CHAPTER III

RESULTS AND DISCUSSION

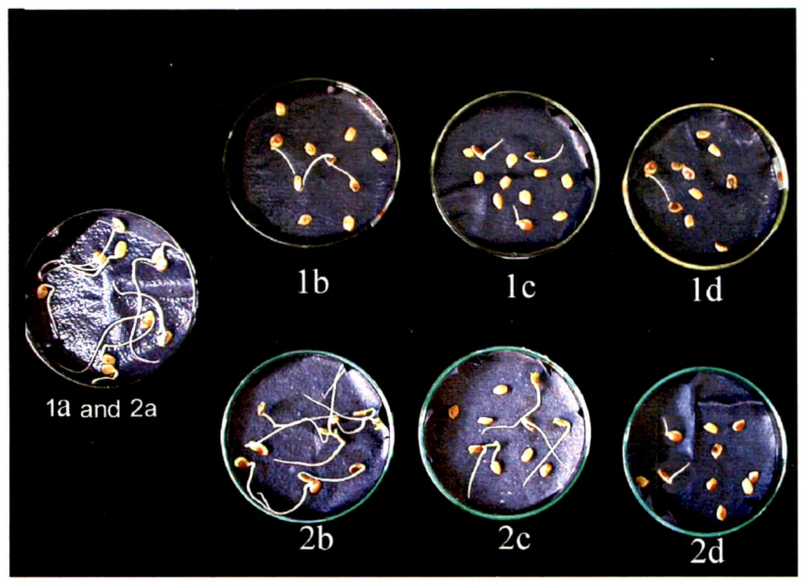


Plate: II: Effect of Sodium Chloride and Sodium Sulphate salinity on seed germination of *Prosopis juliflora.*

1: Treatment of Na_2So_4

1a: Control; **1b:** 100 mM; **1c:** 200mM; **1d:** 300 mM

2: Treatment of NaCl

2a: Control; **2b:** 100 mM; **2c:** 200mM; **2d:** 300 mM

A) Seed Germination

Effect of salinity on seed germination percentage and germination rate of *Prosopis juliflora* seed is recorded in fig. No.1. It is observed that the process of germination is delayed during early hours of germination. However at latter stage germination is some what improved at 120 hr stage. There is more than 50% reduction in seed germination due to 300 mM NaCl and Na_2SO_4. Among the equimolar quantities of two salts, sodium sulphate salinity is found to be more inhibitory than sodium chloride salinity. For emergence of radical which has resulted in greater drop in germination percentage. Seed germination can be regarded as sensitive and very critical phase in the life cycle of every plant which is governed by various endogenous as well as environmental factors. The hard seed coat of *Prosopis* is broken or weakened to allow the water absorption by the seed and for germination to occurs (Catalan and Balzarini,1992). Seed coat is also degraded over time and older seed that is still viable tends to germinate without pre-treatment (Pasiecznik and Felker,1992). Seeds are less likely to germinate if left in the pod.

The predominance of sodium chloride and sulphate ions in saline soil posses difficulties for germination of crop seeds. According to Ayer (1952), the delay or inhibition of germination is proportional to an increase of the osmotic potential. Although germination of seed is usually retarded by high concentration of salt solution in glycophytes as well as halophytes. Both interspecific and

intervarietal difference with respect to salinity tolerance at germination has been recorded and these are of significance in selection and breeding for salt tolerance. Most of the work on seed germination of *Prosopis species* has been carried out by using only NaCl salt. (Lopez Villagra and Galera, 1992). Reasons behind using NaCl as a sole salinizing agent in salinity studies is due to the fact that generally NaCl is the main component of soluble salt mixture present in saline soil (Fan *et al.,* 1993). Seeds under natural condition from seed banks and remain dormant during such adverse condition and whenever the favorable changes in the soil environment occurred the phase of germination. In many halophyte seeds this adaptive feature is noticed (Ungar, 1998).

The seeds of *Prosopis juliflora* have got hard seed coat which can help in overcoming the soil stress. Tobe *et al.,* (2000) reported that the effect of salinity on the germination of *Prosopis flexuosa* seeds from habitats differing in soil salinity level were similar. This was not correlated with the result reported for *Prosopis farcata* by Dafni and Neghi (1978). There was no correlation between salinity and the habitat where seed originated and various salt tolerance criteria studied at germination. In contrast, Bazzaz (1973) found difference in the germination behavior of seeds of the same species which originated in different habitats and suggested that they belong to different 'salt eco types'. Perez and Tambelini (1995) reported a significant reduction in *Prosopis juliflora* germination at (130 mM) NaCl, while Scifres and Brock (1969) showed no germination occurred at -1.62 MPa.

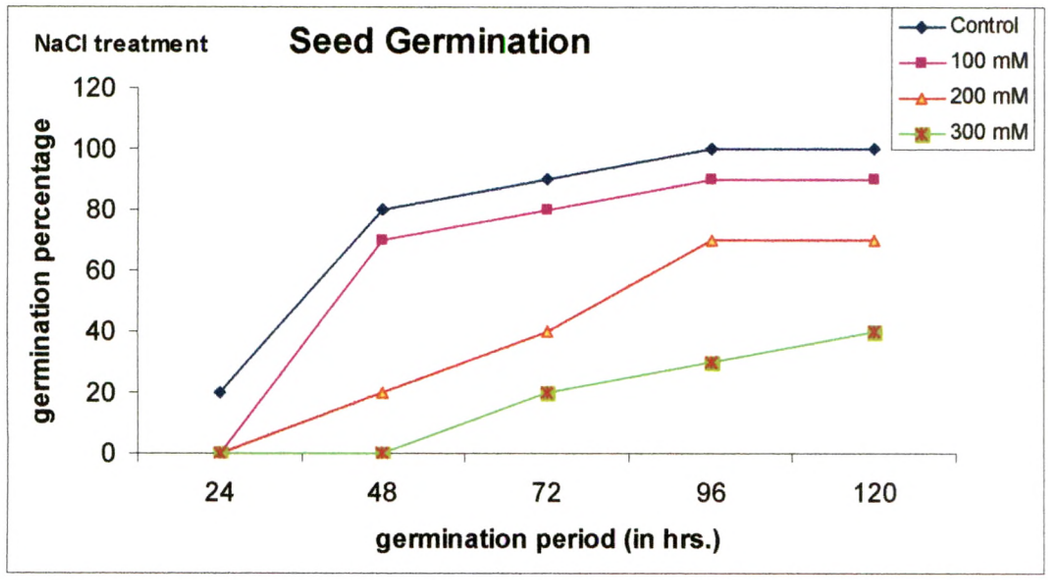
Germination in a UAE population was reduced at 200mM and completely inhibited at 400 mM at 40^0C and 600 mM NaCl at 25^0 C (El- keblawy and Al-Rawai, 2005). According to Khan and Ungar (2002), salinity induced the synthesis of phenols that inhibit germination in *Atriplex triangulars.*

In the present investigation a greater salinity tolerance of *Prosopis juliflora* is noticeable since there is more than 60% seed germination is seen even at 200 mM NaCl treatment. Hence, the seeds from plant growing in saline habitat have been used for germination studies and perhaps this may be one of the reason for display of greater germination potential under saline condition. It has been proposed that seed germination in salt affected soil is influenced by total concentration of dissolved salt (or the osmotic pressure) as well as the type of salt involved (Ryan *et al.,* 1975). Ungar (1978) reported that inorganic ions were not more inhibitory than manitol and polyethylene glycol (PEG) in several halophytes indicating that seed are mainly affected by osmotic stress rather than specific ion toxicities. The result of Egan *et al.,* (1997) on the effect of different sodium and potassium salt on the germination and early growth was primarily due to an osmotic effect and not to a specific ion toxicity of either the chloride or sulphate salts. Few studies have recently focused on the effect of Na_2SO_4 on plant growth in spite of the fact that their comparison with NaCl effect is still of high interest (Grattan and Grieve, 1992). Sosa *et al.,* (2005) observed significantly toxic effect of SO4 on *Prosopis strombulifera* plant which has been cultured hydroponically with increasing concentration of Na_2SO_4 as sole salinizing agent. They showed

strong growth inhibition, lower leaf number, chlorosis and precocious senescence compared with control plant. It has been reported recently that H+ / SO_4^2 transported in the root cell is the first step for SO_4^2 uptake from many different plant species (Buchner *et al.*, 2004). The availability of clones of some of there gene has enabled expression of the high affinity SO_4^2 transport SHST-1 and SHST-2. It is considered that if such regulatory mechanism were universal, permeability to SO_4^2 would blocked and SO_4^{-2} inhibition of germination in salt solution could be explained by the rapid buildup of SO_4^2 in the cell wall of the seed causing deleterious effect on water uptake.

Among the isomolar concentration of two salts Na_2SO_4 and NaCl, Na_2SO_4 appears to be inhibitory for seed germination of *Prosopis juliflora* than NaCl. In addition to greater concentration of sodium in Na_2SO_4 than NaCl which causes toxicity. This may also be due to the fact that isomolar concentration and osmotic pressure of Na_2SO_4 is greater than that of NaCl.

Perez and Tambelni (1995) studied the germination in *Prosopis juliflora* in monosaline solution of NaCl, $CaCl_2$ and Na_2SO_4 at $\psi 0$ from -0.3 to 1.5 MPa and they observed that germination percentage was more affected by Na_2SO_4 than NaCl. Joshi and Hingalajia (1999) worked on some problem and investigated effect of chloride and sulphate on seed germination of *Prosopis juliflora* and recorded that germination percentage was more affected by NaCl than Na_2SO_4. Based on our collected data on seed germination Na_2SO_4 is more effective than NaCl.



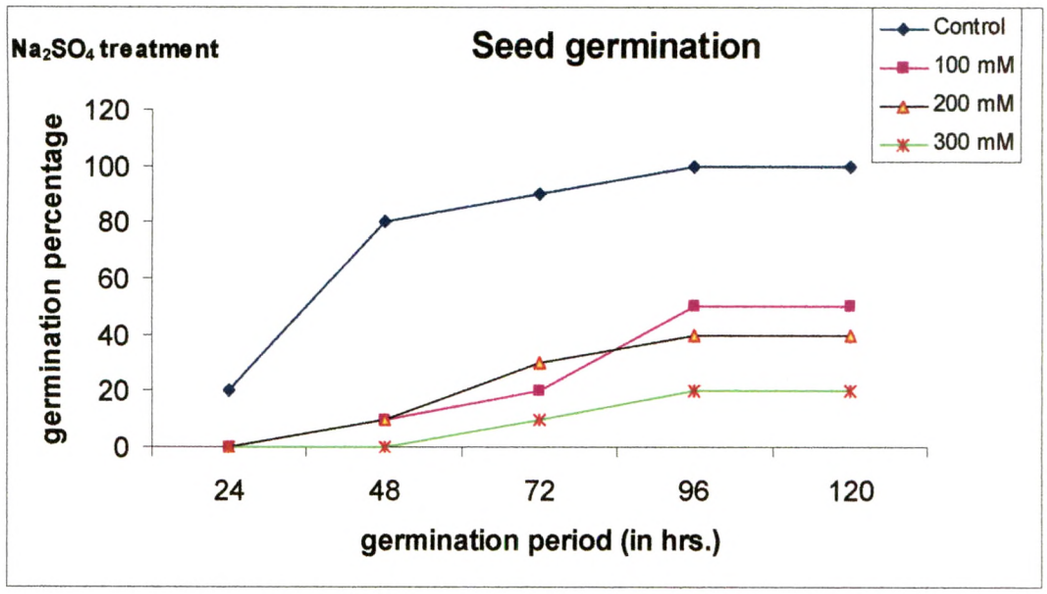


Fig.1. Effect of Sodium chloride and sodium sulphate salinity on seed germination of *Prosopis juliflora* (Swart) DC.

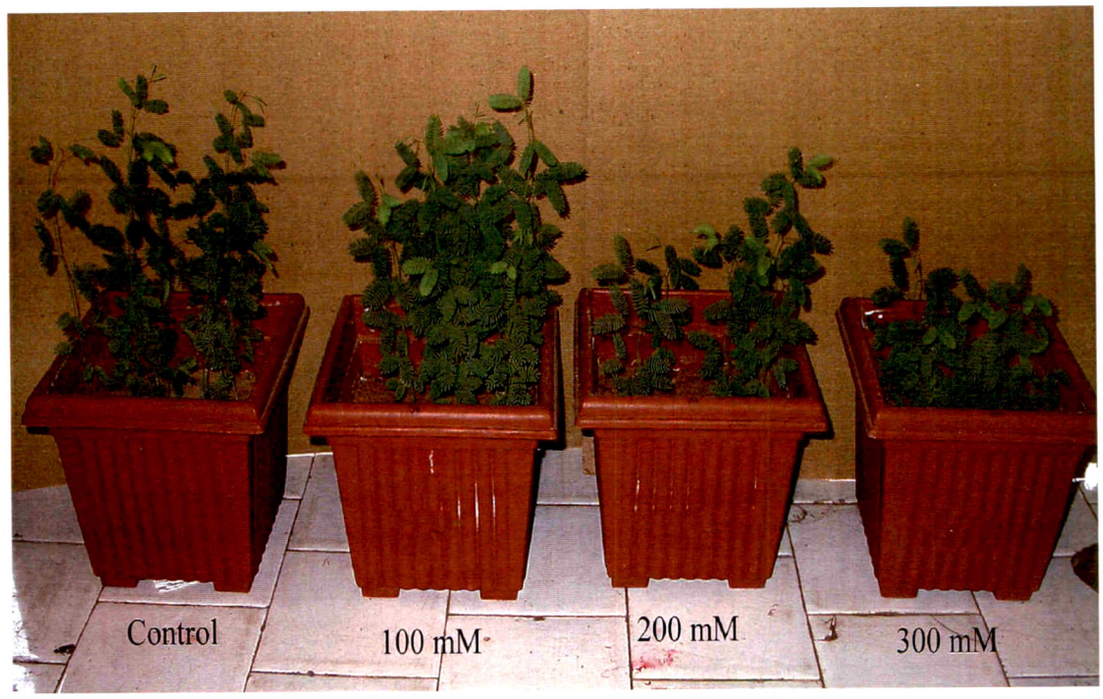


Plate III : Effect of NaCl treatment on *Prosopis juliflora*

Our observations indicate that the seed population from plant growing in salt affected region of Digraj (Sangli district) is more sensitive to sulphate salinity than chloride salinity at germination stage.

B) **Total Fresh Weight and Dry Weight**:-

Fresh weight:-

Effect of NaCl treatment on average fresh weight of 1 month old *Prosopis* plants is shown in the figure.2. It is observed that salinity treatment has caused marked decrease in fresh weight at higher concentration but at lower concentration there is enhancement in fresh weight. The fresh weight of the plant is an important parameter for assessment of growth since it represents a total biomass and considerable portion of this is due to water present in the tissue. Various factors contribute to salinity induced reduction in plant growth is both due to osmotic and specific ion effects. Salinity creates physiological drought and affects water relations in plants which in turn can lead to decline in water status and fresh weight of salt stressed plants. At the same time some halophytes belonging to chenopodiaceae develop succulent nature to bring about salt dilution (Waisel, 1972). In such plants a definite increase in fresh weight under saline conditions will evident. But this will not be the case with salt sensitive glycophytes to which category most of the crop plants belong.

In such plants reduction in biomass is directly co-related with the rate of stress applied (Storey and Wyn Jones, 1978). Karadge and Chavan (1983) stated that low salt level increases biomass in *Sesbania* it is due to restricted N uptake

but it decreased with increasing salinity. Increase in fresh weight of *Sorghum* took place in low salinity level and fresh weight increases with further increment of salt level has been noticed by Jagtap (1991). Fresh weight of Celery shoot was enhanced by low NaCl level (Everad *et al.,* 1994). Venketsen *et al.,* (1997) observed an increase in fresh weight of *Ipomea* plant up to 200mM NaCl. Reduction in fresh weight under salt stress in treated plants was evident in the experiments of Tawfic *et al.,* (1975) in barley plant, Tazel *et al.,* (1980) in alfalfa, Jefferey and Critchley, (1985) in *Phaseolus vulgaris* .

In gene transferred wheat plant salt stress reduced shoot fresh weight with increasing salinity (Abebe *et al.,* 2003). Camara *et al.,* (2004) studied the effect of NaCl on one year old Citrus seedling and they noticed reduction in plant fresh weight due to salinity. In rice variety IR28 fresh weight decreased with increasing salinity and increases with exogenous GB application in salinity stress (Demiral and Turkan, 2006). There is greater reduction in fresh weight in *Vigna radiata* when there is increase in salinity (Sumithra *et al.,* 2006). At the same time lower salinity levels are found to increase fresh weight of some plants.

In *Prosopis juliflora* also an increase in biomass in plants subjected to 100 mM NaCl is noticed. However at very high concentration (300 mM NaCl) about 60.44 % decline in fresh weight is evident. But still at this concentrations the plant survival is seem. Since *Prosopis juliflora* is perennial tree species and the present analysis is performed at 1 month stage, it is quite probable that after initial

hardening, the plant may become highly salt tolerant at latter growth stage and show better biomass production.

Dry weight:-

Influence of NaCl salinity on dry matter production in different parts of *Prosopis juliflora* plant is depicted in fig. 2. It is evident from the fig. that dry weight of leaf and shoot is increased in plants subjected to 100 mM NaCl while at higher concentrations there is decline. The dry weight of stem tissue also shows a similar pattern. In contrast to shoot tissue the dry weight of root is higher than control in plants subjected to 100 and 200mM NaCl and a decline in dry weight of this is noticed at 300 mM NaCl concentration.

Dry matter production in a plant represents a balance between total photosynthesis and respiration. The increase in photosynthesis contributes to greater productivity while respiratory losses results in decrease in dry matter production. This is particularly true for plant organs like leaf while in stem and root the assimilates transported from leaf tissue contribute to dry matter production. Salinity is reported to inhibit photosynthetic CO_2 fixation in many plants (Bruria and Feigin, 2005). Further there is increase in rate of respiration and photorespiration in salt stressed plants (Downton, 1977). Both these factors contribute to decline in dry matter production in salt stressed plants. In most of the plant, salinity results in reducing dry matter except in halophytes. But in some glycophytes moderate salt stress enhanced dry matter production as noticed by Passera and Albuzio (1978) in wheat.

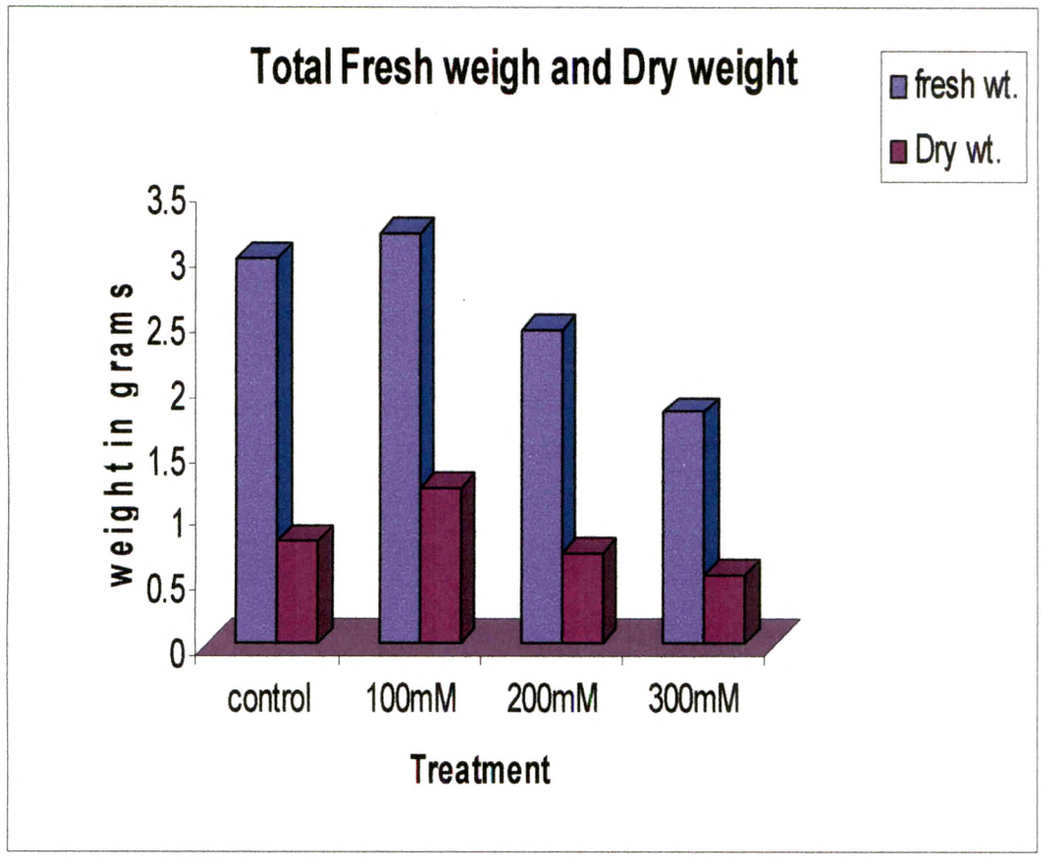


Fig. 2. Effect of Sodium chloride salinity on total fresh weight and dry weight of ***Prosopis juliflora*** (Sw.) plant.

Dwiverdi *et al.*,(1980) in wheat, Patil *et al.*, (1983) in *Phasoleus vulgaris* , Rawson *et al.*, (1988) in wheat, barley and *Triticale* Nukaya *et al.*, (1982) found decrease in dry weight of soyabean whole plant by NaCl salinity. In rice there is no marked difference in dry weight before and after salt treatment(Wakinchi *et al.*, 1982).

According to Karadage and Chavan (1983), the dry matter production in sesbania was affected only at the higher salt concentration. Bottacin *et al.*, (1985) stated that dry matter production was stimulated up to 150 mM NaCl concentration in *Pennisetum americanum* while increasing concentration more than 150 mM leads to decrease in dry matter production. Krishnamurthy (1991) observed that inhibition of dry matter production in salt grown rice. Gaikwad (1995) viewed that salt tolerant in *Amaranthus caudatus* but in *Amaranthus paniculatus* dry mass increases at low salinity level and was decline at higher salinity.

Venkateshan *et al.*, (1997) observed that, in *Ipomea* salinity caused disturbance in dry matter production only at higher level. A marked reduction in dry weight in *Vigna radiata* treated with increasing soil salinity was evident in the experiments of Sumithra *et al.*, (2006). Pessarakli *et al.*, (2005) reported that in saltgrass grown under salt stress the dry weight at 200mM and 400mM is more than that of control plant. In case of *Prosopis juliflora* the dry matter production is reduced by 68.41 % in 300 mM NaCl treated plants, while at 100 mM dose the dry

matter production is stimulated .The dry weight of root tissue is maintained above control level in plants treated with 200mM NaCl and this is certainly an important feature which contribute to salinity tolerance of this species.

C) Study of influence of sodium chloride salinity on metabolism:-

1) Mineral Nutrition:-

i) Sodium and chloride:-

Influence of NaCl salinity on the contents of different mineral elements in leaves and roots of *Prosopis juliflora* plant is depicted in fig.3 to7 . It can be seen from the figure that due to NaCl treatment the level of sodium and chloride in both leaves and roots is increased. Among the two elements the chloride content is increased to a greater degree than sodium. Leaves accumulate relatively more sodium and chloride than the root tissue in the salt stressed plants. The K status of the root tissue is reduced due to 100 and 300mM NaCl treatment while at 200mM there is no effect. The Calcium content in both root and leaf tissue is increased due to salt stress. On the other hand phosphorus content in leaf and root tissue is lowered with increasing in NaCl dose.

Salinity affects normal absorption of mineral nutrition NaCl mainly influence the distribution of essential nutrients in plants. Essenensiality of nutrient varies from plant to plant and regulated by internal ionic status of root. Sodium is dominant cation in saline soil, which is essential micronutrient. In different plant parts it is passively accumulated.

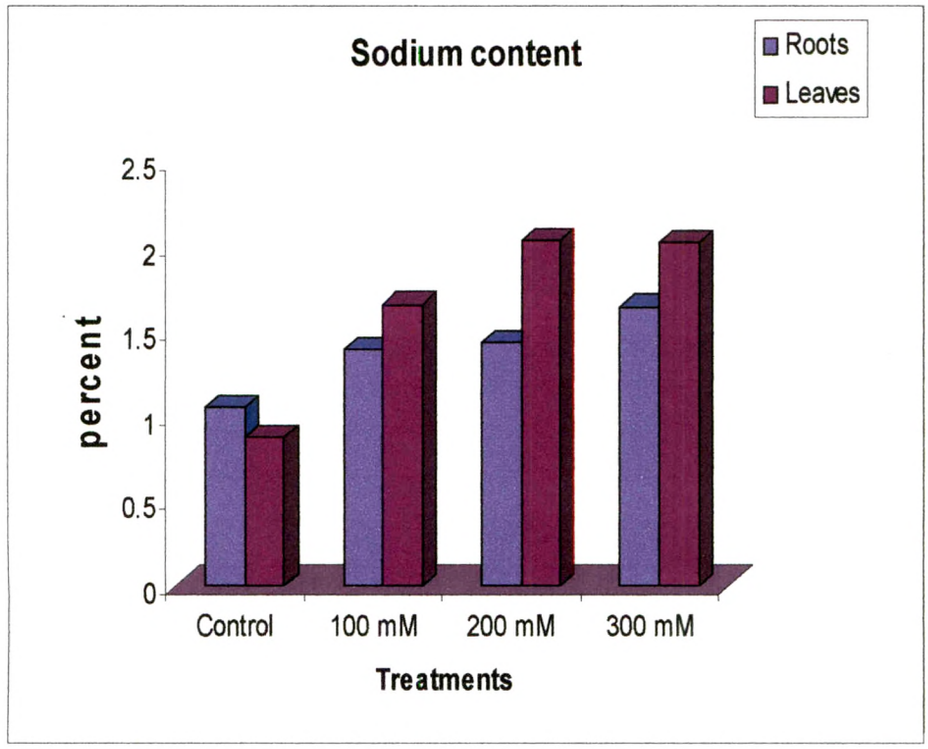


Fig.3. Effect of Sodium chloride salinity on the sodium content in the roots and leaves of *Prosopis juliflora* (Sw.).

Qudar *et al.,* (1980), noticed death of rice seedling due to high accumulation of Na. The high concentration of Na adversely affects many metabolic reactions including nutritional balance, osmoregulation, stomatal behavior and assimilation capacity (Mass and Hoffman, 1977; Mass and Nieman, 1978). Sodium has been reported to act as a substitute for potassium in some metabolic processes (Marschner et al, 1981). While Rain (1972) suggested role of sodium in maintaining favorable water balance. Na acts as activator for some enzyme system (Evans and Sorger, 1966). Kylin (1973) reported the stimulation of enzyme ATPase in the presence of Na.

The real problems of Na^+ nutrition for plant arise when Na^+ concentration exceed the normal limit on the soil medium and becomes a dominating cation. During this situation different plant species adopt various strategies of ion regulation depending upon their genetic makeup and environmental adaptability. Detailed studies of Na^+ and Cl^- distribution within root, especially those which are based on electron probe X-ray micro- analysis of individual cell, have reveled several mechanism which depends on either the progressive exclusion of Na^+ and Cl^- across the radial path way from the epidermis to the xylem Na^+ in barley.

Since sodium is one of the most dominant cation in saline soils accumulation of this element in plants growing in saline habitat is reported by number of workers. However the degree of accumulation of this element varies in different plant species. Similarly the site of accumulation also varies considerably

in different species. Phills *et al.,* (1979) found that the salt sensitive taxa of *Lycopersicon* and *Solanum* were more effective at excluding Na uptake to the leaves. Ashraf and McNeilley (1990) reported there was no constant pattern of Na in root at high salinity treatment. Kawasaki *et al.,* (1983) observed that in beans, maize and sorghum sodium accumulated to a greater extent in shoot region than in root region with increasing salinity. In *Amaranthus caudatus* sodium accumulated in leaf tissue, while in *Amarnthus paniculatus* Na accumulates in the root zone in response to salt stress (Gaikwad, 1995). Robert and Tester (1997) also reported similar accumulation of Na in maize root and shoot. Bottacin *et al.,* (1985) noted that the pattern of ion distribution in the root of *Pennisetum americanus* genotypes was same. But the level of tolerant genotype showed higher exclusion capacity of Na and Cl. According to Loupassaki *et al.,* (2002) in all cultivars of olive plant the higher Na % dm was recorded in the roots followed by shoot, mature and young leaves. Emphasis, which had the highest Na% dm in mature leaves (Yeo *et al.,* 1977). Removal of Na^+ from the xylem element by K/Na exchange mechanism located in xylem parenchyma cell at the base of root in bean (Kramer *et al.,* 1977). According to Schachtman and Munns (1992), an ability to limit Na accumulation in the leaves might be an important mechanism in salt tolerance of *Triticum species*. Sharma (1995) noticed accumulation of Na in all parts of wheat plant under saline condition, which was remarkable in root.

Meier and Kaiser (1988) recorded high capacity of spinach leaves to sequester Na^+ salt in vacuoles. The ratio between Na^+ concentration in the leaf

symplast and apoplast was higher in *Malus zumi* than in *Malus baccata* indicating that *M. zumi* had a higher compartmentalizing capacity and compartmentalization was its main salt tolerance mechanism (Ma-Li-quing *et al.*, 2006). Ramoliya *et al.*, (2006) analyzed the mineral uptake in *Prosopis cineraria* plants exposed to salt stress. These workers noticed that the seedlings in case of *Prosopis juliflora* in contrast to above findings greater accumulation of sodium in the leaf tissue in compare to the root tissue is evident. Sodium is the most essential microelement for halophytes (Marschner, 1986). Sodium also plays a role in osmotic adjustment in halophytes. Since *P. juliflora* belongs to a category of xerohalophytes (Aronson, 1989) besides playing a positive role in metabolism sodium might be also contributing to osmotic adjustment under saline conditions.

Chloride is dominant anion in saline soil. This element serves as an important micro-nutrient required for photosynthetic light reaction in higher plant. During photosynthesis chloride ions are involved in the splitting of water molecule at the oxidizing cite of photo system II. It is assumed by Kelley and Izawa (1978) that chloride acts as a cofactor of manganese containing O_2 evolving system. The normal concentration of chloride in the plant is between 200 to 2000 mg/100 g but chloride requirement of plant for optimal growth is between 34 to 120 mg/ 100 g dry weight which is in the range of micronutrient level (Marschner, 1986). According to Waisel (1972), sodium and chloride are taken up by root of

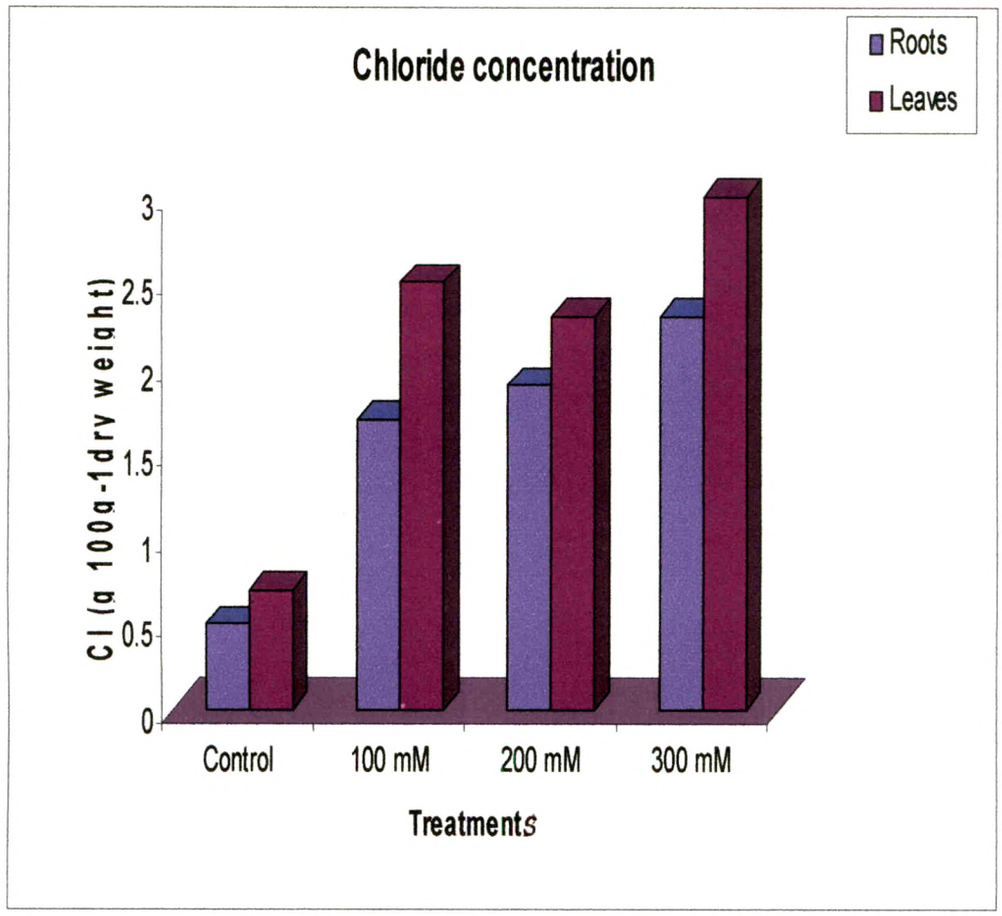


Fig.4. Effect of Sodium chloride salinity on the chloride concentration in the roots and leaves of *Prosopis juliflora* (Sw.).

halophytes in non-equivalent quantities and they also differ in the pattern of their distribution within plant organs. But the physiological requirement of Cl^- is very low and the chloride requirement for optimal growth is between 10 and 30 m mols per Kg dry weight. According to Mengel and Kirby (1982) the plants which take up large amount of Cl^- usually have high water content.

Since the Cl^- is an important osmoticum. Greenway *et al.,* (1966) suggested the presence of 2 types of Cl^- uptaken by plant one is an active absorption which is affected by temperature and other is passive absorption which is affected by transpiration pull. Cl^- is accumulated in ascending order maximum in the stem and leaves and minimum in the root. Though Cl^- is required in very less amount and many plants absorb it more than requirement.

Nassery *et al.,* (1979) observed that seasum roots did not accumulate Cl^-. Na^+ and Ca^+ readily but the leaves accumulates them to some considerable extent and there was no evidence of Cl^- toxicity. According to Yousef and Sprent (1983) Cl^- level in shoot of *Vicia faba* plant were higher than in the root. Gaikwad (1988) observed accumulation of Cl^- in all cultivars of millets, according to him salt filteration mechanism probably operates in millets, this mechanism is more efficiently working in their salt tolerance and chlorides are mostly accumulated in leaves tissue under saline condition. Salt tolerance has been particularly linked to the regulation of Cl^- and Na^+ and it decreases availability of nutrients arising from lower uptake the competition between nutrient such as K^+ and NO_3^- with Na^+ and Cl^- respectively (Bottacin *et al.,* 1984).

The chloride concentration in root and leaves of *Prosopis juliflora* is found to increase along with increasing salinity treatment similar to sodium. The accumulation of this element is more pronounced in leaf tissue than the root tissue. Thus along with sodium probably chloride is also contributing to osmotic adjustment in this xerohalophyte species.

ii) Potassium (K^+)

Potassium is considered as a most essential macronutrient and its uptake is highly selective and closely coupled with metabolic activity. At all level it is highly mobile element in plant. In cells, tissue as well as long distance transport via xylem and phloem. K plays an important role in activation of about 60 plant enzymes, protein synthesis, stomatal movement photosynthesis and osmoregulation (Marschner 1986). It also plays an important role in photophosphorylation (Pfluger and Mengel, 1972) and starch formation (Hawker *et al.*, 1974).Under potassium deficiency, phloem transport is affected, NR activity is declined, protein metabolism is disturbed and amino acids and soluble organic nitrogenous compound are accumulated. The tips and margin of the leaves scorch stem become weak and lodge under K deficiency. The critical concentration of k is in the range of 0.5 to 2% in dry matter and K requirement for optimum growth of plant is 2-5%. High K concentration in cytoplasm and chloroplast is necessary to neutralize the soluble and insoluble macromolecule anion and to stabilize the pH between 7-8 which is optimum for enzyme reaction.

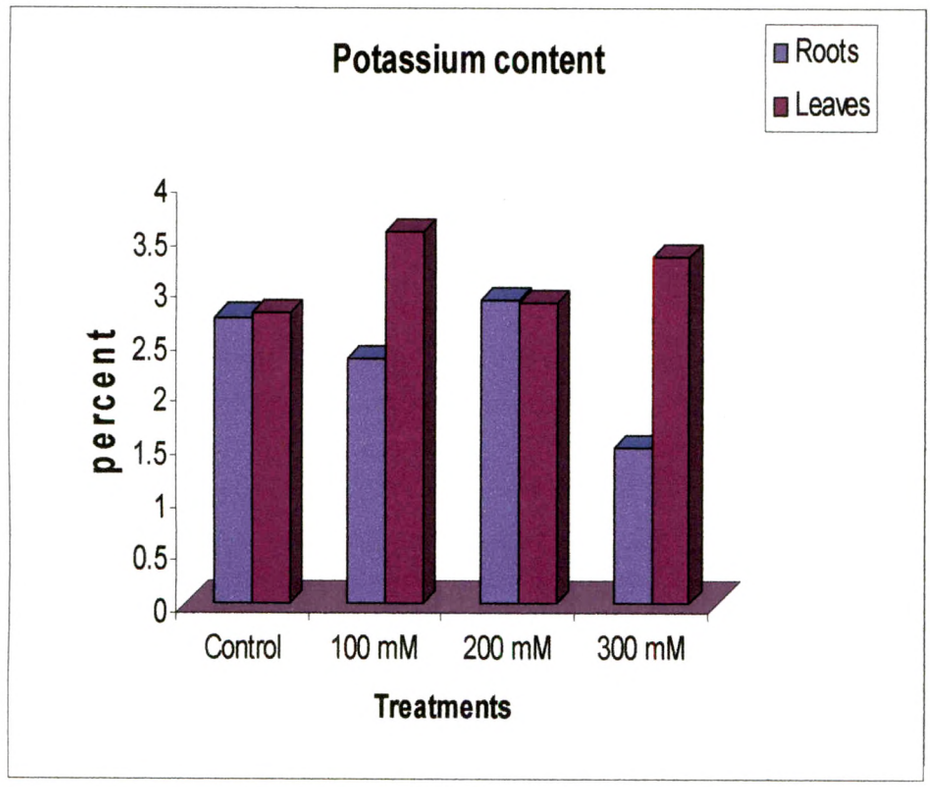


Fig.5. Effect of Sodium chloride salinity on the potassium content in the roots and leaves of *Prosopis juliflora* (Sw.).

According to Ortiz *et al.*, (1994), K is the main inorganic osmoticum in plant because energetically it is cheeper than other solutes. In view of Garbquriioo and Dupont (1988), plant growing in saline soil must maintain a much higher K/Na ratio in their cytoplasm than that present in the surrounding. In nonhalophytes potassium is reported to stimulate Na efflux across root plasma membrane (Jesckhe, 1973). Most of the osmotic adjustment in the leaves was due to the potassium. Weimberg (1986) reported that Na + k concentration and Na: K ratio in the leaves were the function of ionic composition of salt in the growth medium and osmotic potential of the medium. Accumulation of K^+ in the leaves was studied by number of workers in monocotyledon halophytes by Albert and Pope (1977) and Gorham and Wyn-Jones (1980). This is due to an alkali ion uptake and system which exist extremely high affinity to K even at high Na level (Epstein, 1969). Since sodium is dominant monovalent cation, sodium compets with potassium for common uptake, a decline in K uptake is observed by many workers in roots. Kardage and Chavan (1983) reported decrease in K content in root of salt stressed *Sesbania aculeta.* The reduction in the intracellular concentration of potassium might be attributable to inhibition of the influx of potassium as well to stimulation of efflux of potassium (Nakamura *et al.,* 1990). Salim (1989) also reported that decrease in potassium concentration in root of Mung bean, red kidney bean. He and Cramer (1993) noticed decline in the potassium concentration in the *Brassica curinata.* Watad *et al.* (1991) also stated that K uptake appears to be linked H^+ pumping by the plasma membrane, H^+

ATPase. The enhanced capacity of the plasma membrane K^+-ATPase in NaCl adapted cell may be link to the greater K uptake to occur as a function of a salt adaptation. A preferential potassium uptake mechanism has been considered as one of the important mechanism of salt tolerance in marine algae and mangroves (Joshi, 1976). It is indicated by Niu *et al.,* (1995) increased k^+/Na^+ selectivity of potassium uptake system might represent a significant adaptation to high concentration NaCl in *Prosopis juliflora.* In the present investigation it is noticed that *Prosopis juliflora* plants are able to maintain potassium level above on optimal level (1%) in both leaves and roots over the entire range of the salt treatment. The leaf potassium appears to contribute to salinity tolerance since in this plant there is elevation in potassium content in response to salinity.

iii) Calcium:-

Calcium plays a major role in cell wall stabilization. In contrast to other macronutrient, a high proportion of the total Ca^{++} in plant tissue is located in the cell wall at two distinct areas the middle lamella were Ca^{++} is bound with pectin and exterior surface of plasma membrane (Bussler, 1963). Calcium plays important role in cell division and extension, Ca^{++} regulates the spindle activity. Calcium is also involved in pollen tube growth. Fundamental role of Ca^{++} is to maintain membrane stability and cell integrity. Calcium stabilizes cell membrane by binding phosphate and carbonate group of phospholipids (Caldwell and Haug 1981). Clarkson and Hanson (1980) also viewed that Ca is important in pectin synthesis. According to Swami and Reddy (1991) Ca maintains level of super oxide dismutase and catalase, which may be controlling lipid peroxidation.

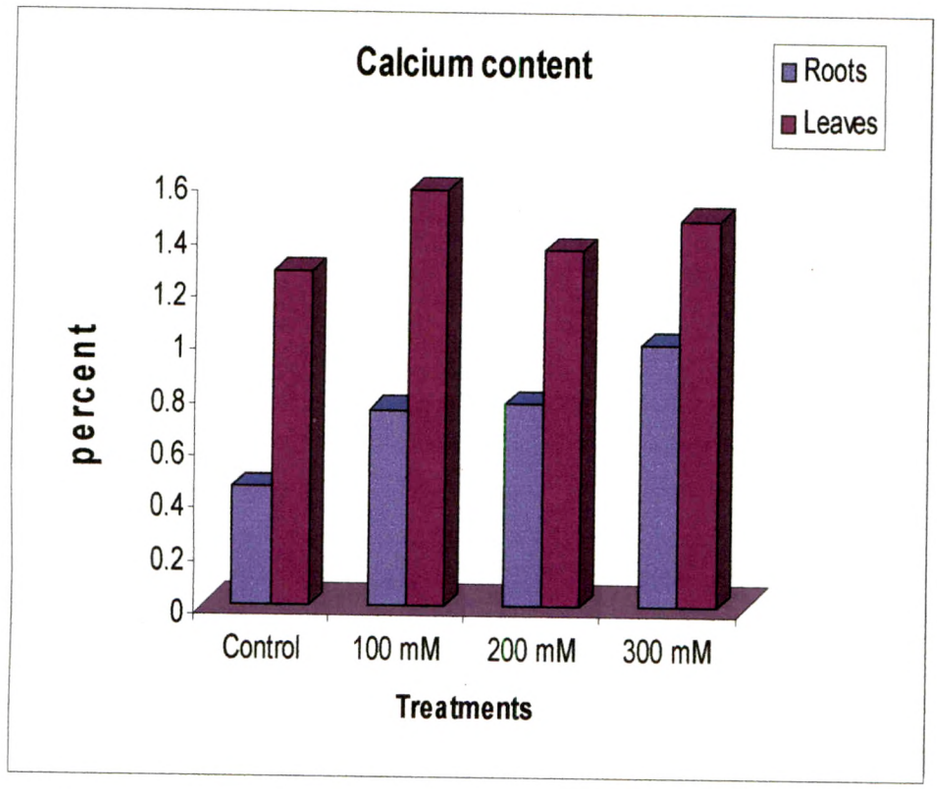


Fig.6. Effect of Sodium chloride salinity on the calcium content in the roots and leaves of *Prosopis juliflora* (Sw.).

Ca competition with Mg for binding sites and it influences enzyme and precipitation of Pi. Many enzymes has been reported to be stimulated or inhibited by Ca such as ATPase, Protien kinase, Pyruvate kinase, Nuclease, α- amylase, Pectin esterase and Lipoxigenase (Clark, 1984). Calcium has recently been recognized as transducer of normal and environmental signals to responsive element of cell metabolism (Hepler and Wayne, 1985) and in this respect changes in cytosolic calcium levels play a crucial role. Both calmodulin and calcium dependent protein kinase contribute to the role of calcium as second messenger. According to Rains (1972), Ca protects injurious effects of H^+ and other toxic ions. A decline in calcium content under saline conditions is reported to create a condition of calcium deficiency in salt sensitive plants (Bernstein, 1975) on the other hand in many plants salinity is found to promote calcium uptake. There are many reports of increasing Ca^{++} content in some plant species under saline condition (Joolka *et al.,* 1977; Ayub, 1974 and Karadge and Chavan, 1983). Calcium is essential for structural and functional integrity of plant membrane under saline conditions Epstein (1969). Many specific effects occur without actual membrane damage, where ion interfere directly with each other uptake such interactions may, competitive or noncompetitive (Fitter and Hay, 1987). In absence of Ca, Na efflux and K influx are not well controlled while in presence of Ca, Na efflux and K influx are enhanced (Marschner, 1986). Ortiz *et al.,* (1994) reported that Ca enhance, the net absorption of potassium. According to Morabito *et al.,* (1996), Ca is protective maintains membrane integrity and avoids K leakage under

salt stress. Ramoliya *et al.,* (2006) observed that calcium content increased in leaves seedlings of *Prosopis cineraria* subjected to salt stress.

In case of *Prosopis juliflora* also calcium might be playing a similar role since calcium nutrition is not at all affected by salt stress and the calcium level in both leaves and roots is elevated in salt stressed plants.

iv) Phosphorus:-

Phosphorus is an important macronutrient essential for all living organisms. It is structural constituent of genetic elements nucleic acid, DNA and RNA. It play major role in energy transfer during plant metabolism like respiration, photosynthesis in the form of ATP, NADP and also in cell division and cell expansion. Phosphorus is involved in the formation of cell membrane lipids, which play a vital role in ionic regulation (Bieleski and Ferguson, 1983). Thus it is involved in heredity, storage and transport of energy phosphorylation, enzymatic actions etc. Plant absorbs P as $H_2PO_4^{2}$ at pH below 7.6 while as $H_2\ PO_4^{-}$ at pH above 7.0. It is present in the plant cell as inorganic P or phosphate or energy rich phosphate bound. There are two forms of phosphate pool i.e., metabolic pool and non metabolic pool. The cytoplasmic pool contains phosphate enters while vacuolar pool inorganic phosphate. The inorganic P (ip) is a substrate as well as product of number of enzymatic reaction and hence compartmentalized to regulate metabolic reaction of cytoplasm. According to Marschner (1986), the phosphorus requirement of plant species is in the range of 0.3 to 0.5 % dry weights during vegetative growth.

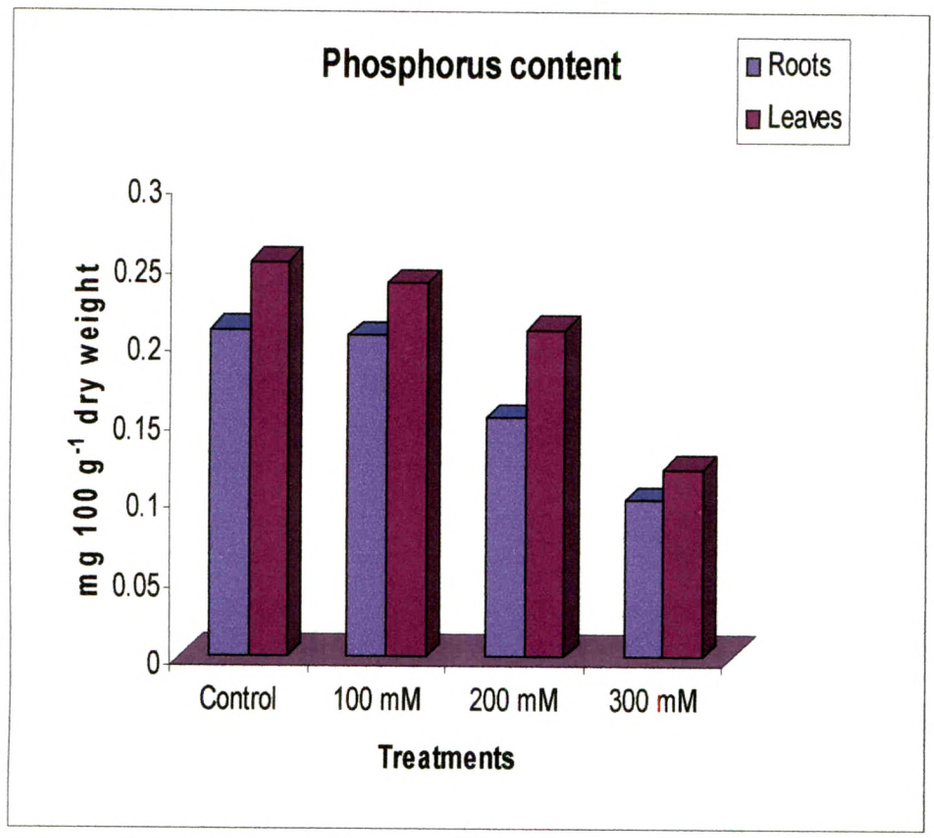


Fig.7. Effect of Sodium chloride salinity on the phosphorus content in the roots and leaves of *Prosopis juliflora* (Sw.).

The P uptake is pH dependent and at low pH enhances P uptake. It is also controlled by the internal status of the root and age of the plant. According to Jacob and Lawlor (1992), plant with low P had low photosynthesis rate and less carboxylation efficiency than plant with ample P. Deficiency of phosphorus leads to less total soluble protein and Rubisco/ unit area.

Although there are many reports indicating suppression of P uptake due to salt stress (Strogonov, 1964; Ravikovitch Porath, 1967; Fageria, 1985; Indulkar and More,1985). Nieman and Clark (1976) also found depression of total P in corn leaves by salinity at low pi in nutrient solution. Helal and Mengel (1979) noticed that salinity hardly affects P content of root and shoot of salt grown barley. In case of *Prosopis cineraria* seedlings Ramoliya *et al.,* (2006) noticed that phosphorus content was significantly decreased in leaves with increase in soil salinity while P gradually decreased in stem and root tissue. Decrease of P in root tissue and increase in leaf tissue with increasing salinization in *Poncirus trifoliata* was evident in the experiments of Tozly *et al.,* (2000).

Prosopis juliflora plants show a pattern similar to that of *P. cineraria* since in both root and leaves a decline in P status is evident in plants exposed to salt stress. According to Gibson (1988), phosphorus deficiency induced by salinity could reduce the cellular ability to accumulate optimum concentration of ion without reduced growth. Thus in contrast to Calcium and Potassium nutrition which appear to be quite stable during salt stress in this species. The phosphorus nutrition in *P. juliflora* is sensitive to salt stress. The disturbance in P nutrition can

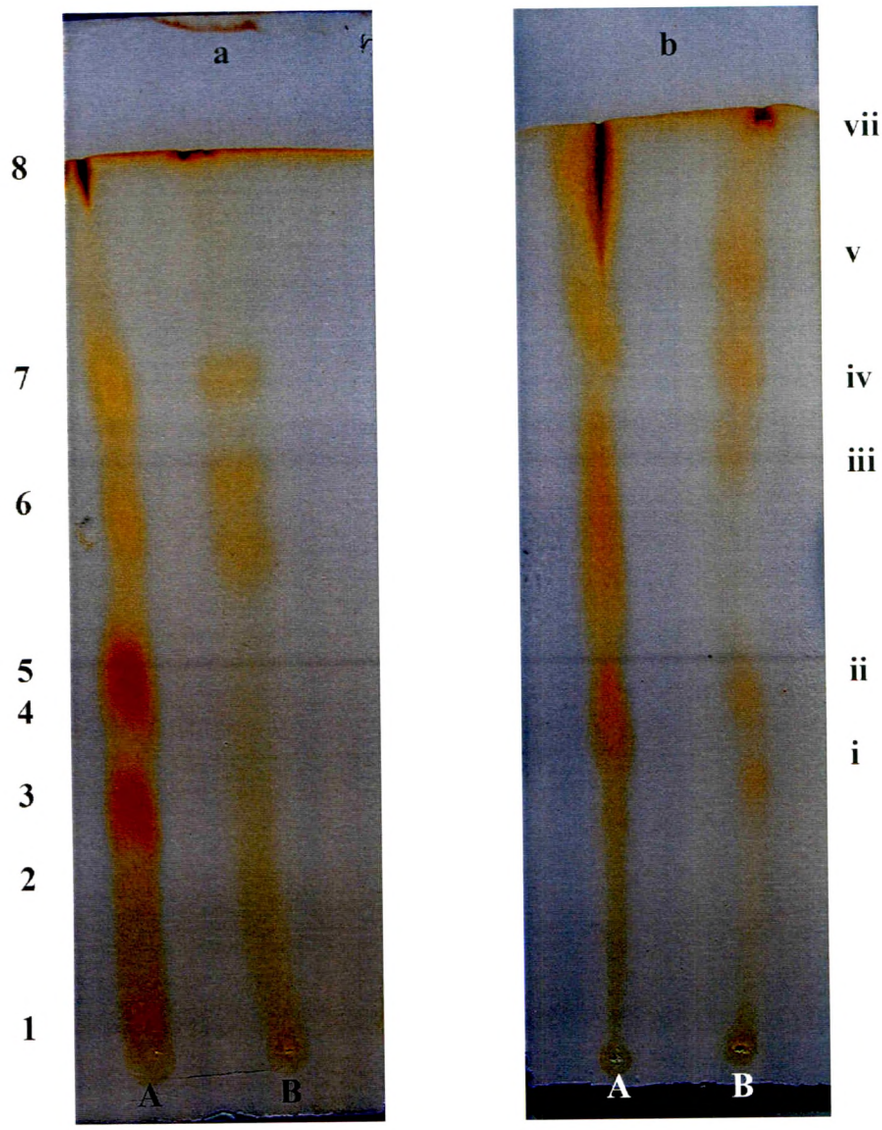


Plate: VI: Influence of salt stress on lipid compostion of roots and leaves of *Prosopis juliflora* (Sw.) DC.

a. control b. 300 mM NaCl treatment.

A. Leaf B. Root.

A. Probable identification of lipids detected in leaf tissue.

1. Phosphatidyl Insiotol.
2. Sulphoquinovosyl diglyceride.
3. Phosphatidyl choline.
4. Digalactosyl diglyceride.
5. Phosphatidyl glycerol.
6. Phosphatidyl ethanolamine.
7. Sterol glycoside.
8. Neutral lipids.

B. Probable identification of lipids detected in root tissue.

i. Sulphoquinovosyl diglyceride.
ii. Phosphatidyl choline.
iii. Phosphatidyl glycerol.
iv. Phosphatidyl ethanolamine.
v. Sterol glycoside.
vi. Neutral lipids.

have significant effects on overall plant metabolism in view of a key role of this element in cellular biochemistry.

2) Qualitative changes of lipid composition:-

Effect of salt stress on lipid composition of root and leaves of *Prosopis juliflora* (Sw.) is depicted in plate.

In leaf tissue of control plant following lipids 1) Phosphatidyl inositol 2) Sulphoquinovosly diglyceride 3) Phosphatidyl choline 4) Phosphatidyl glycerol 5) Digalactosyl diglyceride 6) Phosphatidyl ethanolamine 7) Sterol glycoside 8) Neurtal lipids are detected on TLC. In the leaves of plant treated with 300 mM NaCl following lipids 1) Phosphotidyl choline 2) Digalactosyl diglyceride 3) phosphatidyl glycerol 4) Sterol glycoside 5) Neurtal lipids have been detected on TLC.

The lipids detected on TLC of root extract of control plants are 1) Digalactosyl diglyceride 2) Phosphatidyl glycerol 3) Sterol glycoside 4) Neurtal lipids. TLC of lipid profile of root tissue of plants treated with 300mM reveals presence of 1) Sulphoquinovosly diglyceride 2) Phosphatidyl choline 3) Phosphatidyl glycerol 4) Phosphatidyl ethanolamine 5) Monogalactosyl diglyceride 6) Neurtal lipids.

Lipids refer to a structurally diverse group of molecules that are preferentially soluble in a nonaqueous solvent such as chloroform. Lipid includes a wide variety of fatty acids derived compounds as well as many pigments and

secondary compound that are metabolically unrelated to fatty acid metabolism (Brower *et al.*, 2000).

These metabolites serve many functions in plants. It is in the form of major component of biological membrane. Phospholipids provide structural backbone to the membrane on one hand and dynamic fluidity to the membrane on the other hand. It acts as hydrophobic barrier that is critical to life. Biological membrane not only separate cell from their surrounding but also separate the content of cell organelles. Polar lipids play important role in cellular compartmentalization forming a bilayer which prevent free diffusion of hydrophilic molecules between the cell organelles and prevents diffusion in and out of the cell.

Fats and oils are efficient storage forms of reduced carbon, particularly in seeds. Fats and oils exist mainly in the form of triacylglycerols in which fatty acids molecules are linked by ester bonds to the three hydroxyl group of glycerol. Fatty acids in plants are usually straight chain carboxylic acid having an even number of carbon atoms. Oils are liquid at room temperature, primarily because of presence of unsaturated bond in their component fatty acids (Ohlorogge and Jaworski, 1997). Triacylglycerols in most seeds are stored in the cytoplasm of cotyledon or endosperm cell in organelles known as oil body. The main structural lipid in membrane are the glycerolipids in which the hydropholic protein consist of two 16- carbon or 18 carbon fatty acids esterified to position 1 and 2 of glycerol back bone. Triacylglycerols are frequently referred to as neutral

lipids because of their non polar nature found primarily in seeds. Neutral lipids are not soluble in the aqueous phase of cells, they do not contribute in the osmotic potential of the cell. This is of unique importance as they do not disrupt the maintenance of normal cellular osmolatity (Moore, 1993).

Plant leaf phospholipids have more limited range with very few fatty acids greater than C_{18}. In leaf palmitic, oleic and linoleic acids are predominant. Phospholipids are basically mixed esters of fatty acids and phosphoric acid the alcohols gycerols phospholipids are ubiquitous in animal and plant cell and often undergoing break down and resynthesis. Biosynthesis of complete phospholipid molecules (*de novo*) occurs by two different modes. One by transferring a phosphorus base from water soluble nucleotide-cytidine disphosphodiglyceride to the base. Second by completed molecules may be altered to suit the cell requirements by exchange reactions glycerophosphate arising mainly from the glycolysis. In physical properties they bridge the gap between the completely water insoluble neutral lipid and molecules which form true aqueous solution (Kenedy, 1961; Marienetti, 1967).

Besides playing a role of membrane backbone and energy store house, lipids also play important role in the signalling process. Plants use membrane lipids as precursor for compounds that are used for intracellular or long range signalling (Stintzi and Brower, 2000). Phosphotidylinositol-4, 5bisphosphate (PIP_2) is the most important of several phosphorylated derivatives of Phosphotidylinositol known as phosphoinositide. The action of IP_3 in releasing

Ca^{2+} into the cytoplasm and there by regulating cellular process has been demonstrated in several plant system including the stomatal guard cells (Schroeder *et al.,* 2001).

The response of plant to salt stress is complex and involves changes in their morphological, physiological and metabolism. Alteration in the structure and function of the cell membrane is the first deteriorating change during stress injury. In many plant, alteration in lipids particularly in phospholipids and sterols were observed as a result of water stress (Liljenberg, 1992) and salt stress (Kuiper, 1984). Higher salinity increases the overall level of the n-3 major polyunsaturated fatty acids in *Ulva* where as low salinity increases the level of fatty acid, palmatic acid, oleic acid and the essential fatty acids (Floreto, 1993). Kuiper (1968) studied relationship between the lipid composition of the roots of grape wine root stocks nd chloride translocation to the leaves, he noticed that monogalactose diglyceride content of the root was directly related to the chloride accumulation in the leaves, and a striking negatively correlation was observed between the phosphatidyl choline and cl^- content.

In case of *P. juliflora* the preliminary qualitative analysis of lipids reveals marked changes in lipid composition in both leaf and root in response to salinity. Thus the concentration of neutral lipids is lowered due to salt stress in both roots and leaves. Similarly the concentration of phospholipids is also lowered but at the same time some new lipids are detected in the chromatogram of roots of salt stressed plants. The root cell is the key site of the plant interaction with salt in

the surrounding medium (Lacan and Durand, 1995). Changes in phospholipids, sterols and fatty acids may contribute in the membrane functions such as bilayer permeability (Schuler *et al.,* 1991), carrier-mediated transport (Deuticke and Haest, 1987) and the activity of membrane-bound enzyme including ATPase activity (Cooke and Burden, 1990).

Since the physical properties of lipids are changed due to either low or high temperature, membrane damage has been noticed during low as well as high temperature stress. Accordingly unsaturated fatty acids and saturated fatty acids in the membrane lipids are reported to play a key role in cold tolerance and heat tolerance respectively (Taiz and Zeiger, 1998). Phospholipids act as the selective barrier between living cell and their environment, the plasma membrane plays a key role in the perception and transmission of extra information upon osmotic stress change in phospholipid composition are detected in plant (Munnik *et al.,* 1998). However, during exposure to stress the major role of phospholipid, the back bone of cellular membrane, may be to serve as precurssor for the generation of secondary messenger molecules. Where as the relative cleaving enzyme are the phospholipase. Phosphotidylinositol 4, 5 bisphosphate is itself signal and may be involved in several processes, such as the recruitment of signaling complexes to specific membrane location and their assembly (Martin, 1998).

Eventhough *P. juliflora* belongs to a group of salt tolerant plants; preliminary TLC analysis indicates that the lipid metabolism in both leaves and roots is altered due to salt stress. Only a detailed analysis of individual lipids and the biosynthetic path way would reveal the exact significance of the changes in the lipid composition.

3) Carbohydrate Metabolism

a) Enzyme Invertase:-

Salinity induced changes in the activity of enzyme invertase in leaves and root of *Prosopis juliflora* are shown in fig. 8. It is observed that the activity of enzyme invertase is increased with increasing NaCl salinity in leaves and roots. This increase is more significant in leaves of plants treated with 300 mM NaCl.

Invertase is considered as key enzyme of carbohydrate metabolism as it brings about breakdown of sucrose into glucose and fructose. Such inversion leads to mixed product of glucose and fructose in ratio (1:1). Based on glycosidal sequence classification this enzyme is included in family GH32. The molecular organization of enzyme protein has not been deeply investigated (Alberto *et al.*, 2004). According to Strum (1996) and Tymowska-Lalannae and Kries (1998), different isoform of invertase are present showing different biochemical properties and subcellular localization. The entry of sucrose into different utilization pathway is controlled by isoforms.

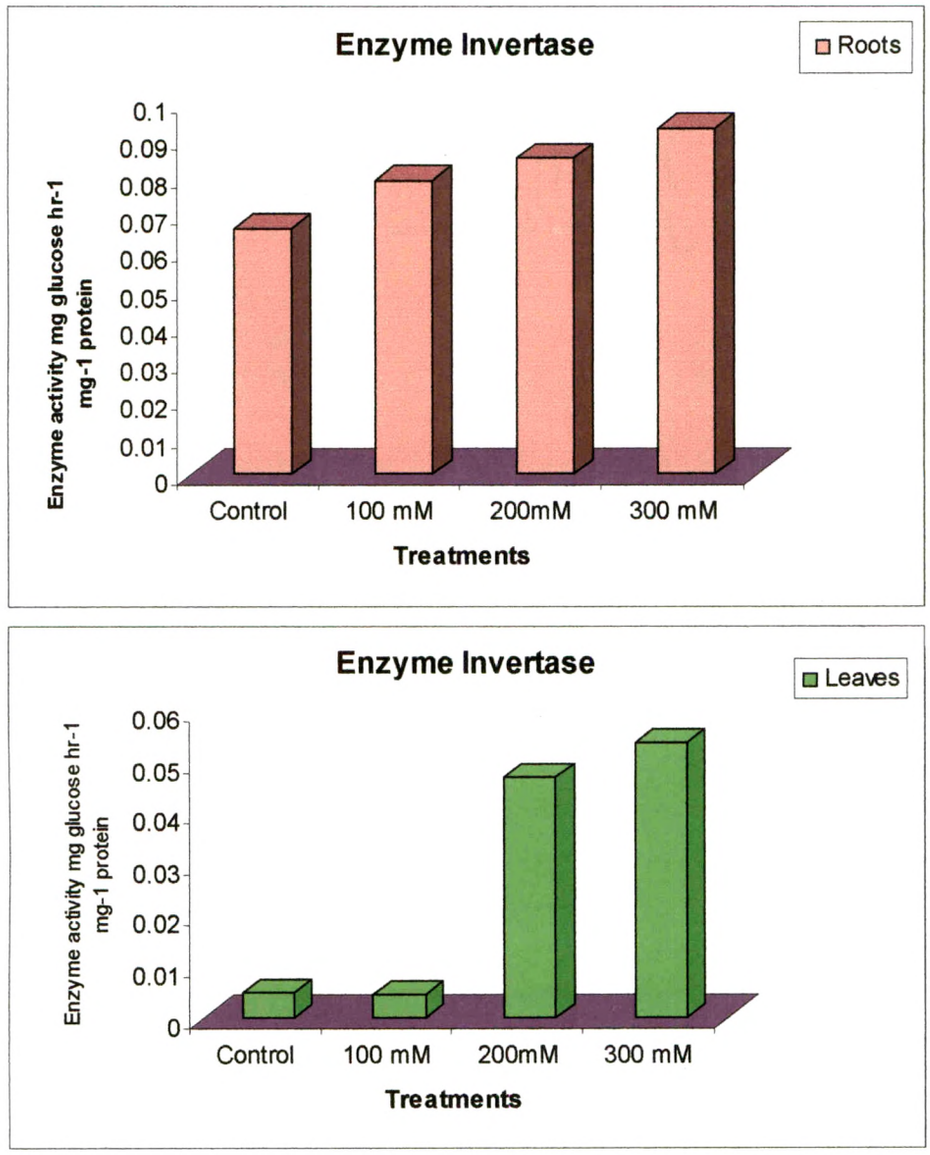


Fig.8. Effect of Sodium chloride salinity on the activity of enzyme Invertase in the roots and leaves of *Prosopis juliflora* (Sw.).

Different forms of invertase isoforms are accumulated in apoplast, cytoplasm and vacuole of plant cell. Hexoses have different fates and functions, which are generated by the activity of this enzyme (Strum, 1999). Invertase converts sucrose into hexoses which supplies energy and carbon for synthesis of different compounds. Breakdown of sucrose result in the formation of glucose and fructose which increases osmotic pressure for cell elongation and plant growth, thus invertase play indirect role in cell differentiation and plant development (Gibeaut *et al.,* 1990). Eschrich (1980) is of the view that invertase play important role in transport of sucrose from site of phloem loading and unloading.

Roitsch *et al.,* (2003) reported that extracellular invertase which is important enzyme of an apoplastic phloem unloading pathway and is involved in hydrolytic cleavage of the transport sugar, sucrose released into the apoplast. According to them the control of extracellular invertase represent a common response to biotic and abiotic stress such as pathogen infection and salt stress. It was noticed by Zeng *et al.,* (1990) in root tip of maize seedling expression of invertase gene and the invertase sucrose synthase ratio was reduced due to low oxygen level. Wiel *et al.,* (1994) observed that cell wall invertase activity was inhibited at pH 4.5 due to small polypeptides of 17 KD, which plays significant role in the regulation of enzyme activity. The activity of acid invertase is restricted by heavy metal ions such as Hg^{2+} and Ag^{2+} and by their reaction products like glucose and fructose.

Rathert (1982) reported that invertase activity was inhibited due to Cl^- and SO_4^{-2} salinity in leaves of salt sensitive cotton var. Dondara, while activity of enzyme was increased in the leaves of salt tolerant var. Giza-45 due to salinity. According to Grierson *et al.,* (1991), salt stress induced high level of invertase activity in n-tomato mutant plant. Fernandes *et al.,* (2004) studied effect of (50 mM and 150 mM NaCl) on acid and alkaline invertase in leaves of *Lupinus albus* L., they found that acid invertase activity was higher at 50 mM NaCl and decreased to control level at 150 mM NaCl. On the other hand alkaline invertase activity was increased due to salt treatment. In case of *P. juliflora* also it is noticed that in the leaves as well as roots the activity of invertase (pH 7.5) is stimulated due to salt stress at 50 mm, 100 mM and 200 mM NaCl treatment. Hawker and Walker (1978) noted that reduction in invertase activity contribute to reduction in the expansion rate of leaves of salt stressed bean, maize and barley plants. Since in *P. juliflora* leaf tissue the enzyme activity is stimulated due to salt stress there may not be any limitation of this factor on the leaf expansion rate.

b) Enzyme α-amylase:-

Effect of salt treatment on the activity of enzyme α-amylase in leaves and roots of *Prosopis juliflora* is shown in the fig. 9. It is evident from the figure that enzyme activity in roots was slightly increased due to 100 mM NaCl concentration, while it was decreased with increasing salinity treatments. In leaf tissue the activity of enzyme α-amylase was found to be decreased with the

increasing salinity treatments and this decreased was more prominent in 100 mM NaCl treatments.

A metal protein hydrolytic enzyme Alpha-amylase is one of the most important enzyme of carbohydrate metabolism. This enzyme also has got a great commercial significance in industrial biotechnology. Grager *et al.,* (1975) showed that α-amylase consist of a single polypeptide chain containing 475 residues with 2 SH groups, 2 disulfide bridges and tightly bound Ca^{2+}, While enzyme stability and activity is maintained due to presence of Ca^{2+} per molecules. Enzyme α-amylase randomly hydrolyse amylose and amylopectine after breakdown of α (1-4) linkage to produce oligosaccharides which further hydrolysed by α-glucosidase to produce glucose and maltose. Starch is a major carbohydrate reserved in majority of seeds. For the germination process and seedling development energy was supplied by converting this polysaccharide into simple sugar. The starch is a major product of photosynthetic CO_2 fixation and it is temporarily deposited during day in chloroplast. During night hours this assimilatory starch is degraded and resultant glucose molecules serves as respiratory substrate. The breakdown of starch also provides carbon skeleton for synthesis of several metabolites in plant cells (Jacobson *et al.,* 1970; Higgins *et al.,* 1976 and Munoz *et al.,* 1990). In germinating cereal grain α-amylase is involved in the conversion of insoluble starch granules into soluble fragments which are suitable for further degradation.

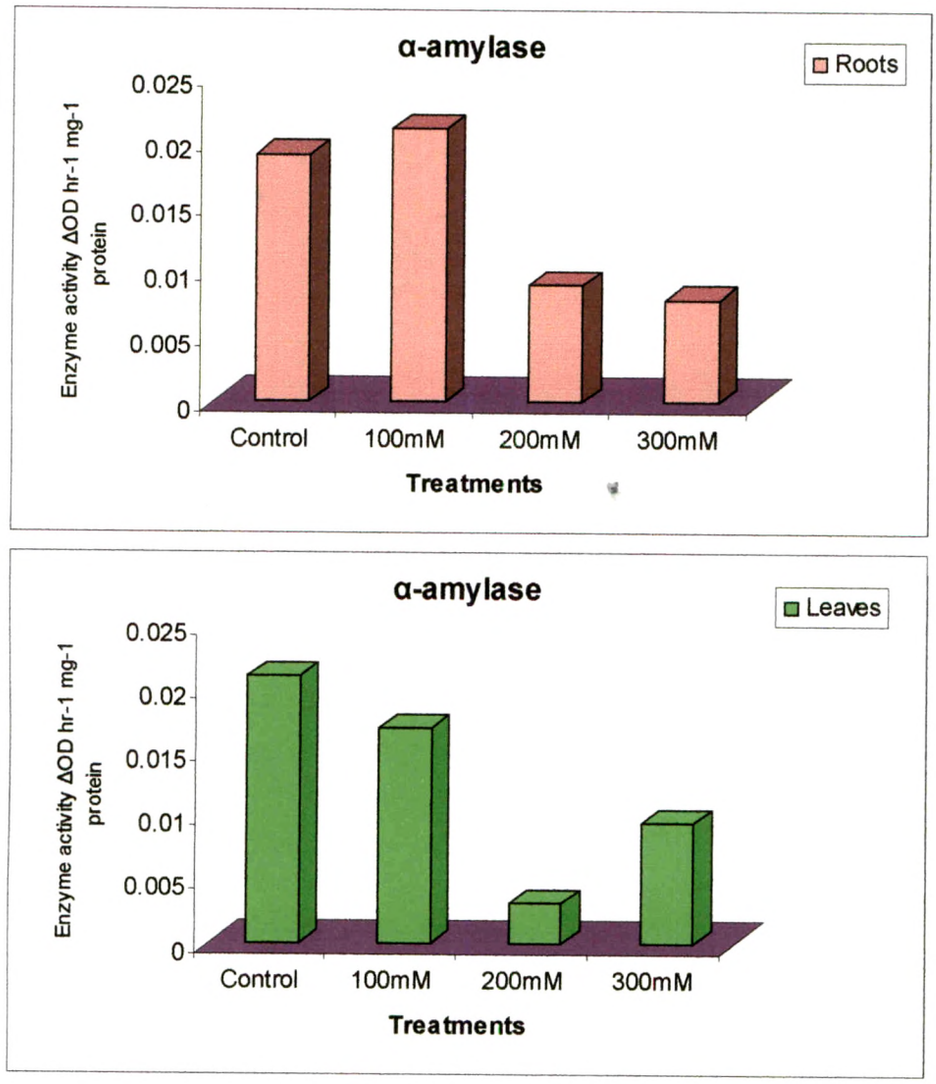


Fig.9. Effect of Sodium chloride salinity on the activity of Enzyme α-amylase in the roots and leaves of *Prosopis juliflora* (Sw.).

According to Muthukumarsamy and Pannerselvam (1997), inhibition of α- amylase activity in salt stressed radish seedling was either due to lowering of the enzyme protein or due to an inhibition of enzyme activity. The work of Kashem *et al.,* (2000) indicates an increasing chloride concentration there was a continuous decline in α-amylase activity.

Sangwan *et al.,* (1997) noticed NaCl salinity decreased the accumulation of the activity of α-amylase. El-Floury and Jung (1970) also noticed activation of amylase by NaCl salinity. Binet (1971) observed inhibition of amylogenesis in leaves of *Aster tripolium* by NaCl. The type of salinity also seems to exert specific influence on amylase activity. In sugar beet leaves the activity of amylase was not affected as a result of Cl^- salinization but decreases by SO_4^{-2} as concentration increases (Rathert and Doering, 1981). Sanchez *et al.,* (2004) recently noticed that the activity of α-amylase in the leaves of *Amaranthus hypochondriacus* is significantly increased by salt stress. An increase in α-amylase in the leaf tissue during day time can affected the deposition of photoassimilated starch in the plastids thereby causing disturbance in the carbohydrate metabolism. Such situation would not observed in leaves of salt stressed *P. juliflora* plants since there is a decline in α-amylase activity. Not much information is available about influence of salinity on α amylase activity in root tissue. Muthukumarsamy and Panneerselvam (1997) observed that in the roots of radish seedlings α-amylase and β-amylase activities were inhibited due to salinity. In case of

P.juliflora roots the activity of α-amylase is slightly stimulated by 100 mM NaCl treatment while higher doses caused an inhibition. Since such biosynthesis and degradation are not major metabolic activities in the root tissue in contrast to leaf tissue, the alterations in α-amylase activity may not have any significant role in overall metabolic picture of salt stressed roots.

4) Phosphorus Metabolism

a) **Enzyme Acid Phosphatase**:-

Effect of sodium chloride salinity on activity of enzyme acid phosphatase in leaves and roots of *Prosopis juliflora* is recorded in fig. 10. It is evident from the figure that the enzyme activity in both root and leaves is stimulated by salt stress except 300 mM NaCl treatment which has caused inhibition of the enzyme in roots.

Due to their sedentary mode of life, plant resort to many adaptive strategies in response to different abiotic stresses such as high salt, dehydration, cold and heat which ultimately affect the plant growth and productivity (Gill *et al.*, 2003). Against these stress plant adapt themselves by different mechanism including changes in physiological and biochemical responses (Bohnert *et al.*, 1995). Among these enzymes phosphatase, which are believed to be important for many physiological process including regulation of soluble phosphorus (pi) (Yan *et al.*, 2001). Phosphatases are traditionally classified as being acid and alkaline depending on their optimum pH for enzyme activity above and below pH 7.0

(Barret- Lennard *et al.,* 1982). Free soluble phosphatase play vital role in energy transfer, metabolic regulation, degradation of important structural constituent of biomolecules like phytin bodies and uptake of phosphorus from soil through solubilization of rock phosphatase. Although some abiotic stresses like salt, osmotic and water have been reported to increase phosphatase activities by maintaining a certain level of inorganic phosphate in plant cells (Olmos and Hellin, 1997). Acid phosphatase is mainly involved in hydrolysis of wide range of orthrophosphate monoesters and exhibit a pH optimum value below 6.0 (Hollander, 1971). In plant acid phosphatases are present in tissues like seeds (Kawarasaki *et al.,* 1996) roots (Penheiter *et al.,* 1997), leaves (Staswick *et al.,* 1994) and tubers (Gellathy *et al.,* 1994). All plant cell vacuoles are having acid phosphates activity compartmentation of acid phosphatase within the cell vacuoles. Acid phosphatase does not show absolute substrate specificity.

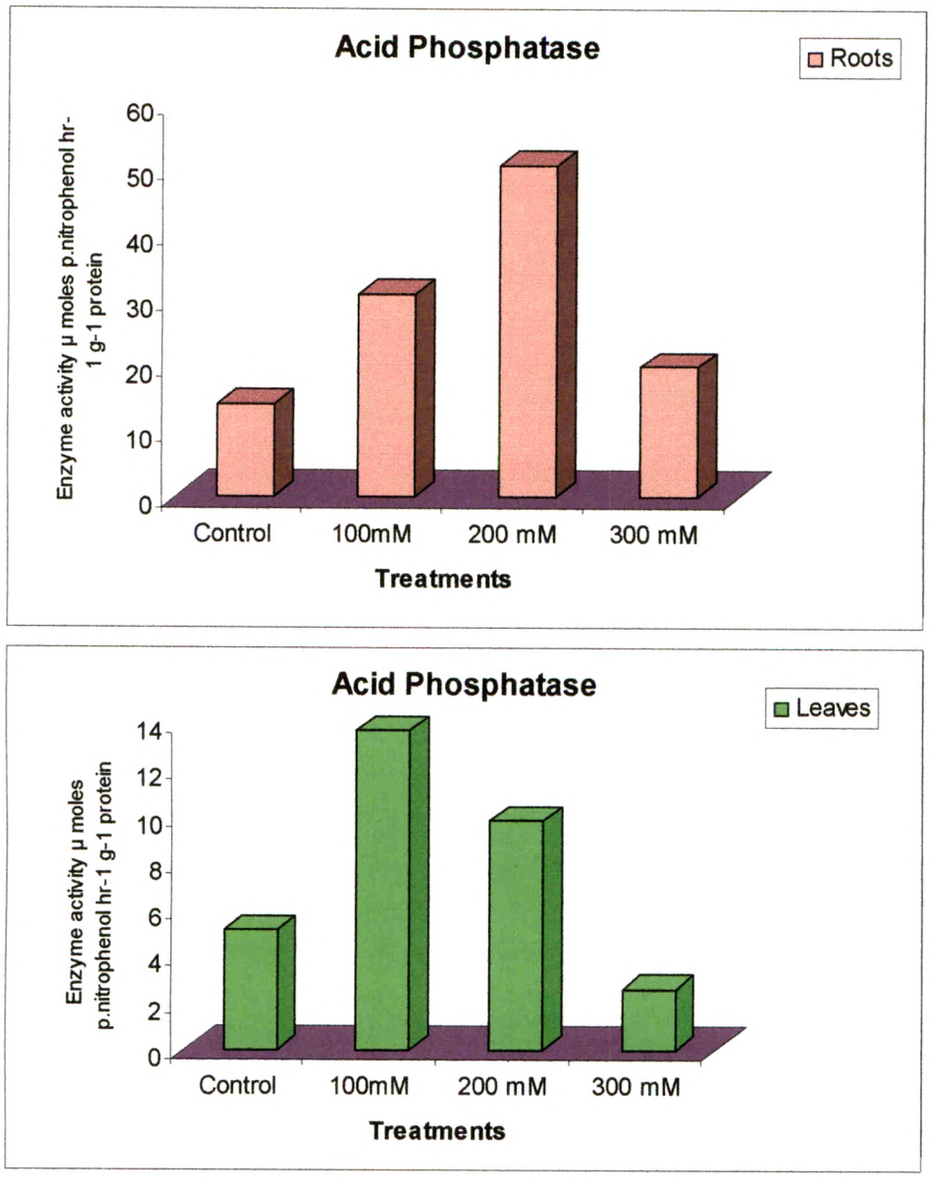


Fig.10. Effect of Sodium chloride salinity on the activity of Enzyme Acid Phosphatase in the roots and leaves of *Prosopis juliflora* (Sw.).

On the basis of relative substrate selectivity the acid phosphatase are divided in two categories as non- specific and specialized acid phosphatses. They are involved in the production, transport and re-cycling of pi. The specialized acid phosphatase is 3-p-glycrate phosphatase of maize leaves (Randall and Tolbert, 1971). It was reported by Duff *et al.,* (1991) that acid phosphates are monomeric or dimeric glycoproteins, which have subunit molecules masses of approximately 50 to 60 k Da. It was concluded by Tamura *et al.,* (1982) that during early phase of seed germination the enzyme play vital role in phosphorus metabolism by changing the level of inorganic phosphorus. Positive correlation between leaf acid phosphatase activity and total phosphorus uptake of wheat has been observed by McLachlan and De Marrco (1982). The activity of AP-1 is checked by Ca^{2+}, Fe^{3+}, MO^{6+} and Zn^{2+}. Acid phosphatase has both phosphatase and pyrophosphatases activity and it is highly stable even at 50^0C. Influence of salt stress on acid phosphatase activity has been investigated by some workers. Detailed study of influence of salinity on enzyme acid phosphatase in spinach leaf was performed by Pan (1987). He noticed enhancement of the enzyme activity due to salt stress. According to him an increased high molecular weight acid phosphatase play role in osmotic regulation and regulation of ion contents.

Flowers (1972) observed that sodium chloride of 333 mM has no significant effect on acid phosphatase activity from *Pisum.* Similar enhancement has been reported by Ahmed and Huq (1974) and Karadge and Chavan (1983) in halophytic *Spinach* and *Sesbania* respectively. Mittal and Dubey (1992) stated that

in endosperm and embryo axis of seeds of salt tolerant rice varieties CSR-1 and CSR-3, mitochondrial acid phosphatase activity was increased due to salinity. Lila Arab and Ehsanpour (2006) measured acid phosphatase activity in leaf and stem of *in vitro* grown *Medicago sativa* plant under saline condition. The enzyme activity increased due to increasing salt concentration. Rapid increase of vacuolar volume in response to salt stress in *Bruguiera sexangula* (L.) shows rapid increase in the vacuolar volume was an active process which followed the activation of the tonoplast H+ ATPase and Vacuolar acid phosphatase under salt stress (Mimura *et al.,* 2003). Chakrabarti and Mukharji (2003) found that salt stress stimulated acid phosphatase enzyme activity in leaf and root of mung bean over the control set. Acid phosphatase activity was increased by salt and osmotic stress in alfalfa (Ehsanpour and Amini, 2003). Flasinki *et al.*, (1989) reported that water and salt stress caused two to four fold increase in acid phosphatase activity in leaves of oilseed rape plant. Parida and Das (2004) studied effect of various levels of salinity (0, 100, 200, 400mM) on the activity of acid phosphatase in *Bruguiera parviflora* plant growing under hydrophonic culture. Their experiment revealed stimulation of acid phosphatase activity due to salinity.

Similar to this mangrove species in *Prosopis juliflora* also the acid phosphatase in roots and leaves is stimulated due to salt stress and it might be playing a role in redistribution of Pi utilize the plant.

b) Enzyme Alkaline phosphatase:-

Effect of NaCl treatment on the activity of alkaline phosphatase in leaves and roots of *Prosopis juliflora* is depicted in the figure.11. From the figure it is evident that the activity of enzyme is decreased in root and leaves with increasing salt concentration.

Enzyme alkaline phosphatase has pH optima in alkaline range. Enzyme alkaline phosphatase in plants is relatively less studied than acid phosphatase. Singh *et al.*, (2006) studied alkaline phosphatase (Apase) both phosphomonoesterage (PMEase) and phosphodiesterase (PDEase) in the Cyanobacterium *Anabaena oryazea* and also concluded that alkaline phosphatase (PMEase and PDEase) are quite stable enzyme and require a narrow range of pH (10-10.2) and temperature (35-40^{0}C) for their optimal activity. According to Duff *et al.*, (1994), plant alkaline phosphatase generally display an absolute substrate specificity and the example of this class of phosphatase in plants are cytosolic fructose 1-6 biphosphatase and sucrose 6 Phosphatase. Coleman and Gettins (1983) that alkaline phosphatase is a Zn (II) metalloenzyme and it catalyzes hydrolysis of phosphate monoester through the formation of a phosphosenyl intermediate.

Alkaline phosphates is a periplasmic dimeric enzyme and each monomer is long polypeptide chain of a relatively hydrophobic N-terminal extension or single peptide (Chung *et al.*, 1980). He also stated that monomer contain a single unglycosylated polypeptide chain of 499 amino acid residues having molecular weight of 47029 or a native dimmer of molecular weight of 94,058. Each

monomer contain an active centre with 3 distinct metal. The subunits are related by two fold rotational symmetry in two crystal form. In trigonal form the two fold axis is crystallographic and in the orthorhombic from the two folds axis is in a general position with one dimmer in the crystallographic asymmetric unit (Wyckoff *et al.,* 1983). The enzyme activity was stimulated due to low phosphatase concentration in the medium and continued protein synthesis. Spencer *et al.,* (1981) noticed that the enzyme was found to be associated with the cytoplasmic membrane and also found as a soluble enzyme in the periplasmic region.

Some attempts have been done to find out influence of salinity on behavior of this enzyme. Weimberg (1975) noticed decrease in alkaline phosphatase in pea seedlings due to NaCl salinity. A contrasting behavior of acid and alkaline phosphatase under saline condition was noticed by Ahmed and Huq (1974) in halophytic spinach under saline condition. In case of horsegram only lower concentration 25 mM of NaCl caused the real increase in alkaline phosphatase activity Nigwekar (1988). Parida and Das (2004) noticed that in mangrove *Bruguiera parviflora* plant the enzyme activity was increased due to varying level of salinity (0, 100, 200, 300mM). The effect of salt stress on alkaline phosphatase was studied by Pan (1983) in *Spinach* and concluded that alkaline phosphatase was inhibited by salinity (> 150 mM NaCl). In case of *Prosopis juliflora* a trend more or less similar to Spinach and pea is evident in both root and leaf tissue and the enzyme activity shows decline in salt stressed *Prosopis* acid

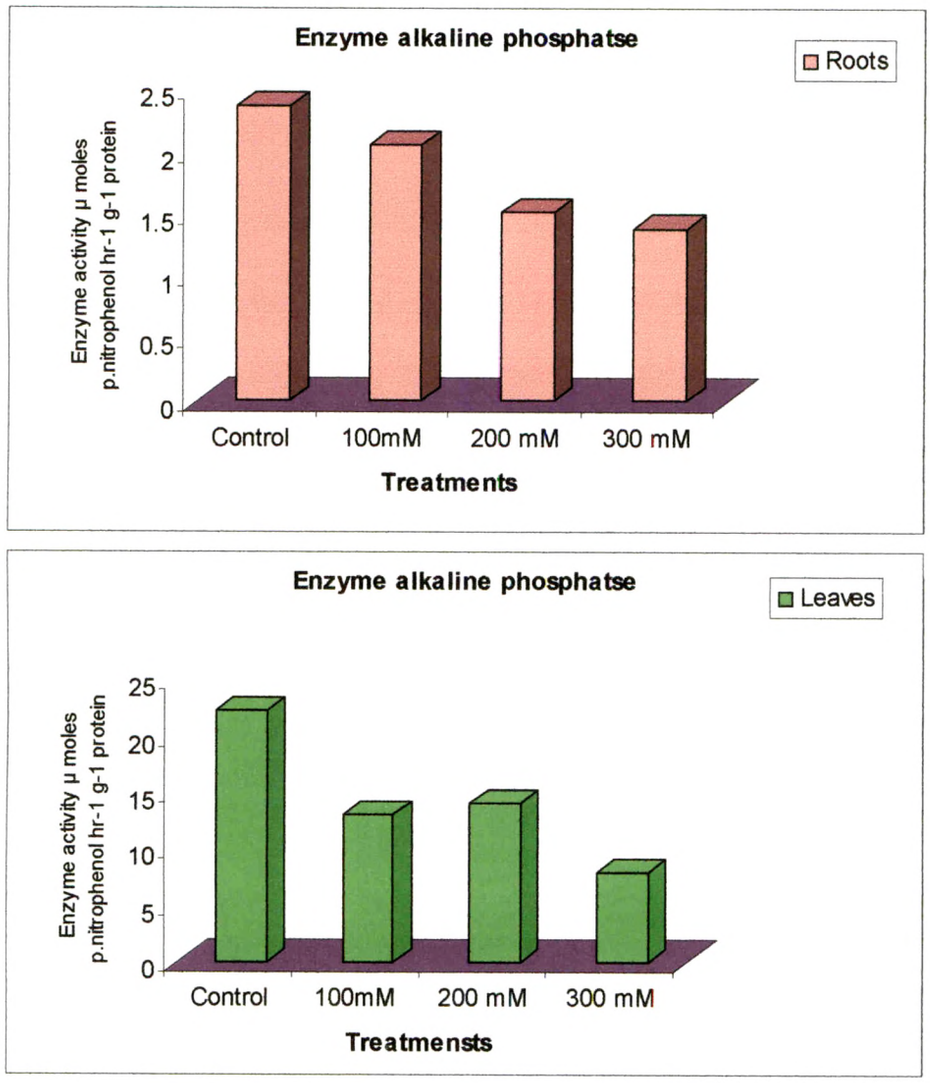


Fig.11. Effect of Sodium chloride salinity on the activity of Enzyme alkaline phosphates in the roots and leaves of *Prosopis juliflora* (Sw.).

phosphatase and alkaline phosphatase in the root and leaves tissue show an opposite trend. A difference in ionic balance and resulting in a shift in cellular pH might be a reason for such alterations.

c) Enzyme ATPase:-

Effect of NaCl salinity on enzyme ATPase in leaves and roots of *Prosopis juliflora* is shown in figure.12. It is evident from the figure that the activity of enzyme ATPase in root increases with increasing NaCl treatment upto 200 mM and decreases significantly at 300 mM NaCl treatment. While in leaf tissue the enzyme ATPase increases with increasing NaCl treatment.

The plasma membrane of higher plant has H+ ATPase as its major ion pump. According to Matsumoto and Yamaya (1984), ATPase enzyme is present at every site of energy transfer. This enzyme is involved in ion transport process and in formation of a subunit of the of the membrane structure. The enzyme play a key role in the nutrition and growth of the plant (Serrano, 1989). The plasma membrane H+ ATPase generates on electric potential and pH gradient across the plasma membrane by extrading protons from the cell. The energy bond in this electrochemical gradient is thought to be the driving force for solute carries and channels that are responsible for nutrient uptake and maintenance of cell turgor (Palmgren, 1991). The native H^+ ATPase of fungi and higher plant is likely to exist as a oligomer of the 100 k Da polypeptide (Dufour and Goffeau, 1980; Chadwick *et al.,* 1987) V-ATPase is a multi-subunit complex composed of two

functional domains, the peripheral V cytoplasmic domain and the membrane integral Vo domain. The molecular mass of V1 domain is about 500 k Da and it is responsible for ATP hydrolysis.

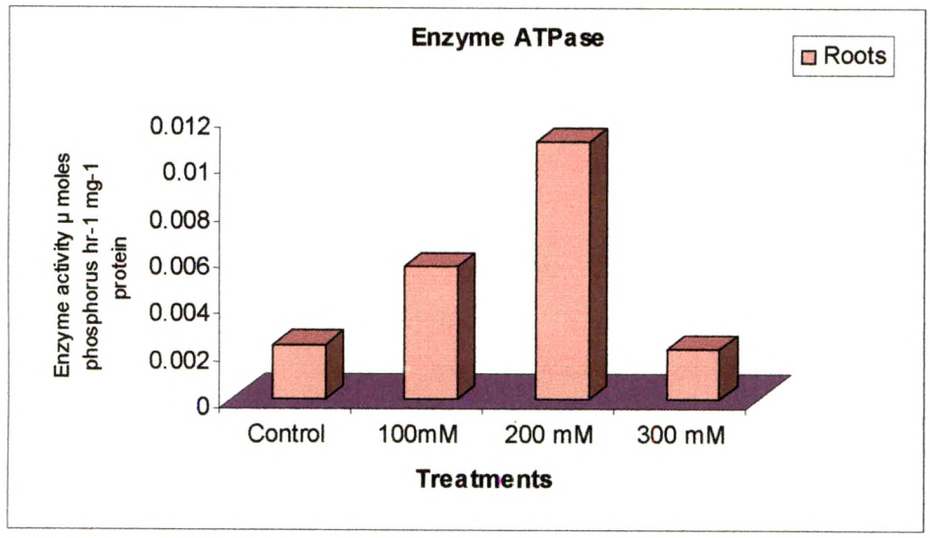
It consists of at least eight different subunit of molecular weight 13-70 k Da (Senthilkumar *et al.*, 2005). The primary active transporter process in the tonoplast is the accumulation of H+ by the action of two H+ translocating enzyme i.e. H- ATPase and H-PPase. The vacuolar H+ ATPase (V ATPase) is a universal component and it is involved in acidification of intracellular component of eukaryotic cells. The V ATPase pump proton from the cytoplasm to the lumen of the vacuole against the electrochemical gradient using the energy released by ATP hydrolysis and regulates the cytoplasmic pH (Senthilkumar *et al.*, 2005). The C-terminus of the plasma membrane H+ ATPase can be removed specifically by incubation of the enzyme with protease (Palmgren *et al.*, 1991). The C-terminal domain of plasma membrane H-ATPase has been identified to be an auto inhibitory domain. Cleavage of the C-terminal with trypsin resulted increasing H-ATPase activity in ATP hydrolysis and H pumping. Proteolytic treatment of membrane vesicles with trypsin resulted in increased H- ATPase activity and this was comparable to the activation of H- ATPase activity by fusicoccin (Johansson *et al.*, 1993). The self inhibitory domain seems to be involved in the fusicoccin activation of H- ATPase in spinach leaves (Johansson *et al.*, 1993), radish seedling (Rasi *et al.*, 1993) and oat roots (Lanfermeijer and Prins, 1994). For the C-terminal domain to have a physiological role eg. Interact with the ATP

binding site of the H+ ATPase, it has to be exposed on the cytoplasmic side of the plasma membrane. The regulation of ions across the plasma membrane is thought to be achieved by an electrochemical gradient generated by plasma membrane H+ ATPase (Sze, 1985) plasma membrane H- ATPase is regulated by light and hormones and is sensitive to cell turgor change (Romani *et al.,* 1983; Curti *et al.,* 1993 and Michelet and Boutry, 1995). Acidic phospholipids activate the plasma membrane H+ ATPase from higher plant (Memon *et al.,* 1989). Also acidic phospholipids mimic the stimulatory effect of calmodulin on this particular pump (Niggli *et al.,* 1981). It has been suggested that the stimulatory effect of phosphotidylserine on the plasma membrane Ca^{2+}-ATPase is linked to the ability of this acidic phospholipids to promote Ca^{2+} binding (Hermoni-Levine and Rahamimoff, 1990). Lysophospholipids, which are natural detergents produced by the action of phospholipase A2 on phospholipids are highly specific activators of the plasma membrane H+ ATPase (Pedehenko *et al.,* 1990). The plant hormone auxin seems to activate this enzyme (Scherer and Andre, 1989).

Plasma membrane Ca^{2+}-ATPase activity might be stimulated by self association of enzyme molecules into oligomers (Kosk and Bzdega, 1988). The oligomeric plasma membrane Ca^{2+}- ATPase has a higher specific activity than the monomeric enzyme. Calmoduline stimulates the Ca^{2+}- ATPase activity of monomeric Ca^{2+}- ATPase, but it is neither stimulates the Ca^{2+}- ATPase activity of oligomers nor promotes oligomerization (Kosk and Bzdega, 1990). Calmoduline binding to the enzyme increase the maximum transport velocity and the Ca^{2+} affinity and induces a high degree of cooperation in the Ca^{2+} activation (Schatzman, 1983).

As found for plasma membrane and vacuolar ATPase from other plant tissue, the pH 8.0 ATPase associated with beet vacuoles was Mg^{2+}- dependent (Hodges, 1976). This probably indicates that Mg-ATP is the true substrate (Balke and Hodges, 1975). In the presence of Mg^{2+}, activity was further stimulated by KCl which is also characteristic of many membrane bound ATPase in plants (Hodges, 1976). Analysis of the ion stimulation properties of the ATPase suggest that it is more sensitive to anions than to cations (Walker and Leigh, 1981). Among various environmental factors the influence of salinity on ATPase activity have been more thoroughly studied by several workers. Weimberg (1975) found that in seedling of pea which grown under highly saline media, activity of ATPase was slightly reduced. Kuiper *et al.,* (1991) noticed that the activities of Mg^{2+} dependent ATPase was increased due to increased mineral level in root of wheat seedling and juvenile plants of *Plantago major.*

The substrate specificity, the sensitivity to inhibitor and the pH optimum of the enzyme were not affected by ionic strength. Mittal and Dubey (1992) noticed that salinity causes increase in mitochondrial ATPase activity in embryo axis of rice seeds where as enzyme activity was decreased in endosperm and embryo axis in sensitive cultivars of rice. Lin *et al.,* (1997) noted that the activity of H+ ATPase was increased due to 75 mM NaCl in seedling of cotton.



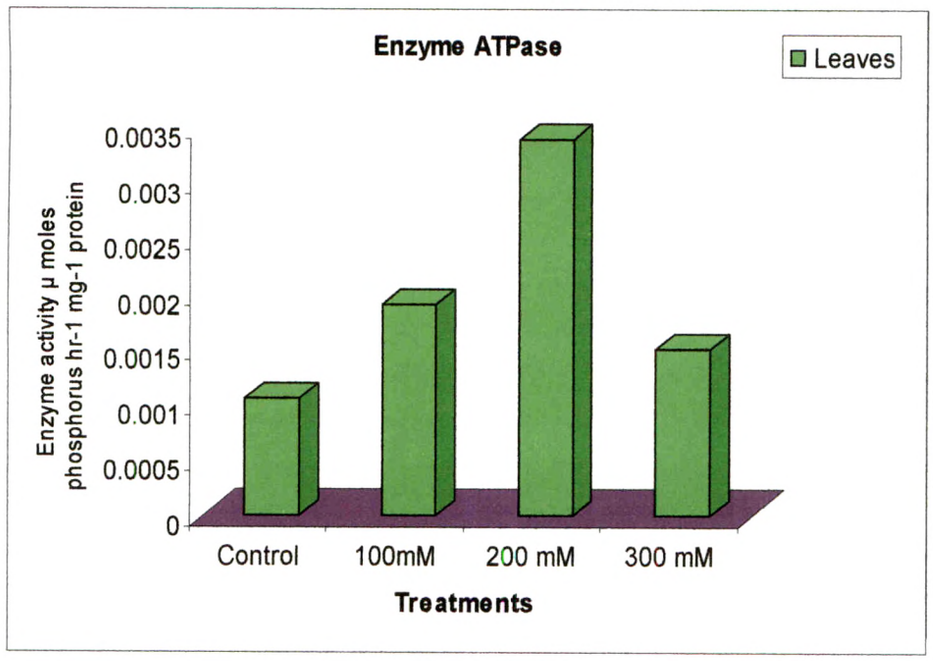


Fig.12. Effect of Sodium chloride salinity on the activity of Enzyme ATPase in the roots and leaves of *Prosopis juliflora* (Sw.).

Horovitz and Waisel (1970) reported that this enzyme is associated with salt tolerance with many halophytes. They also concluded that stimulation of this enzyme in glycophytic bean and carrot root and inhibition of the same in *Atriplex* and *Suaeda* roots after exposure to salt. Larher *et al.*, (1983) reported that in pea leaves activity of ATPase was stimulated at lower salt concentration but inhibited at higher salt concentration. Vacuolar ATPase helps in maintaining the pH of vacuole and thereby plays a crucial role in the functioning of vacuolar sodium proton antiport. Under salinity stress its expression is down regulated in root and upregulated in shoot of pearl millet (Tyagi *et al.*, 2006). Jauh *et al.*, (2006) indicated that this ATPase at participates in the endoplasmic reticulum, Golgi mediated protein sorting machinery for both house kipping function and compartmentalization of excess sodium under high salinity in *Mesembryanthemum.*

Leaf of maize plant treated with 125 mM NaCl shows slightly increase in H+ ATPase according to Zoerb *et al.*, (2005). Kolbus and Janicka (2004) stated that involment of specific calcium. Calmodulin dependent protein kinases helps in the activation of membrane ATPase under salt stress condition from *Cucumis sativus* roots from salt stress. According to Balasubbramaniam *et al.*, (2006), in *Aster* plant Plant-ATPase activity decreases with 3 % NaCl treated plant while F-ATPase activity increases with increase in NaCl concentration. Thus, it is clear that enzyme play important role in salt tolerance process. This increase may help in regulation of ion uptake as well as contribute energy to growth processes.

d) Enzyme Alkaline inorganic pyrophosphatase:-

Effect of NaCl treatment on the activity of alkaline inorganic pyrophosphatase activity of leaves and roots of *Prosopis juliflora* is depicted in the figure.13. It is clear from the figure that the activity of enzyme is decreased with increasing salt concentration in both root and leaf tissue. This trend is quite prominent upto 300mM NaCl treatment.

Pyrophosphate (PPi) is an important product formed in several reactions within a cell. During biosynthesis of proteins, nucleic acids and polysaccharides, pyrophosphate is produced as a side product. Hydrolysis of PPi is carried out by the inorganic pyrophosphatase in the plant. In the leaf inorganic pyrophosphatase in cytosol causes breakdown of pyrophosphate liberated during synthesis of UDPG (sucrose biosynthesis) while in plastid the enzyme catalyzes a similar reaction causing a breakdown of pyrophosphate liberated during synthesis of ADPG (starch biosynthesis). Thus this enzyme plays an important role though indirect in photosynthetic carbon metabolism. Kornburg (1962) has postulated that this enzyme is involved in control of biosynthesis of proteins, nucleic acids and fatty acids. According to Hendrick and Hans (2003), the alkaline pyrophosphatase was largely located in the chloroplast. Krishnan and Gnanam (1988) studied properties of Mg+ dependent inorganic pyrophosphatase in chloroplast of *Sorghum vulgare* leaves. It is a holoenzyme having a molecular mass of 42 ± 1.5 kDa. It was having high specificity for tetrasodium pyrophosphatase and its activity was strongly inhibited by pi and L-malate. Medd *et al.*, (2002) noted that

in lentil seedlings, nucleotide pyrophosphatase activity was enhanced in presence of divalent cations such as Ca^{++}, Mg^{++} and Mn where as Cu^{++}, Zn^{++} and Ni^{++}ions reduced activity of the enzyme.

Hemaltha and Prarasd (2002) recorded that alkaline pyrophosphatase isolated from cotyledon of Sesame was a monomer having molecular mass of 20.8 kDa. Maximum activity of enzyme was noticed at pH 8.6 and the temperature 50^0C in presence of Mg^{++}. The inhibition of enzyme activity was caused by ATP and partially reduced by Mg^{++}. Increase in the activity of acid and alkaline pyrophosphatase in cotyledon during germination of sesame was noticed by these workers. Karlsson (1975) observed that inorganic pyrophosphatase activity from leaf and root of sugarbeet was stimulated by K+, Rb+, Li+. Reilly (1982) have done extensively work on this enzyme and they reported that this enzyme activity showed changes in response to deficiencies to major nutrients. They observed that activity of alkaline pyrophosphatase was significantly enhanced in calcium deficient wheat plant and there was reduction in enzyme activity in absence of some major element. Besides regulating the level of pyrophosphate and supplying Pi for various reactions requiring Pi in the cell, inorganic pyrophosphatase is known to play some other roles due to the fact that breakdown of PPi is exergonic reaction.

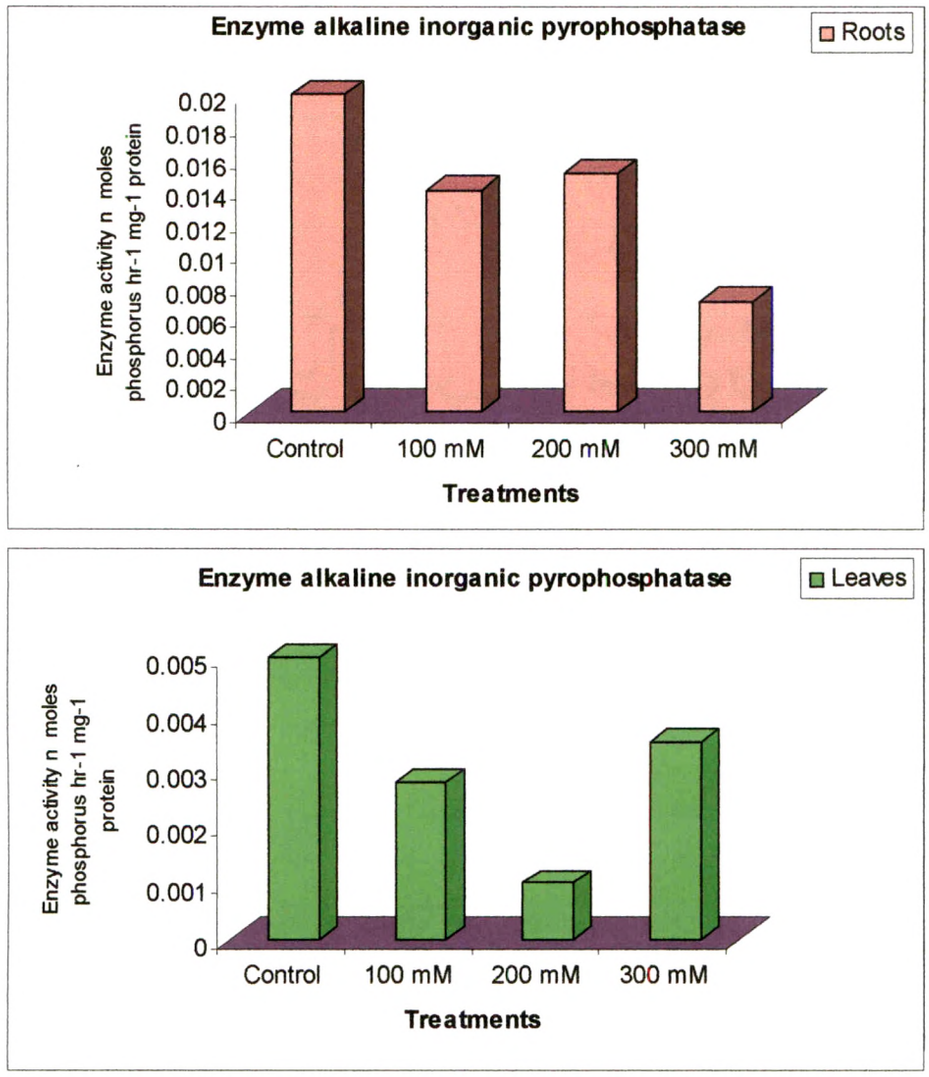


Fig.13. Effect of Sodium chloride salinity on the activity of Enzyme alkaline inorganic pyrophosphatase in the roots and leaves of *Prosopis juliflora* (Sw.).

Rea and Sander (1987) reported that inorganic pyrophosphatase can also acts as proton pump across the tonoplast membrane. Vianello and Macri (1999) noted that in higher plants, cell membrane bound proton pumping pyrophosphatase are present. In addition to vacuolar H+ PPiase three moitochondrial H+ PPiase are noticed which are present in the inner surface of inner mitochondrial membrane and involved in the specific hydrolysis of PPi coupled to proton transport. Simmons and Butter (1969) indicated that high activity of this enzyme in certain plant is directly related to high photosynthetic efficiency.

Murumkar and Chavan (1990) reported that in the leaves of salt sensitive legume chickpea a stimulation of inorganic pyrophosphatase was evident under saline conditions. In salt sensitive plants such an increase may play same role in energy dependent processes because ATP level is affected due to salt stress. But in salt tolerant *Prosopis juliflora* such situation perhaps may not occur which demands greater breakdown of PPi when ATP level becomes limiting.