J. Indian bot. Soc. ISSN - 0019 - 4468 Vol. 95 (1 & 2) 2016 : 142-147

IN VITRO ALLEVIATION OF NaCl INDUCED INHIBITION OF ACTIVITIES OF CERTAIN ENZYMES BY PHYTOHORMONES

Shweta Tyagi and Sanjeev Kumar

Department of Botany, DAV (PG) College, Muzaffarnagar

E-Mail : shewtatyagi@gmail.com

When crude enzyme extracts of water imbibed seeds of Phaseolus aureus cv. K-851 were treated with 1×10^{-1}M NaCl, inhibited activities of alpha amylase, beta amylase, acid phosphatase, alkaline phosphatase and protease were noted. But, when enzyme extract containing 1×10^{-1}M NaCl, was subjected to $1x10^{-10}$M (0.1NM) IAA,GA and Kn, the activities of these enzyme was increased, being maximum at the treatment of a mixture of $1x10^{-10}$ M of each IAA, GA and Kn.

Key words : Amylases, protease, acid and alkaline phosphatase, Phytohormones, NaCl.

Salt stress have indicated that inhibition of seed germination and seedling phase of development (Gulzar *et al.* 2003) and growth, dry matter accumulation, yield (Sultana *et al.* 1999). Dry mass of plants is also reduced in proportion to the increase in salinity (Romero-Aranda *et al.* 2001). Reduced shoot dry mass, cob yield, total kernel yield, chlorophylls "a" and "b" and relative water content in the maize plants and increased proline accumulation, activities of the key antioxidant enzymes superoxide dismutase, peroxidase, catalase and polyphenol oxidase and electrolyte leakageis noticed (Kaya *et al.* 2013).

Interestingly, foliar application of 15 ppm IAA to the sodium chloride stressed plants have shown an alleviating effect on crop yield. (Gurudevi *et al.* 2012). Gibberellic acid (GA_3) is known to induce the synthesis of a α-amylase in embryo-less rice seeds (Palmiano and Juliano 1972). Inhibition of α-amylase activity under salt stress is also reduced by GA (Lin and Kao, 1995). In present study, we reports the effect of growth hormones on *in-vitro* activities of certain enzyme in salinity stressed crude enzyme extract of water imbibed seeds of *Phaseolus aureus cv K-851.*

MATERIAL AND METHODS

The certified seeds of *Phaseolus aureus* cv. (K-851) were obtained from IARI, New Delhi. Seeds of uniform size, shape, colour and weight as far as possible, were surface sterilized with 0.1% $HgCl_2$ solution and washed with distilled water. These seeds were imbibed in distilled water. Enzyme extract of these imbibed seeds was prepared after homoginizing 1 gm material in 10 ml TMB buffer and centrifuge at 6000 rpm for 20 minutes to get a clear supernatant and volume made to 20 ml with buffer. Doses of NaCl (1×10^{-1} M) and growth hormone (1×10^{-10}M IAA, 1×10^{-10}M GA and 1×10^{-10} M Kn) were prepared. There after, extract of crude enzyme treated with NaCl/ growth hormones (9 doses of enzyme extract : 1 dose of NaCl / growth hormone) and the combination of doses of all the growth hormones and activities of certain enzymes i.e β- amylase, acid phosphatase and alkaline phosphatase were measured. A common Tris - malate buffer at 6.8 pH was prepared. (Vimala 1983). This was used as extraction cum assay medium for amalyses and proteases. Crude enzyme was extracted by homogenising 1 gm material in 10 ml Tris-malate buffer and centrifuging the extract to get a clear supernatant, which was made to 20 ml with the buffer. The preparation constituted the crude enzyme extract. Further, each enzyme was assayed as per the method given here under :

α-amylase activity : Take 1ml of enzyme extract and 1 ml substrate i.e. starch (0.15%) added to it and then incubate it at room temperature for 10 minutes. Now add 3 ml of

Received on April 25, 2016 www.indianbotsoc.org Accepted on June 19, 2016

quinching reagent and read O.D. at 620 nm. Total activity was determined in term of mg starch degraded per minute per gm fresh weight (Filner and Varner 1967).

β-amylase activity : Pipette 0.5 ml of respective enzyme dilutions into a series of numbered test tubes. Incubate a blank with 0.5 ml distilled water. Incubate the tubes at 25°C for 3 to 4 minutes to achieved temperature equilibrium. After that, add 0.5 ml starch solution (1%) and incubate exactly 3 minutes and add 1ml DNS color reagent to each tube. Incubate all tubes in a boiling water bath at 100ºC for 5 minutes and kept at room temperature and mix well then read absorbance at 540 nm. Total activity was determined in term of mg maltose degraded per minute per gram fresh weight. (Bernfeld 1955).

Protease activity : 1 ml of enzyme extract was incubated for 1 hr at 40^{O}C with 1 ml substrate (4mg/ml casein in buffer). The reaction was quenched by addition of 2 ml of TCA and chilling for 3 hr. The supernatant was collected by centrifugation, made slightly alkaline by addition of 1 ml 1.5 N NaOH and final volume made to 5 ml with buffer. 1 ml of this was mixed with 5 ml of copper sulphate reagent and after 10 minutes, 1 ml Folin's reagent (Lowry *et al.* 1951) was added to the reaction mixture, kept for 30 minutes and then take O.D. at 620 nm. (Yomo and Varner 1973). Total activity was expressed as mg or µg tyrosine released / h / gm fresh weight.

Acid and alkaline phophatase : Crude enzyme was extracted by homogenizing 50 mg plant material in extraction buffer and centrifuged the extract at 6000 rpm for 15 minutes to get a supernatant. Now, 50 µl of sample and 25 µl of pNpp were added in it and then made the volume by 2.925 ml of acetate buffer (pH-5) for acid phophatase and tris buffer (pH-7.5) for alkaline phosphatase. Then, incubated at 37ºC for 30 minutes. After incubation, 2 ml of 0.1 N NaOH was added in it. After that O.D. was taken at 430nm with the help of spectrophotometer. The activity of phosphatase was determined (Lea 1990, Prince *et al.* 1982, Wilson *et al.* 1996 and Sawhney 2007) in terms of pNpp as a substrate at 430nm. Total activities was determined in term of mg pNpp degraded/min / gm fresh weight.

RESULTS AND DISCUSSIONS

Table 1 exhibit the effect of NaCl dose ($1x10^{-1}$M) and growth hormone doses ($1x10^{-10}$M IAA,$1x10^{-10}$M GA and $1x10^{-10}$ M Kn) on *in - vitro* activities of enzymes. At the treatment of NaCl, a decline in enzyme activity is observed i.e. the total activity of α-amylase is 78% of control, β-amylase activity is 82% of control, protease activity is 85% of control, acid phosphatase activity is 79% of control and alkaline phosphatase activity is 86% of control. At the treatment of IAA, the total activity of α-amylase is 106% of control, β-amylase activity is 108% of control, protease activity is 128% of control, acid phosphatase activity is 126% of control and alkaline phosphatase activity is 121% of control. At the treatment GA, the total activity of α-amylase is 111% of control, β-amylase activity is 111% of control, protease activity is 86% of control, acid phosphatase activity is 139% of control and alkaline phosphatase activity is 125% of control. At the treatment of Kn, the total activity of α-amylase is 104% of control, β-amylase activity is 106% of control, protease activity is 110% of control, acid phosphatase activity is 117% of control and alkaline phosphatase activity is 109% of control. This indicate that NaCl reduce the activities of amylases, proteases, and phosphatises. While, growth hormone individually stimulate.

Table 2 show the Interactive effect of NaCl dose ($1x10^{-1}$M) and growth hormone doses (1×10^{-10} M IAA, 1×10^{-10}M GA and 1×10^{-10} M Kn) on *in-vitro* activities of enzymes. When enzyme extract is treated with NaCl+ IAA, the total activity of α-amylase is 113% of control, β-amylase activity is 110% of control, protease activity is 86% of control, acid phosphatase activity is 133% of control and alkaline phosphatase activity is 132% of control. Enzyme extract treated with NaCl+ GA, the total activity of α-amylase is 119% of control, β-amylase activity is 114% of control,

Table - 1 : Effect of NaCl dose ($1x10^{-1}$M) and growth hormone doses ($1x10^{-10}$M IAA,$1x10^{-10}$M GA and $1x10^{-10}$ M Kn) on *in -vitro* activities of certain enzymes in *Phaseolous aureus* (K-851)

Total activities of enzymes (Per gm fresh weight ± SD)	Treatment (9 ml enzyme extract : 1 ml NaCl / Hormones)				
	Control	NaCl	IAA	GA	Kn
α-amylase activity (mg starch degraded/min.)	49.36±3.82	38.47±2.99	52.34±5.23	54.59±5.39	51.19±4.78
β- amylase activity (mg maltose degraded/min.)	45.78±3.10	37.38±3.71	49.39±3.56	50.64±4.23	48.44±3.85
Protease activity (mg tyrosine released/hr.)	2.34±0.46	1.99±0.10	2.99±0.29	2.01±0.47	2.58±0.78
Acid Phosphatase activity (mg pNPP degraded/min.)	9.28±1.28	7.37±0.91	11.67±6.12	12.93±5.39	10.89±2.38
Alkaline Phosphatase activity (mg pNPP degraded/min.)	7.38±1.11	6.32±0.89	8.91±2.65	9.23±2.49	8.02±1.38

protease activity is 96% of control, acid phosphatase activity is 128% of control and alkaline phosphatase activity is 139% of control. Enzyme extract treated with NaCl+ Kn, the total activity of α-amylase is 119% of control, β-amylase activity is 117% of control, protease activity is 90% of control, acid phosphatase activity is 136% of control and alkaline phosphatase activity is 150% of control. Enzyme extract treated with NaCl+ IAA+ GA+ Kn, the total activity of α-amylase is 132% of control, β-amylase activity is 125% of control, protease activity is 100% of control, acid phosphatase activity is 146% of control and alkaline phosphatase activity is 158% of control.

Salinity have influenced plants in different ways also such as osmotic effects, specific-ion toxicity and/or nutritional disorders (Läuchli and Epstein 1990). Higher level of salt stress caused reduction in the germination of seeds while lower level of salinity induced a state of dormancy (Khan and Weber 2008). Many-fold effects of salinity on the germination process may be due to that it alters the imbibitions of water by seeds having lower osmotic potential of germination media (Khan *et al.* 2004), besides it the cause of toxicity may be due to changes in the activities of enzymes of nucleic

Table 2 : Interactive effect of NaCl dose ($1x10^{-1}$M) and growth hormone doses ($1x10^{-10}$M IAA,$1x10^{-10}$M GA and $1x10^{-10}$ M Kn) on *in - vitro* activities of certain enzymes in Phaseolous aureus (K-851)

Total activities of enzymes (Per gm fresh weight ± SD)	Treatment (9 ml enzyme extract : 1 ml NaCl / Hormones)				
	Control + NaCl	NaCl + IAA	NaCl + GA	NaCl + Kn	NaCl + IAA + GA + Kn
α-amylase activity (mg starch degraded/min.)	**36.47±1.99**	41.28±4.21	43.37±4.29	43.25±3.34	47.98±5.23
β- amylase activity (mg maltose degraded/ min.)	31.38±3.11	34.37±2.78	35.78±3.21	36.67±2.89	39.35±3.89
Protease activity (mg tyrosine released/hr.)	2.19±0.50	1.89±0.23	2.11±0.89	1.98±0.56	2.20±0.23
Acid Phosphatase activity (mg pNPP degraded/min.)	8.17±1.01	10.89±1.29	10.43±1.12	11.11±2.32	11.89±1.56
Alkaline Phosphatase activity (mg pNPP degraded/min.)	7.12±0.94	9.38±0.69	9.89±0.99	10.68±1.02	11.26±0.86

acid metabolism (Gomes-Filho *et al.* 2008), alter action in protein metabolism (Yupsanis *et al.* 1994, Dantas *et al.* 2007), disturbance in hormonal balance (Khan and Rizvi 1994) and

it reduces the utilization of seed reserves (Promila and Kumar 2000, Othman *et al.* 2006). Similarly, Amirjani 2010 reports that when NaCl concentration increase in plant then the photosynthetic pigment, soluble sugar and proteins decrease. While, Ahmad (2006) has observed in *Pisum sativum* that the sugar content and proline concentration increase with increasing salinity but chlorophyll decrease. Gurudevi *et al.* (2012) have shown that increased salinity cause decrease protein content in *Phaseolus mungo.* Similar in soyabean, NaCl decrease content of protein. (Moussa 2004). High salinity caused a decrease in nitrate reductase activity and increase peroxidase and catalase activity in *Phaseolus mungo* (Gurudevi *et al.* 2012). High amylolytic enzyme activity in seeds of *P. aureus* grown under saline condition has been reported by Thimmaiah *et al.* (1989) and a decrease in nitrate reductase activity with increasing NaCl (Ahmad 2006, Moussa 2004).

The exogenous application of plant growth regulators, auxins (Khan *et al.* 2004), gibberellins (Afzal *et al.* 2005), cytokinins (Gul *et al.* 2000) produces some benefit in adverse effects of salt stress and also improves germination, growth, development and seed yields and yield quality (Egamberdieva 2009). Hormones generally decrease viscosity of cytoplasm and increase diffusion of water into the cell. Not only, decreasing the viscosity of the cytoplasm, the hormones may induce growth by production of substances within the endosperm prior to radicle emergence, which may as well increase the osmotic potential of the cell. (Dias *et al.* 1993). The role of phytohormones under salinity stress is critical in modulating physiological responses that eventually lead to adaptation of plants to an unfavorable environment (Iqbal *et al.* 2013). The primary effects of salt stress are caused by the presence of ions in rhizosphere limiting extraction of water by roots and reduced plant growth, while the secondary effects are caused by ionic disequilibrium resulting in inactivation of enzymes, nutrient starvation, ionic toxicity in tissues and oxidative stress. Phytohormones have also been shown to influence salinity tolerance through modulating several physiological processes and biochemical mechanisms (Fatma *et al.* 2013). Through decreasing the production of O_2 and H_2O_2 such products can also alleviate the stress by controlling the likely oxidative damage, similar to the effects of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) on plant growth under various stresses (Song *et al.* 2006, Tian and Lei 2006, Tseng *et al.* 2007, Li *et al.* 2008, Tuna *et al.* 2008, Zheng *et al.* 2009, Sajedi *et al.* 2011). However, the exact mechanism of hormone action to suppress the adverse effect of NaCl is not clear. It needs further analysis and experiments.

Thanks are due to Prof. Vimala, Y., Botany Deptt., C.C.S. University, Meerut for her valuable suggestions.

REFERENCES :

Afzal I, Basra S and Iqbal A 2005 The effect of seed soaking with plant growth regulators on seedling vigor of wheat under salinity stress. *J Stress Physiol Biochem* **1** 6-14.

Ahmad P 2006 Growth and antioxidant responses in mustard (*Brassica juncea* L.) plants subjected to combined effect of gibberellic acid and salinity. *Arch Agron Soil sci* **51** 665-672.

Amirjani MR 2010 Effect of NaCl on some physiological parameters of rice. *EJBS* **6** 16.

Bernfeld P 1951 Amylase α and ß. In : *Methods in Enzymology* Colowick SP, Kaplan NO (Eds.). Academic Press New York. **1** 149-158.

Dantas BF, Ribeiro LDS and Aragao CA 2008 Germination, initial growth & cotyledon protein content of bean cultivars under salinity stress, *Revista Brasileira de sementes* **29(2)** 1-7.

Dias P, Wilson KA and Tan-Wilsson AL 1993 Immunocyto chemical analysis of proteolysis in germinating Soybean. *Photochemistry* **33** 961-968.

Egamberdieva D 2009 Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat. *Acta Physiol Plant* **31** 861-864.

Fatma M, Khan MIR, Masood A and Khan NA 2013 Coordinate changes in assimilatory sulphate reduction are correlated to salt tolerance : involvement of Phytohormones. *Annual Review and Research in Biology* **3** 267–295.

Filner P and Varner JE 1967 A test for de novo synthesis of enzyme. Density labelling with H_2O^{18} of Barley, alpha amylase indused by gibberellic acid. *Prac Notl Acad Sci* **58** 1520-1526.

Gomes-Filho E, Machado Lima CRF, Costa JH, da Silva AC, da Guia Silva Lima M, de Lacerda CF and Prisco JT 2008 Cowpea ribonuclease : properties and effect of NaCl-salinity on its activation during seed germination and seedling establishment. *Plant Cell Rep* **27** 147–157.

Gul B, Khan MA and Weber DJ 2000 Alleviation salinity and dark enforced dormancy in *Allenrolfe aoccidentalis* seeds under various thermo periods. *Aust J Bot* **48** 745–752.

Gulzar S, Khan MA and Ungar LA 2003 Salt tolerance of a costal salt marsh grass comm. *Soil sci plant Anal* **34** 2595-2605.

Guru Devi R, Pandiyarajan V and Guru saravanan P 2012 Alleviating effect of IAA on salt stressed *Phaseolus mungo* with reference to growth & biochemical characteristics. *Recent Research in Sciences & Technology* 4 (3) 22-24.

Iqbal N, Masood A and Khan NA 2013 Phytohormones in salinity tolerance: ethylene and gibberellins cross talk. In : Khan, N., Nazar, R., Iqbal, N., Anjum, N.A. (Eds.), *Phytohormones and Abiotic Stress Tolerance in Plants*. Springer-Verlag, Berlin, Heidelberg.

Kaya C, Ashraf M, Dikilitas M, Tuna AL (2013) Alleviation of salt stress induced adverse effect on maize plant by exogenous application of IAA & inorganic nutrients. *Australian Journal of crop science* **7(2)** 249-254.

Khan MA, Gul B and Weber DJ 2004 Action of plant growth regulators and salinity on seed germination of *Ceratoides lanata. Can J Bot* **82** 37-42.

Khan MA and Rizvi Y 1994 Effect of salinity, temperature and growth regulators on the germination and early seedling growth of *Atriplex griffithii* var. Stocksii. Can *J Bot* **72** 475–479.

Khan MA and Weber DJ 2008 *Ecophysiology of high salinity tolerant plants (tasks for vegetation science)*, 1st edn. Springer, Amsterdam.

Läuchli A and Epstein E 1990 Plant responses to saline and sodic conditions. In K.K. Tanji (ed). *Agricultural salinity assessment and management*. ASCE manuals and reports on engineering practice No, 71. pp 113–137 ASCE New York.

Lea PJ 1990 *Methods in plant biochemistry. Vol. 3 Enzymes of primary metabolism.* Acadmic press, New York.

Li QY, Niu HB, Yin J, Wang MB, Shao HB, Deng DZ, Chen XX, Ren JP and Li YC 2008 Protective role of exogenous nitric oxide against oxidative-stress induced by salt stress in barley (*Hordeum vulgare*). *Colloids, Surface B: Bio interface* **65** 220–225.

Lin CC and Kao CH 1995 NaCl stress in rice seedlings: starch mobilization and the influence of gibberellic acid on seedling growth. *Bot Bull Acad Sin* **36** 169-173.

Moussa HR 2004 Amelioration of salinity – induced metabolic change in soybean by weed exudates. *International Journal of agriculture & biology* **6(3)** 499-503.

Othman Y, Al-Karaki G, Al-Tawaha AR and Al-Horani A 2006 Variation in germination and ion uptake in barley genotypes under salinity conditions. *World J Agric Sci* **2** 11–15.

Palmiano EP and Juliano BO 1972 Biochemical changes in the rice grain during germination. *Plant physiol* **49** 751-756.

Prince NC and Steven L 1982 *Fundamentals*

of Enzymology, Oxford University Press, Oxford.

Promila K and Kumar S 2000 *Vigna radiata* seed germination under salinity. *Biol Plant,* **43** 423–426.

Romero A, Soria RT and Cuartero 2001 Tomato plant-water uptake and plant-water relationship under saline growth conditions. *Plant Sci* **160** 265-272.

Sajedi N, Ardakani M, Madani H, Naderi A and Miransari M 2011 The effects of selenium and other micronutrients on the antioxidant activities and yield of corn (*Zea mays L.*) under drought stress. *Physiol. Mol. Biol. Plants* **17** 215–222.

Sawhney SK and Singh R 2007 *Introductory Pratical Biochemistry.* Narosa Publishing House, 135-142. New Delhi.

Song L, Ding W, Zhao M, Sun B and Zhang L 2006 Nitric oxide protects against oxidative stress under heat stress in the calluses from two ecotypes of reed. *Plant Sci*. **171** 449–458.

Sultana N, Ikeda T and Itih R 1999 Effect of NaCl (salinity) on photosynthesis and dry matter accumulation in developing rice grains. *Environ exp Bot* **42** 211-220.

Thimmaiah SK, Viswanath DP, Vyakarnahal BS and Hunshal CS 1989 Effect of salinity on yield, seed quality & Biochemical characteristics in *Setaria italic* L. *Amerian association of cereal chemists* **66** 6.

Tian X and Lei Y 2006 Nitric oxide treatment alleviates drought stress in wheat seedlings. *Biol Plant* **50** 775–778.

Tseng MJ, Liu CW and Yiu JC 2007 Enhanced tolerance to sulfur dioxide and salt stress of transgenic Chinese cabbage plants expressing both super-oxide dismutase and catalase in chloroplasts. *Plant Physiol Biochem.* **45** 822–833.

Tuna A, Kaya, Cengiz K, Dikilitas M and Higgs D 2008 The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. *Envorin Exp Bot* **62** 1–9.

Vimala Y (1983) Change in the certain enzymes accompanying natural and induced loss of seed viability, 6th Bot. Conf. *J. Indian Botanical Science* (Soppl.) **62** 71-X-12.

Wilson K and Walker J 1996 *Pratical Biochemistry principles and technique*. 4edu, Cambridge University Press, London.

Yomo H and Varner JE 1973 Control of the formation of amylase and protease in the cotyledons of germinating peas. *Plant Physiol* **51** 708-713.

Yupsanis T, Moustakas M and Domiandou K 1994 Protein phosphorylation-dephosphorylation in alfalfa seeds germinating under salt stress. *J Plant Physiol* **143** 234–240.

Zheng C, Jiang D, Liub F, Dai T, Liu W, Jing Q and Cao W 2009 Exogenous nitric oxide improves seed germination in wheat against mitochondrial oxidative damage induced by high salinity. *Env Exp Bot* **67** 222–227.