients. Salt stress increase soybean seedlings Na+ content but decrease K+ content . By virtue of having high spectral spatial, temperature and radiometric resolution, remote sensing offers unique potential to address both the structural and functional attributes of agriculture ecosystem more appropriately, including the fragility of dry land agriculture system (**Rao, 1995).** The measurements of reflectance in the red and near infrared (NIR) is being used to determine the differences in crop canopy densities, "vegetational indices" and crop growth measurement. Microwaves are sensitive to both soils and crop characteristics detects the differences in crop types, crop growth stage, crop height, biomass and leaf area index. Measurement of radiance in the thermal area can be used to derive the surface temperature of the crops. As the water transpires from the plants, its leaves are cooled. In absence of enough water, the surface temperature increases which is indicative of water status & crop water stress (**Jackson *et al*. 1986)**, Hyper spectral remote sensing has the potential to identify & measure specific carotenoids, weed species, crop water status, soil organic matter, soil texture & soil conservation.

The current satellite remote sensing capabilities for irrigation and water management include end-of-season evaluation of canal command areas at the disaggregated level and diagnostic analysis of the problem of distribution to enable follow up connective management.

It is possible to discriminate between irrigated and unirrigated crops. Further the irrigated areas have been differentiated on the basis of their source of irrigation (**Panigrahi & Ray 2004).** Since vegetation indices (vegetation indices are arithmetical combinations of spectral reflectance in different wave lengths especially red and near infrared) are indicators of crop growth, these can also be used for irrigated crop yield forecasting at different spatial scales. Remote sensing, through synoptic and repetitive coverage can monitor irrigated land degradation and salinization. From images of pre monsoonal period, agricultural salt-affected lands can be delineated based upon high reflectance, lack of vegetation or patches with poor crop growth. Water logged areas can be identified by using pre & post monsoon data because of their darker tone as compared to their surroundings.

Irrigation scheduling is a critical point in command areas. Proper scheduling of irrigation potential can be determined by a rapid non contact method of evaluating crop water status.

RS based method for timing of irrigation is based on stomatal closure as a result of water stress which results in elevated temperatures. Leaf air temperature difference may be an indicator of crop water stress One common measure of stress is Stress Degree Day (SDD) which is the delay value of the conopy-air temperature difference (Te-Ta) measured at the time of maximum surface temperature. TC can be measured by RS & Ta cam be obtained from meteorological data. Generally Te-Ta will be near zero or negative for canopies with adequate water and positive for water stressed conopies.

Net irrigation requirement is computed from crop water requirement after removing the contributions due to effective rain fall, ground water and available soil moisture. Crop water requirement can be expressed as :

$$\mathbf{ET = {}_{K}ET}$$

Where $_{K}$ is the crop coefficient which accounts for the effects of crop characterstics on crop water requirement and its value depends upon the crop charatistatics, time of planting or sowing stages of crop development and general climatic conditions (**Allen *et al.*, 1998**). ET is the reference crop evapotranspiration which can be estimated from meteorological variables like temperature, relative humidity and wind speed. RS of soil moisture depends upon the measurement of radiations that has been reflected or emitted from the surface. Variation in the intensity of this reflection depends on either its dielectric properties or its temperature or a combination of both . Moist soils have high absorption of radiation, Microwaves offer the best capability for soil moisture estimation where in the emission of the target is measured. In this method soil moisture determination is based on the contrast between the dielectric properties of liquid water (~ 80) and dry soil (~ 4). As the moisture increases the dielectric constant of the soil water mixture increases Remote sensing has also been used in hydrological modelling integrating RS derived soil map, crop planting period and leaf area index values in a hydrological model MIKESHE (**Singh *et al* 2003).** Increased temperature and higher rate of evapotranspiration may also result in lowering of groundwater table at some places. Change in rainfall volume and frequency and increasing events of intense wind may alter the severity, frequency and extent of soil erosion.

Modifying crop management practices, improving water management, adopting new resource conserving technologies (RCTs), crop diversification, improving pest management, better weather forecasting and harnessing the indigenous technical knowledge of farmers can mitigate the effect of climate. An urgent effort is required to improve crop nutrition and soil fertility management in India, integrating agro-ecological and socio-cultural aspects of the problem to avert worsening of a situation that is already desperate. Although the productivity has steadily increased to three times in the last 50 years but it remained stagnant during the last 15 years creating a plateau. Green revolution has turned agriculture more intensive by exploiting moisture & nutrients from the soils by high yield crop varieties. While arable land marginally increased (130 Mha to 140 Mha), productivity went up to three times. Destructive phase started with irrigated command area turning saline / water logged and rain fed areas witnessing massive soil erosion. Introduction of high yielding crop genotype coupled with inefficient and indiscriminate use of water, inorganic fertilizers and pesticides / weedecides has resulted in disruption of agro ecosystem.

Farmers are using unusually high levels of nitrogen fertilizers. There is also a negative trend of organic carbon depletion resulting in set back in production potential and soil health. Imbalanced use of nutrients has increased the production losses and has led to severe environmental consequences.

The agricultural sector has registered a very low or negative growth in 2000-01 and 2002-03. The major failure was the unavailability of adequate rain fall. In 1990-91, The annual growth rate of gross domestic products (GDP) of the primary sector which included agriculture was 4.6, then to a negative -1.1 in 1991-92, 0.6 in 1999-2000, 0.2 in 2000-01 and 2.3 in 2005-06.

For each of cereal grains harvested, 20-27 Kg nitrogen, 10 to 19 Kg of phosphorus and 22 to 48 Kg of potash and a sizeable amount of micronutrients are removed from the soil. There is a deficit of about 10 million tones of nitrogen, phosphorus and potash per year in the country. Almost similar trend is prevailing in Bundi. The majority of the Indian soils are already low to medium in available nitrogen and phosphorus. Zinc deficiency is widespread. Sulphur deficiency has also emerged in the last few years primarily due to shift from single super phosphate to DAP and due to little application of the organic manure. The magnitude is related to the introduction of high yielding varieties, neglect of application of bulky organic

manures, imbalanced use of chemical fertilizers indiscriminately. There is also a negative trend of carbon depletion resulting in setback in production potential and soil health.

Agriculture in Bundi covers less than 2% of the cultivated area of Rajasthan. Pulses contributes less than 2% of the State production. Oil seeds of course, have contributed to more than 3% of the state production. Almost 10% of the land is usar and 5% in not usable for agriculture. This may further increase due to soil salinity & soil sodicity in irrigation command area. Extraction of more nutrients from soil than that being replenished and disprotionate and indiscriminate use of fertilizers is creating nutrient imbalance in the soil.

Canal command area irrigation has resulted in soil and water salinity due to a number of factors such as amount of soluble salts, soil type, rainfall water table condition and water management practices. Salinity adversely affects the plant growth through osmotic effect of excessive salts in the soil and through excessive concentrations , adsorption of the individual ions which prove toxic to the plants. Most critical stages are germination and early seedling establishment, followed by phase changes from vegetative to reproduction.

Saline irrigation adversely affects plant growth through osmotic affects of the excessive salts in the soil solution and through excessive concentration & absorption of individual ions which may prove toxic to plants and / or retard absorption of other essential plant nutrients. An interplay of factors like nature and content of soluble salts, soil type, rainfall, water-table conditions, nature of crops grown and water management practices, governs resultant salinity build-up vis-à-vis crop performance.

Accumulation of salts is nearly one-half that of the irrigation water in coarse textured soils. It is equal to that of irrigation water in medium textured sandy to loam soils, and more than two times in fine textured soils.

It is necessary that use of saline water should be avoided in initial stages of crop growth.

Crop productivity must be stabilized against climate change by adoption of all possible measures, including better water management on the one hand and induction of agricultural technologies that meet the challenges of warmer temperature and local scarcity on sudden excess of water, on the other hand. There is need for new crop varieties to meet the climate changes.

Since rainfalls are spatially very variable. A large number of rainfall based techniques for quantification have been used, which varies from analysis of rainfall data to hydrological

balance indicators. But use of Advanced Very High Resolution Radio meter (AVHRR) for regional drought assessment using temporal pattern of Normalized Difference Vegetation Index (NDVI) has given a near close approximation . Data from other sensors such as IRS, Wide Field Sensor (WIFS) . FAO has started producing a ten-day (decadal) global vegetation index .

A comparison of decadal SPOT-vegetation composite NDVI, across three years 1999-2000, 2000-2001 and 2001-02, for East Rajasthan revealed both higher and lower levels of vegetation being significantly more than that observed in 1999-2000 and 2000-01. Early increase in slope of the vegetation curve was ascribed to early on set of monsoon in 2001-2002. The peak in NDVI in July was ascribed to maximum rainfall during the month. Early cessation of monsoon in 2000-01 resulted in significantly poor ensuing rabi season. Intermittent rainfall till late october had sustained to appreciable 1999-2000 rabi season.

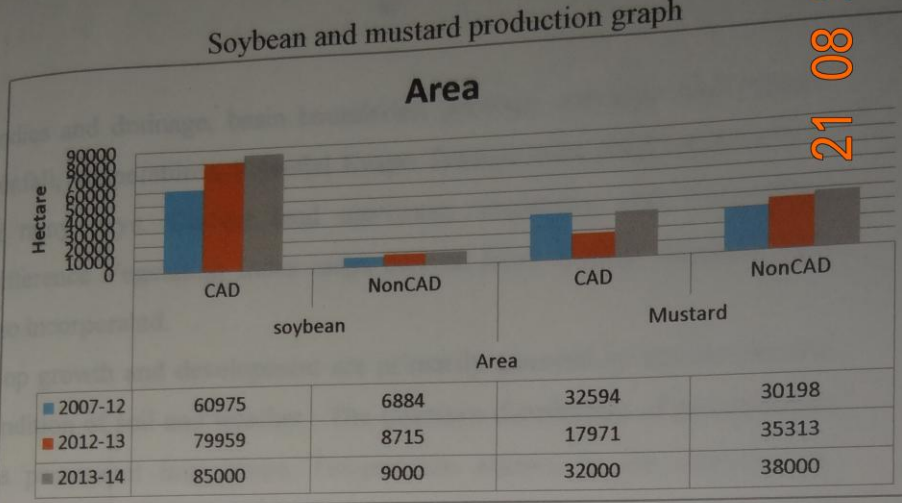
A Biogeographical Information System (BGIS) has been developed ,which provides detailed information on bio resources and their environment. The approach employed is to map independently the environmental attributes of a biological species and its spatial distribution over space and time. Thematic maps on geographical and environmental parameters in BGIS are prepared based on survey of India (SOI) topographic sheets. The themetics include altitude / contour, water bodies and drainage, basin boundaries, geology, soil type and texture, rainfall, temperatures, Potential Evapo Transpiration (PET) and number of rainy days. Current land use/cover, vegetation and Normalized Difference Vegetation Index maps derived from satellite imagery were also incorporated.

Crop growth and development are primarily governed by environmental condition of soil and weather . The quantum distribution of precipitation has paramount importance. Temperature regime in the reproductive phase becomes critical. Coefficient of variation (CV) indicates that CV values are large exactly in the area where annual rainfall is least. It exceeds 40% to 80% in the arid region of Rajasthan.

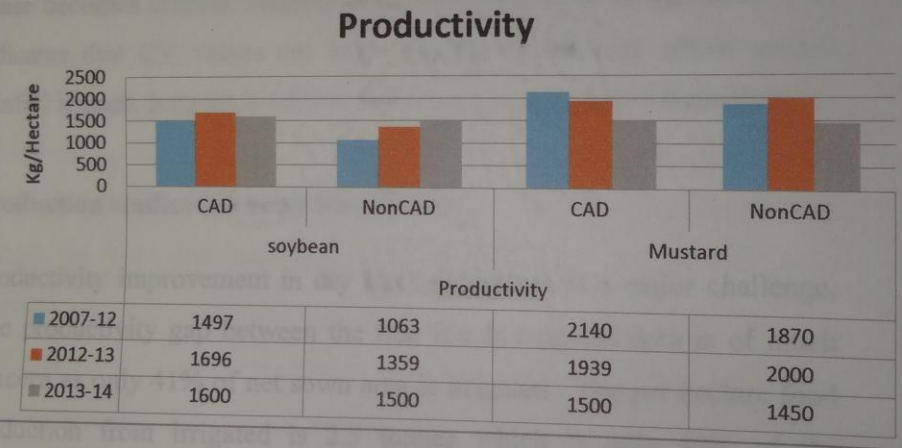
Productivity improvement in dry land agriculture is a major challenge. The productivity gap between the rain fed & irrigated area is of much concern as only 41% of net sown area is irrigated . The per hectare food production from irrigated is 2.5 tonnes which is only 40% of the realizable potential. The productivity in rain fed area is just one tonne.

Figure: 16

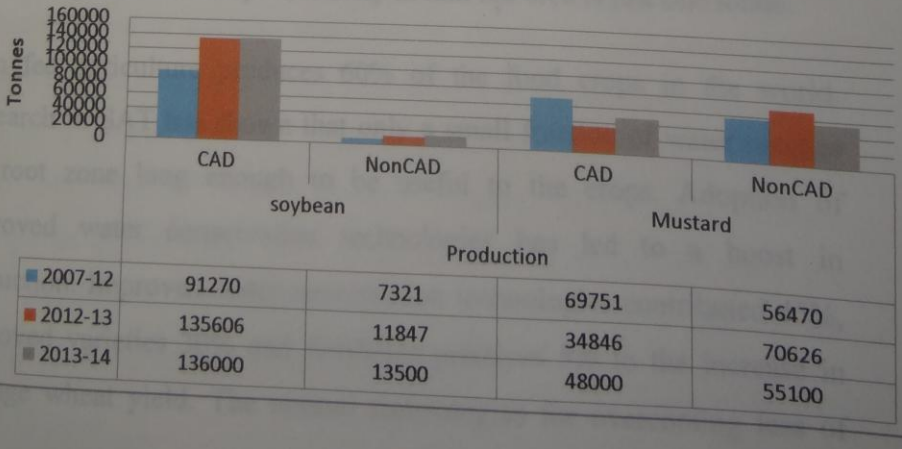
Soybean and mustard production graph



2007-12	60975	6884	32594	30198
2012-13	79959	8715	17971	35313
2013-14	85000	9000	32000	38000



2007-12	1497	1063	2140	1870
2012-13	1696	1359	1939	2000
2013-14	1600	1500	1500	1450



2007-12	91270	7321	69751	56470
2012-13	135606	11847	34846	70626
2013-14	136000	13500	48000	55100

Rain fed agriculture produces 60% of the food crops in the world. Research has shown that only a small fraction of water reaches the root zone long enough to be useful to the crops. Adoption of improved water conservation technologies has led to a boost in production. Improved water conservation technologies contributed 45%, improved varieties 30% and fertilizers practices 5% to the increase in average wheat yield. The normal technologies for overcoming loss of rain fed agriculture are the well known soil , water and soil conservation techniques. The principal requirement is to improve infiltration of water holding capacity and water uptake efficiency in plants. Sub soiling coupled with manure could lead to four told increase in yield of crops.

The moisture storage capacity of soil and hence the choice of crops, isopleths of normal moisture index, which takes in account normal rainfall and normal potential evapotranspiration and normal isotherm of soil maps is essential for the choice of production of crops .

Hydrophysical properties of soil, moisture retention characteristics and hydraulic conductivity decide about the availability of water to plant roots. As moisture content decreases, hydraulic conductivity decreases more steeply in coarse textured soils. The greater the prolification and density of roots, the lesser is the sensitivity of the crop to soil water depletion.

Future Strategies For Soybean Genomics

Analysis of SSR, RFLP, and SNP markers indicate that these breeding efforts have captured approximately 70% of the genomic diversity of soybean (Gene-rich regions have been observed in soybean using hypomethylated RFLPs as signatures of genic regions as compared to SSRs. Of more than 2,000 BAC-end sequences examined, RFLP-associated sequences had only one-half as many repetitive sequences and 50% more genic sequences compared with SSR-associated sequences. Based on one segment of soybean sequence more than 330 kb in length, estimated gene density to be as high as 1 gene/5 kb .

Current mapping data has been compiled from numerous populations from across several maturity groups. The maps need to be expanded by the development of 1,000 to 2,000 more sequence-based markers, thus bringing the total number of molecular markers on the soybean map to nearly 4,000.

Such a genetic map of sequence-based markers that could be applied to other legumes would facilitate the translation of genetic information from one species to another. As few as 150 markers would provide the genetic framework to connect genomic regions among select members of the family. Molecular breeders suggest a concept of "breeding by design" as a targeted, efficient, and comprehensive strategy to elevate the salt tolerance of soybean (**Peleman and Dvander Voort 2003; Wan 2006).**

Summary-

Introduction

There are about 8.6 mha salt affected soils in the country. Soil salinity is a highly dynamic soil condition varying in time and space depending on factors such as rainfall and irrigation. Salinity has become a matter of concern in irrigation command areas. A computerized data based in digital format for salt affected soils of Rajasthan in a scale of 1 : 250000 using Integrated Land & Salt Management (ILSM) software. Spatial decision support system (SDSS) models were validated to suggest the optimal decision of land and water allocation for improving water productivity in water logged saline situations.

Computerized data base of salt affected area show that in Rajasthan they occupy 3.75 lakh ha and are primarily located in the arid plains (269,177 ha) and mostly saline (39%) and saline sodic (59%) in nature. Physiographically, alluvial plains comprise 19698 ha, aelofluvial/aeolian 269177 ha and others 86067 ha respectively **(Mandal & Sharma, 2005**). In some of command areas, there are seasonal changes in their salt composition.

Salt affected soils have great potential for carbon sequestration. Green manuring and farm yard manure improve the micro biological property such as dehydrogenase activity and microbial biomass carbon in the alkali soil (**Chawla & Chabara 2005).**

An experimental study using saline ground waters along with organic matter (FYM) was conducted with wheat & guar (**Sharma & Singh 2005**). Saline ground water used were of 3.5-4.3 and 7-9 ds m^{-1} respectively. Grain & straw yield of wheat decreased with the increase of irrigation water salinity. Assuming wheat yield with best saline water irrigation as the potential value (100%) the mean relative grain yield of wheat irrigated with highly saline water was 78.6%. Substitution of best available saline water at first post-plant irrigation and

applying there-after only high salinity water increased the yield to 83.1%. Organic matter application had a significant effect on the grain yield of the wheat. Use of saline water during the winter season increased the soil profile salinity but the accumulated salts were leached down during the ensuing monsoon period.

For an effective agricultural production in irrigated area through the LMC of CAD, it is necessary that the following practices be adopted:

(i) Extensive soil survey including morphological and laboratory studies of soil profiles. (ii) Measurement of water tables and analytical results of water samples from CAD and ground water (iii) Soil erosion survey (iv) Mapping and delineation of areas on profile and soil conservation unit basis (v) Classification of the area into land capability classes and (vi) Modernise agricultural practices.

Agriculture productivity in areas with conjunctive use of canal and ground water is dependent on several factors including the quality and quantity of ground water. Canal command irrigation has resulted in soil and water salinity due to factors like soluble salts, soil type, rain fall and water management practices.

At present , most of agriculture farming is being done through ground water .Indiscriminate exploration of ground water have not only severely depleted the ground strata but has resulted in water salinity .

Growth of population, massive urbanization, rapid rate of industrialization has led to water pollution. Effluents from industries contain organic and inorganic compounds, acids, alkali, suspended salts and other materials. Such discharges have high biological oxygen demand (BOD) and chemical oxygen demand (COD).

Mushroom population growth has generated huge amount of sewage which contain potential toxic elements, contaminants and pathogenic bacteria. Farmers are forced to use industrial effluents and sewage for agricultural production. Attempts has, therefore, been made to study the effect of salinity , industrial effluent and sewage on the germination of seeds of soybean *(Glycine max).* Certain biochemical and other aspects of the germination and plant growth have also been investigated. The following experiment have been conducted .

1. The effect of different molar concentrations of saline water on the germination.

2. Effect of industrial effluent, sewage and activated charcoal treated effluent and sewage on germination has been studied.

3. Enzymes involved in energy tranduction and transamination during the process of germination.Changes in the electrophoretic protein patterns during germination.

4 Absorption spectra of germinating seeds in ultra violet range.

5 Effect of phyto-hormone with conjunctive use of saline water on seed germination.

6. Histological changes in germinating seeds due to salinity .

7. Effect of saline water irrigation and different proportion of sodium and potassium fertilizers on the growth and associated character of the plants – A pot culture study .

8. General production studies of the field data: Study of soybean vis-à-vis other crops in Bundi district.

Pot Culture Study :

Effect of saline water on growth of soybean in integrated P and K fertilizers grown on swell-shrink soil of Hadoti region was studied .

A completely randomized design was used with three replicates and a total of twelve treatments. Non saline irrigation water served as control , the levels of salinity comprised of Ec 0.24 dsm $^{-1}$ (control) ,7.8 dsm $^{-1}$ and 14 dsm^{-1} , two levels of $P_2 O_5$; 20 and 40 kg ha $^{-1}$ and two levels of K_2O ; 15 and 30 Kg ha^{-1} . Growth studies included shoot weight gain , root weight gain , ash content of the root, shoot and leaves on 30 ,45 and 60 days . After sowing of seeds exchanging sodium potassium (ESP), electrical conductivity (EC) , pH and the available P2 O5 and K2O in the soil was determined . Increased salt concentration over 0.24 dsm-1 retarded the growth of root and shoot length response of applied $P_2 O_5$ and K_2O from 20 to 40 Kg- Ha -^{1} and 15 to 30 kg ha -^{1} had favorable effect on growth. Sodium content increased significantly with increasing EC over 0.24 dsm -1 .

Increasing levels of salt in irrigation water adversely affected the seed germination ,plant growth and restricted the availability of P and K nutrients significantly. Available P and K were maximum under the regime of applied higher doses.

Germination studies

Effect of 10 mM ,20 mM ,50 mM ,100mM ,500mM,750 mM , 1M and 2M NaCl on the germination of *Glycine max* was studied. Distilled water served as control. Length of root, shoot and cotyledon was measured on the day 5 and day 8. There were little differences between the control, 10 mM and 20 mM saline concentrations on the length of the root, shoot and cotyledon, though a slight increase in case of control was noticed.

There was drastic retardation in the length of each parts with higher concentrations of salinity. Not only there was severe retardation, in the length but it also resulted in a significantly reduced germination rates .

Mean germination rates into 2 M, 1M, 750 mM, 500 mM as against to control were 24%, 32%, 64% and 76% respectively.

The length of root and shoot of seed germinated in 2M NaCl were 58% and 30% shorter respectively as compared to control .

Higher saline concentration not only retarded the growth on day 8 as compared to day 5 but also registered as increased mortality.

Effect of phytohormone on germination :

Germination was observed using 1 ppm ,2 ppm of cytokinin and 1 ppm ,2 ppm of auxin in combination with 50mM ,100 mM and 500 mM NaCl. Growth of root and cotyledon was observed on day 5 and day 8 . In another experiment, effect of both the hormone after two spraying on the length of root and cotyledon was recorded at day 8. The first spraying was done on the day 1 and second was on day 5.

At a concentration of 50mM NaCl , there was not much difference in the growth due to auxin and cytokinin spraying.

There was differential response of auxin and cytokinin at 500 mM . Cytokinin was more effective at 100mM NaCl.

The responses of two levels of cytokinin at 500 mM was quite distinguishable .

The effect of two spraying on the growth of root and cotyledon was highly significantly responsive . Growth was increased and profuse in root and cotyledons .

Industrial effluent and sewage

Effect of industrial effluent and sewage on the germination of *Glycin max* on day 5 and day 8 was recorded distilled water served as control . Germination was also studied after treating the industrial effluent ,sewage and control with activated charcoal . Other parameter were recorded in the industrial effluent were Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) ,Total dissolved solid and pH. Electrical conductivity and pH was also recorded in pretreated and post treated industrial effluent and sewage .

It was observed that the mean root length , shoot length and cotyledon length were greater as compare to industrial effluent and sewage .Highly significant difference was observed in case of shoot and cotyledon .

There was significant increase in all these parameters on day 8 as compared to day 5 , however control value was always higher than day 5 in effluent and sewage values. There was a marginal difference in the root , shoot and cotyledon between sewage and industrial effluent on day 5 and day 8 .

Treatment with activated charcoal resulted the best growth in length of root ,shoot and cotyledon in all the treatments (control ,effluent and sewage) .

Activated charcoal has the distinct property of absorbing the suspended solid dissolved substances and heavy metals . A reduction in the size of the root, shoot and cotyledon of activated charcoal treated samples could be due to removal of metals which otherwise would have been beneficial for the growth.

Sewage water , supernatant obtained after treatment with activated charcoal and the residual precipitate subjected to Gram's staining and standard plate count . Sewage and treated sewage contained gram positive rods and non hemolytic bacteria. The residue also contained gram positive cocci .

The bacterial colony counting revealed that bacterial load was drastically reduced. Bacteria remained up to 10^{-3} serial dilution .

Biological Oxygen Demand is a measure of oxygen used by microorganism to decompose wastes. The value of 18 mg per liter in the effluent was within the range of secondary effluents. The chemical oxygen demand measures the chemical oxidation of the effluent / sewage by oxidizing agents . The value of 80 mg per liter appears to be high .There was a change in pH and electrical conductivity in pre and post treated effluent and sewage.

Ultraviolet absorption spectra (UV)

Absorption spectra in ultraviolet range from 240 milimicron to 340 milimicron was recorded in root ,shoot and cotyledon of *Glycine max* germinated in distilled water (control), 10 mM and 20 mM NaCl on day 5 and day 8 . The ratio of λ max of DNA (260 milimicron) and protein (280 milimicron) was calculated for each of the part (λ 260/280) .A λ max of 260 is normally used for the estimation of DNA and for harvesting DNA molecules while λ max 280 is absorption maxima of proteins. A ratio of λ 260/280 provides information on the general trend of changes in nucleic acid and protein metabolism. The perusual of 260/280 ratio revealed that the pace of change in all the parameters and durations were almost similar indicating that the metabolism transformation in proteins and nucleic acid were equal magnitude however this is an approximation only .

The absorption maxima 260 and 280 were ill defined . It was expected since the proteins and nucleic acids were not purified . An interesting finding is the presence of intense λ max at 300 milimicron . The metabolites at 300 milimicron could not be ascertained .

Histological changes :

Histological changes in the seeds germinated in 500 mM and 1M NaCl on the day 5, it was compared to distilled water. Sections of 7 milimicron were subjected to ascending and descending grades of alcohol and after final processing, were stained with hematoxyline , eosine and also with 0.25% toluidene blue. Sections were examined at 10 X and 40 X magnification. There were no discernible differences between the treatments in either of the staining methods.

Enzymes

Mg^{2+} ATPase :

Graphical representation of Mg^{2}+ ATPase revealed a declining trend in the activity of the enzyme. Lowest value was obtained on day 5 specially at 10 mM saline concentration. Similar trend was observed, decreasing trend with the duration of germination in case of Ca^{2}+ ATPase . There were marked differences in the enzyme activity having highest concentration in control followed by 10 mM saline and least in 20 mM saline concentration on the first day. However, there was no difference in the declining phase in the all three treatments .

Ca^{2+} ATPase showed a significant decrease in 10 mM saline as compared to control on day 1 , day 3 and day 5 .

Both calcium and Mg^{2+} ATPase show significant decline on day 1 in 20 mM NaCl concentration .Ca^{2+} ATPase also decreased on day 8 .

It has been succinctly proved that $Na^{+} – K^{+}$ transport ATPase does not exist in *Glycine max* based on the following two evidences :

1 . The activity of the Na^{+}- K^{+} ATPase was negligibly low .

2. The enzyme was not inhibited by cardiac glycoside dioxin which selectively inhibits the $Na^{+} – K^{+}$ transport ATPase in mammalian system .

The role of Mg^{2+} and Ca^{2+} in the process of homeostasis is well documented and the involvement of Ca^{2+} ATPase and Mg^{2+} ATPase in the regulation process is quite likely.

The enzyme L-aspartate : 2-oxoglutarate amino transferase ; GOA (Glutamate-oxalo acetate transaminase ,GOT/AST) catalyzes the transfer of amino group of aspartic acid to α-keto glutaric acid with the formation of glutamate and oxalo acetic acid .

The enzyme α –alanine : 2-oxoglutarate amino transferase (Glutamate pyruvate transaminase :GPT) catalyzes the transfer of amino group of alanine to α-keto glutaric acid with the formation of glutamate and pyruvic acid.

Both the enzymes catalyze reversible reaction and hence the substrates may enter constituent of TCA cycle for further oxidation.

The declining phase in the activity of AST was noticed in the all treatments, that is, control , 10 mM and 20 mM NaCl with maximum decline on day 8 .

While there was a steady decline in the activity of ALT in control samples from day 1 to day 8 , the decline was sharp in case of 10 mM and 20 mM saline . Highest decline was observed in case of 10 mM saline .

Other salient findings were |:

A significant decrease in the enzyme activity at 10 mM NaCl as compared to control on day 3 and highly significant difference on the day 5.

AST level decreased significantly on the day 5 and highly significantly on day 8 at 10 mM concentration .

ALT level was highly significantly decreased on day 5 at 10 mM NaCl .

Significant decline in AST was observed on day three at 20mM NaCl and highly significant decrease on day 5 and day 8.

Highly significant decline on day 5 and day 8 at 20 mM NaCl was observed in case of ALT.

It appears that all the ATPases and transaminases have a significant role in the process of germination since there have been remarkable changes in the activity of these enzymes during the process of germination.

Protein Electrophoresis :

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) was accomplished on seeds germinated in distilled water and saline concentration of 50 mM , 100 mM and 500 mM on day 5 and day 8. Molecular weight analysis was performed using standard marker proteins. Percentage change in the intensity of protein bands was calculated using scion image software .

Proteins were resolved into different bands with varying molecular weight . Some of the bands , however , were diffused . There were differences in the intensity of the bands between

the degree of salinity and duration of germination .Some of the proteins were upregulated while others were down regulated during the process of germination .

There was complete absence of some bands on day 8 of germination indicating the role of regulator , structural and controlled genes under the domain of an operon .

Attempts have been made to assign the values of molecular weight to some putative enzymes .

General production studies and factors affecting production in Bundi district

Soils of tehsils of Bundi ,Kesohrai Patan ,Indergarh and Nainwa were serveyed for the level of nitrogen, phosphorus and potassium. Potassium level was high in all the tehsils. Nitrogen and phosphorus were less as compared to standard requirement in all the tehsils . Deficiencies of phosphorus and nitrogen was observed in most of the areas of Hindoli tehsil .

It was observed that, for an efficient growth and development of associated parameters in soybean level of 20 kg of phosphorus fertilizers and 45 Kg of potassium fertilizers hectare $^{-1}$ was necessary.

Inspite of the variation in the soil type in different tehsils, soybean has been grown in all the tehsils . This south east agroclimatic zone is mainly humid plain zone. Hindoli , Nainwa , Indergarh and Talera tehsils are either rainfed or having medium rain fall , The soil is medium textured . Talera is heavily irrigated and the soil is heavy textured. Keshorai Patan is heavily irrigated but the soil is calcareous. The main types of the soils are black, clay loam , loam and some other types also . Maximum percentage of black soil are in K.Patan and Talera blocks followed by Nainwa and Hindoli. Clay loam soil is highest in Hindoli followed by equal distribution in Nainwa and Talera ,lowest in K. patan .

Soil salinity is present in around 6000 ha of the district. It is mainly located in areas which are irrigated by left main canal. The soil and water salinity is increasing at an alarming rate now . Indiscriminate and over exploitation of the ever increasing number of tube wells have further aggravated the salinity situation.

Highest rain fed cultivation was in Nainwa . There has been consistent fall in ground water level with in the last 22 years. The fall ranges from -3.48 to -6.69 in various regions of the district. The highest per year fall was in Nainwa .

There has been a significant change in the pattern of cropping . A study of a period from 1974-75 and 2003-04 , reveals that there was decrease in rabi crop from 66.4% to 60 % respectively , areas in kharif crop increased from 33.6 % to 40% . The area sown in paddy crop declined from 4.7% to 2.2% . Jowar from 17.9% to 3.1%. Soybean was not known to this area in 1974-75 and it increased to 23.7% in 2003-04 . The area under wheat decreased from 31.4% to 24.8% . Mustard proved to be crop of choice which increased from 0.1 to 22.9% in 2003-04 .

There was significant difference in the trend of productivity (kg/ha) between canal irrigated and rain fed areas .

In 2011 ,wheat ,gram and mustard were grown in far greater areas ,productivity trend was almost 50% higher in wheat as compared to 2003-04 .

There has been remarkable increase in area of cultivation of soybean as compared to mustard . Cultivated area in CAD (command area) was almost 500 times more as compared to non CAD area . It is unfortunate that the productivity of soybean (kg/ha.) has remained constant in the last three years (2011-2014) . Boost in productivity is possible only if converging technologies are employed .A judicious application of fertilizers, use of high yielding varieties and improved technologies can increase the level of productivity.

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