

Influence of salt stress on phosphorus metabolism in the roots and leaves of one month old *Prosopis juliflora* (Sw.) DC seedlings

*Patil, Amol V. and Chavan, P. D.

Department of Botany, Shivaji University, Kollhapur-416 004 (MS) INDIA.

ABSTRACT

A sand culture experiment was designed to study the effect of sodium chloride salinity on phosphorus metabolism in the roots and leaves of one month old *Prosopis juliflora* (Sw.) DC seedlings. It was found that the P level in the roots as well as leaves was decreased with increasing level of salinity in rooting medium. However, the activities of enzymes acid phosphatase and ATPase were increased in both the parts of seedlings grown in saline conditions. The activities of alkaline phosphatase and inorganic pyrophosphatase were found to be decreased in the root and leaves of seedlings grown under saline conditions.

Key words: salinity, phosphorus, enzymes, *Prosopis juliflora*.

INTRODUCTION

The selection and breeding of salt tolerant crops is regarded as one of the main approaches to deal with a serious problem of salt affected soils throughout the world. In order to achieve this strategy it is necessary to identify the mechanisms of salt tolerance in the plant species well adapted to such problem soils. *Prosopis juliflora* is one such plant species which can successfully grow and complete its life cycle in a variety of problem soils. It is noticed that the plant has successfully established in farmlands of Digraj (Dist. Sangli) which are heavily affected by secondary salinization. *Prosopis juliflora* is a multipurpose plant of great economic potential. The ability of this species to grow on the poorest soil, under arid conditions and on saline soil is well known Pasiecznik et al.^[1]

According to Dagar and Tomar^[2] in India about 8.53 million ha land is waterlogged, 5.50 million ha land is saline and 3.88 million ha land is alkaline and more and more land is becoming water logged due to several factors. According to CSSRI these soils can be judiciously utilized for raising forestry, agriculture and horticulture crops. Afforestation programme for saline soil requires the proper selection of

tree species, as the major problems of such soils are high water table, high salinity impeded drainage and less soil aeration for tree growth, Singh^[3]

Phosphorus metabolism occupies a key position in cellular-biochemistry as it is related with energy relation in respiration and photosynthesis. Hence, an attempt has been made to study the phosphorus metabolism in the roots and leaves of *Prosopis juliflora* seedlings grown under salinity stress in laboratory conditions.

MATERIALS AND METHODS

For the experiment, seeds were obtained from the pod of *Prosopis juliflora* plants growing in the salt affected agriculture field in Sangli district in the month of April-May. Mechanically scarified seeds were used to raise the seedlings. After the establishment of seedlings for 5 days, they were treated with increasing concentration of salt (100, 200, and 300 mM NaCl) mix with half strength Hoagland solution. The seedling were grown for one month and then analysed for phosphorus metabolism. The method of Sekine *et al.*^[4] was employed for estimation of Phosphorus from the root and leaves. Fresh leaves and roots were used for the assay of enzymes of Phosphorus metabolism. For enzyme acid phosphatase crude enzyme was prepared in 0.1 M acetate buffer (pH 5) and assayed according to the method of Mclachlan^[5] The activity of enzyme ATPase was determined following the method

*Address for correspondence:
E-mail: prosopis.1912@gmail.com

DOI: 10.5530/pj.2011.25.9

described by Todd and Yoo^[6] and liberated phosphorus was estimated by the standard method^[7] The method described by Weimberg^[8] was employed for the study of activity of enzyme alkaline phosphatase. A method by Kar and Mishra^[9] was employed for the determination of the activity of enzyme alkaline inorganic pyrophosphatase. The soluble proteins in the enzyme preparations were determined according to the method of Lowry *et al.*,^[10]

RESULTS

Phosphorus is an important macronutrient essential for all living organisms. It plays a 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^[11] There are many reports indicating suppression of P uptake due to salt stress.^[12,13] Nieman and Clark^[14] also found depression of total P in the corn leaves due to salinity at low level of inorganic phosphorus in the nutrient solution. In case of *Prosopis cineraria* seedlings Ramoliya *et al.*,^[15] noticed that phosphorus content was significantly decreased in the leaves with increase in soil salinity while that was gradually decreased in the stem and root tissues. A decrease in P content of root tissue and that increase in the leaf tissue of salt grown *Poncirus trifoliata* was evident in the experiments by Tozly *et al.*^[16]

Prosopis juliflora seedlings have shown a pattern similar to that in *Prosopis cineraria* since in both root and leaves a decline in P content was evident in the seedlings exposed to salt stress (Figure 1) According to Gibson^[17] 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

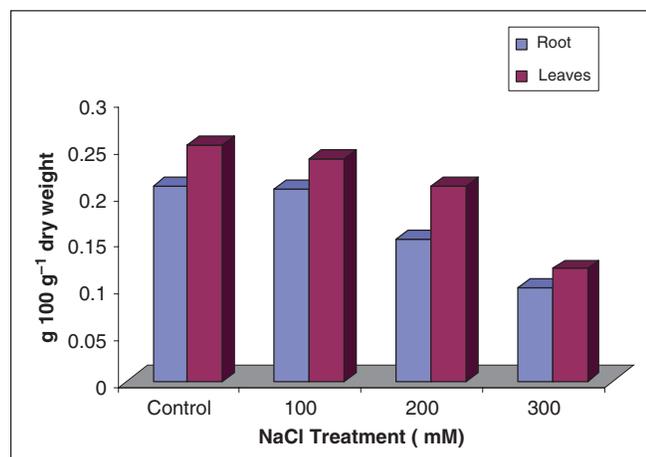


Figure 1: Effect of Sodium chloride salinity on phosphorus content in the roots and leaves of *Prosopis juliflora* (Sw.).

which appears to be quite stable during salt stress in this species, the phosphorus nutrition in *Prosopis juliflora* seems to be sensitive to salt stress. The disturbance in P nutrition can have significant effects on overall plant metabolism in view of a key role of this element in cellular biochemistry.

Effect of sodium chloride salinity on the activity of enzyme acid phosphatase in the leaves and roots of *Prosopis juliflora* is recorded in figure 2(a). It is evident that the activity of this enzyme in both root and leaves is stimulated at all salinity levels except 300 mM NaCl, at which it has decreased in the roots. Enhancement in the activity of acid phosphatase in the leaves of spinach grown under saline condition has been reported by Pan.^[18] Similar observations have been made by Karadge and Chavan^[19] in *Sesbania*. Lila Arab and Ehsanpour^[20] measured acid phosphatase activity in the leaf and stem of *in vitro* grown *Medicago sativa* under saline conditions and found that the activity was increased due to increasing salt concentration. Chakrabarti and Mukharji^[21] have also found that the salt stress caused to increase the activity of acid phosphatase in the leaf and roots of mung bean. Parida and Das^[22] studied effect of various levels of salinity (0, 100, 200, 400mM NaCl) on the activity of acid phosphatase in *Bruguiera parviflora* growing under hydroponic culture. Their experiments also revealed that the salinity causes stimulation of activity of this enzyme.

Effect of NaCl salinity on the activity of enzyme alkaline phosphatase in the leaves and roots of *Prosopis juliflora* is depicted in the figure 2 (b). It is evident that the activity of this enzyme is decreased in the root and leaves with increasing level of salt in the medium. Weimberg^[23] noticed a decrease in the level of alkaline phosphatase in pea seedlings due to NaCl salinity. A contrasting behavior of acid and alkaline phosphatases under saline conditions was noticed by Ahmad and Huq^[24] in halophytic spinach. In the case of horsegram only lower concentration of salt (25 mM of NaCl) caused the real increase in alkaline phosphatase activity.^[25] Parida and Das^[22] noticed that the activity of this enzyme in a mangrove, *Bruguiera parviflora* was increased under varying levels of salinity (0, 100, 200, 300 mM NaCl). The effect of salt stress on alkaline phosphatase was studied by Pan^[26] in Spinach. He found that the enzyme alkaline phosphatase was inhibited by salinity (> 150 mM NaCl). In case of *Prosopis juliflora* a trend more or less similar to that in Spinach and pea is evident in both root and leaf tissues. Acid phosphatase and alkaline phosphatase in the root and leaves of this plant, however have shown an opposite trend. A difference in ionic balance resulting in a shift in cellular pH might be a reason for such alterations.

Effect of NaCl salinity on enzyme ATPase in the leaves and roots of *Prosopis juliflora* is shown in figure 2(c). It is evident that the activity of enzyme ATPase in the root was

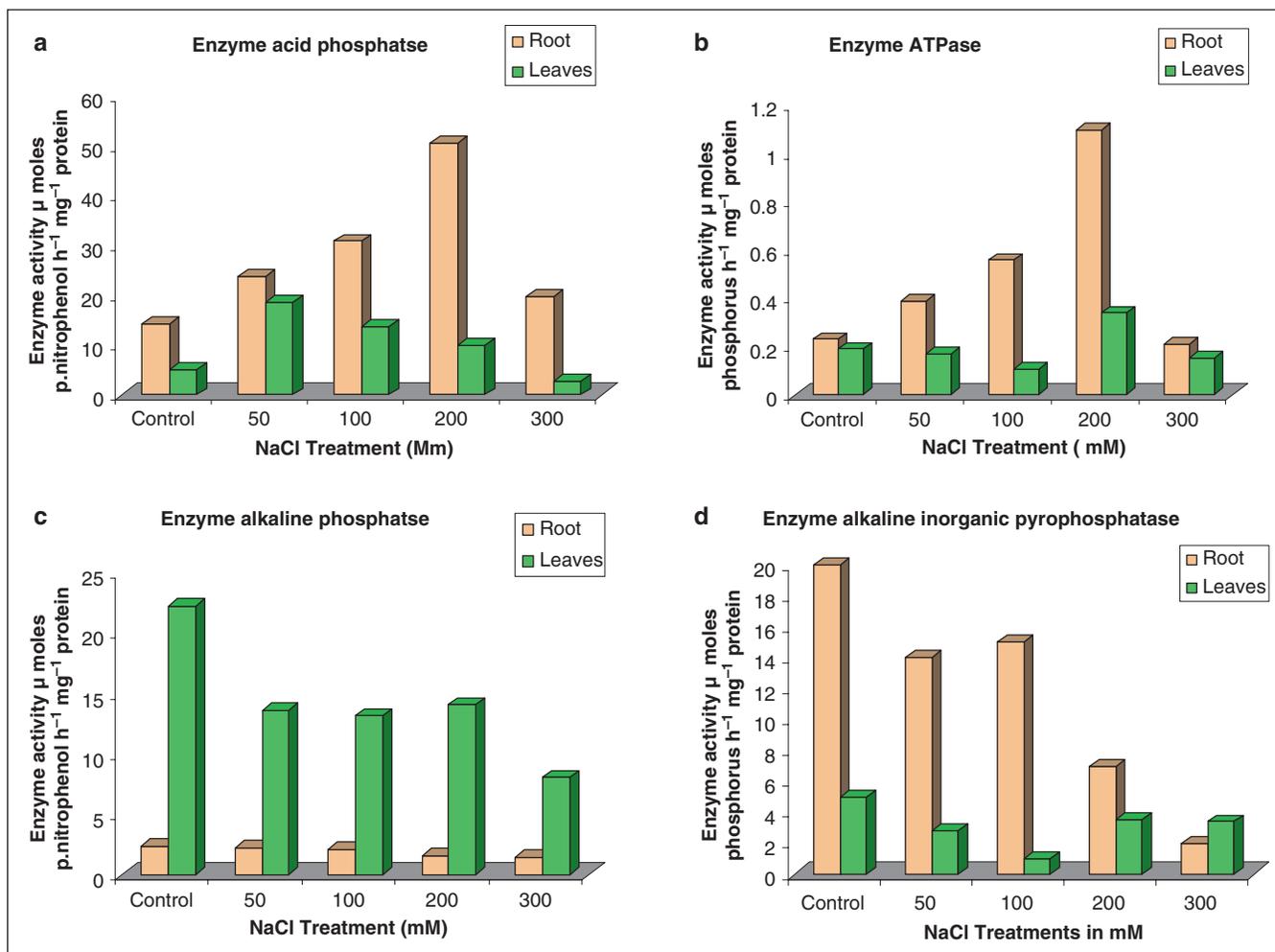


Figure 2: Effect of Sodium chloride salinity on the activity of (a) Enzyme acid phosphatase (b) Enzyme alkaline phosphatase (c) Enzyme ATPase and (d) Enzyme alkaline inorganic pyrophosphatase in the roots and leaves of *Prosopis juliflora* (Sw.).

increases with increasing NaCl treatment upto 200 mM and later decreased significantly at 300 mM NaCl. While, in the leaf tissue its activity was increased with increasing level of salt. Weimberg^[8] found that in the seedlings of pea grown under highly saline media, the activity of ATPase was slightly reduced. Kuiper *et al.*^[27] noticed that the activities of Mg²⁺ dependent ATPase was increased due to increased mineral level in the root of wheat seedlings and juvenile plants of *Plantago major*. Lin *et al.*,^[28] noted that the activity of H⁺ ATPase was increased due to 75 mM NaCl in the seedlings of cotton. Horovitz and Waisel^[29] reported that this enzyme is associated with salt tolerance with many halophytes. They also observed a stimulation of this enzyme in glycophytic bean and carrot root and inhibition of the same in *Atriplex* and *Suaeda* roots after exposure to salt. Under salinity stress its expression is down regulated in root and upregulated in shoot of pearl millet.^[30] Leaf of maize plant treated with 125 mM NaCl showed slight increase in H⁺ ATPase.^[31] Balasubramaniam *et al.*,^[32] reported a decrease in the activity of ATPase in 3 % NaCl

treated *Aster* plant while F-ATPase activity was increased with increase in NaCl concentration. Thus it is clear that this enzyme plays an important role in salt tolerance process. This increase may help in regulation of ion uptake as well as contribute energy to growth processes.

Effect of NaCl salinity on the activity of enzyme alkaline inorganic pyrophosphatase in the leaves and roots of *Prosopis juliflora* is shown in the figure. 2 (d). It is evident that the activity of this enzyme decreases in the root and leaf tissue with increasing salt concentration. This trend is quite prominent upto 300mM NaCl treatment. This enzyme plays important role in regulating the level of pyrophosphate and supplying Pi for various reactions requiring Pi in the cell. Rea and Sander^[33] reported that inorganic pyrophosphatase can also acts as proton pump across the tonoplast membrane. Vianello and Macri^[34] noted that in higher plants, cell membrane bound proton pumping pyrophosphatase and three moitochondrial H⁺ PPiase present in the inner surface of inner mitochondrial

membrane involved in the specific hydrolysis of PPi coupled to proton transport. Simmons and Butter^[35] indicated that high activity of this enzyme in certain plants is directly related to high photosynthetic efficiency. Murumkar and Chavan^[36] reported that in the leaves of salt sensitive legume *Cicer arietinum*, 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

CONCLUSION

In conclusion it can be stated that due to salinity, there is definite changes in phosphorus metabolism in the salt tolerant species *Prosopis juliflora*. Some of these changes are probably related to mechanisms underlying salt tolerance in this species.

ACKNOWLEDGEMENT

Authors are highly thankful to Dr. B. A. Karadge the Ex. Head, Department of Botany, Shivaji University, Kolhapur (MS, India) for his valuable suggestions.

REFERENCES

- Pasiecznik, N., Felker, P., Harris, P.J.C., Harsh, L.N., Cruz, G., Tewari, J.C. Cadoret, K. and Maldonado, L.J. 2001. The *Prosopis juliflora*, *Prosopis pallida* complex: A Monograph. HDRA, Coventry, UK.
- Dagar J.C. and Tomar O.S. Utilization of salt affected Soil and Poor Quality water for Sustainable Biosaline Agriculture in Arid and Semi-arid Regions of India. 12th ISCO Conference Beijing; 2002.
- Singh G. The role of *Prosopis* in reclaiming high pH soils and in meeting firewood and forage need of small farmers. pp.1.3-1.27. In: *Prosopis: Semi-arid Fuelwood and Forage Tree; Building Consensus for the Disenfranchised.* (Eds.) P.Felker and J. Moss. Center for Semi-Arid Forest Resources, Kingsville, Texas, USA, 1996.
- Sekine T, Sasakawa T, Morita S, Kimura T and Kuratom K. cf. laboratory manual for physiological studies of Rice (Eds.) Yoshida, S., Forno, D., Cook, J.B. and Gomez, K.A. Pub. International Rice Research institute, Manila, India: 1972.
- McLachlan K.D. Acid phosphatase of intact roots and phosphorus nutrition in plants. *Aust. J. Agric. Res.* 1980; **31**:441-448.
- Todd G.W. and Yoo B.Y. Enzymatic changes in detached wheat leaves as affected by water stress. *Phyton* (Buenos Aires), 1964; **21**:61.
- Fiske C.H. and SubbaRao Y. The calorimetric determination of phosphorus. *J. Biol. Chem.* 1925; **66**:375-400.
- Weimberg R. Enzyme levels in pea seedlings grown in highly salinized media. *Plant Physiol.* 1970; **46**:466-470.
- Kar M. and Mishra D. Inorganic pyrophosphatase activity during rice leaf senescence. *Can. J. Bot.* 1976; **53**:503-511.
- Lowry O.H., Rosenbrough N.J., Furr A.L. and Randall R.J. Protein measurement with folin phenol reagent. *J. Biol. Chem.* 1951; **193**:262-263.
- Bielecki R.L. and Ferguson I.B. Physiology and metabolism of phosphate compounds (Eds. A. Lauchi and R.L. Bielecki). In: *Inorganic plant Nutrition. Encyclopedia of plant physiology, New series.* 1983; **15**. pp. 422-449. Springer-verlag New York.
- Fageria N.K. Salt tolerance of rice cultivars. *Plant and soil*, 1985; **88**:237-243.
- Indulkar B.S. and More S.D. Interactive effect of nature of salinity and nitrogen on growth and nutrient composition of *Sorghum*. *J. Indian Soc. Soil Sc.* 1985; **33** (8):641-645.
- Nieman R.H. and Clark R.A. Interactive effect of salinity and phosphorus nutrition on the concentration of phosphate and phosphate esters in mature photosynthesizing Corn leaves. *Plant Physiol.* 1976; **57**:157-161.
- Ramoliya P.J., Patel H.M., Joshi J.B. and Pandey A.N. Effect of salinization of soil on growth and Nutrient Accumulation in Seedlings of *Prosopis cineraria*. *J. of Plant Nutrition.* 2006; **29**:283-303.
- Tozly I., Moore G.A. and Gey C. Effect of NaCl concentration on stem elongation dry mass production and micronutrient of *R. Trifoliata*. *Aust. J. of Plant Physiol.* 2000; **27** (1):35-42.
- Gibson T.S. Carbohydrate metabolism and phosphorus /salinity interaction in wheat (*Triticum aestivum* L.). *Plant and Soil.* 1988; **111**:25-35.
- Pan S.M. Characterization of multiple acid phosphates in salt stressed spinach leaves. *Aust. J. Plant Physiol.* 1987; **14**:117-124.
- Karadage B.A. and Chavan P.D. Physiological studies in salinity tolerance of *Sesbania aculeata* Poir. *Biol. Plant.* 1983; **25** :412-418.
- Lila A. and Ehsanpour A. The effect of ascorbic acid on salt induced alfalfa (*Medicago sativa* L.) in *in vitro* culture. *Nigerian Society for experimental Biology.* 2006; **18**(2):63-69.
- Chakrabarti N. and Mukherji S. Growth regulator mediated changes in leaf area and metabolic activity in mungbean under salt stress condition. *Indian J. of Plant Physiol.* 2003; **7**(3):256-263.
- Parida A.K. and Das A.B. Effect of NaCl stress on nitrogen phosphorus metabolism in a true mangrove *Bruguiera parviflora* grown under hydroponic culture. *J. of Plant Physiol.* 2004; **161** (8):921-928.
- Weimberg R. Effect of growth in highly salinized media on the enzymes of the photosynthetic apparatus in pea seedlings. *Plant Physiol.* 1975; **56**:8-12.
- Ahmad R. and Huq Z. Some Physiological and biochemical studies on spinach growing on saline soil. *Pak. J. Bot.* 1974; **6**:49-52.
- Nigwekar A.S. Physiological studies in horse-gram (*Dolichos Biflorus* L.). A Ph.D. thesis submitted to Shivaji University, Kolhapur. India :1988.
- Pan S.M. The effect of salt stress on the betain aldehyde dehydrogenase in spinach. *Taiwania.* 1983; **28**:128-137.
- Kuiper D., Sommarin M. and Kylin A. The effect of mineral nutrition and benzyl adenine on the plasmalemma. ATPase activity from roots of wheat and *Plantago* major ssp *pleiosperma*. *Physiol. Plant.* 1991; **81**:169-174.
- Lin H., Salus S.S. and Schumaker K.S. Salt sensitivity and the activities of H⁺ ATPase in cotton seedling. *Crop Sci.* 1997 ; **37**:190-197.
- Horovitz C.T. and Waisel Y. Different ATPase system in glycophytic and halophytic plant species. *Experientia.* 1970; **26**:941-42.
- Tyagi W., Singla P., Nair S., Reddy M.K. and Sopory S.K. A novel isoform of ATPase subunit from pearl millet that is differentially regulated in response to salinity and calcium. *Plant Cell Reports.* 2006; **25**(2):156-163.
- Zoerb C., Stacke B., Trumitz B. and Denter D. Does H⁺ pumping by plasma membrane limit leaf growth ATPase in maize during 1st phase of salt stress. *Journal of plant nutrition and soil Science.* 2005; **168** (5):550-557.
- Balasubramaniam R., Thilo R., Ahmed B., Ralf S., Burnhard H., Ahlert S. and Jatta P. *Aster tripolium* L. and *Sesuvium portulacastrum* L.: Two halophytes two strategies to survive in habitat. *Plant physiology and Biochemistry.* 2006; **44**(5-6):395-404.
- Rea P.A. and Sander D. Tonoplast energization: Two H⁺-pump, one membrane. *Physiol. Plant.* 1987; **71**:131-141.
- Vianello A and Macri A. Proton pyrophosphatase from higher plant mitochondria. *Physiol. Plant.* 1999; **105**:763-768.
- Simmons S. and Butter L.G. Alkaline inorganic pyrophosphatase of maize leaves. *Biochim. Biophys. Acta.* 1969; **172**:150-157.
- Murumkar C.V. and Chavan P.D. Influence of salt stress on phosphorus metabolism in leaves of chickpea *Cicer arietinum* L. *Indian Bot. Repr.* 1990; **9**(2):56-60.