

Pretreatment Effect of Salicylic Acid on Protein and Hydrolytic Enzymes in Salt Stressed Mung Bean Seedlings

Sadaf Shakeel and Simeen Mansoor

Department of Genetics, University of Karachi, Karachi, Pakistan-75270

Abstract: Salinity is a known abiotic stress that exhibit changes in biochemical and metabolic activities thus responsible for the reduction in crop yield. Salicylic Acid (SA) is a growth regulator that plays an important role in number of abiotic stresses including salt stress. We investigate the effect of salicylic acid on biochemical response of plant to salt stress. In the present study two mung bean genotypes (NM 19-19 and NM 20-21) were taken as experimental material and imbibed in distilled water (d/w) and solution of SA (50, 100 and 1000 μ M) for 13 h prior to salt stress. Then seedlings were treated with 0, 50 and 100 mM NaCl. It was observed that 50 mM NaCl treatment enhanced protein synthesis which was reduced in seedlings treated with 100 mM NaCl as compared to control. However the pretreatment of SA before NaCl treatment enhanced protein synthesis. Salt stress caused reduction in α -amylase activity (AMY) with the promotion in the activity of Acid Phosphatase (AP). The pretreatment of low concentrations of SA (50 and 100 μ M) induced AMY activity as compared to salt treated samples with the reduction in AP activity. Highest concentration of SA (1000 μ M) showed reduction in the activity of both enzymes. It was further observed that the overall biochemical performance of NM 19-19 was better than NM 20-21.

Key words: Acid phosphatase (AP), α -amylase (AMY), hydrolytic enzymes, mung bean, salicylic acid (SA), salt stress

INTRODUCTION

Environmental stresses such as salt (NaCl) affects plant performance and results in the significant reduction in crop yield and quality worldwide (Greenway and Munns, 1980; Boyer, 1982; Bohnert *et al.*, 1995). High concentration of salt causes ion imbalance and hyperosmotic stress to plants due to which it decreases dry matter and leaf area (Amirjani, 2011) and ultimately decreases crop yield. As a consequence of these primary effects, secondary stress such as oxidative damage often occurs. Against these abiotic stresses, plants adapt themselves by different mechanism including change in morphological and developmental pattern as well as biochemical responses (Bohnert *et al.*, 1995). Adaptation to all these stresses is associated with metabolic adjustments that lead to the modulation of different enzymes (Shinozaki and Shinozaki-Yamaguchi, 1996; Ehsanpour and Amini, 2003). Quantitative and qualitative changes in the activity of enzymes isolated from plants subjected to salt stress have been reported (Yu and Rengel, 1999; Benavides *et al.*, 2002).

Salicylic Acid (SA) is an endogenous growth regulator (Sakhabutdinova *et al.*, 2003) and plays an important role in the protection of plants under various abiotic stresses including salt (Shakirova and Bezrukova,

1997). Salt leads to oxidative stress and severe impairment of seedling survival and SA greatly potentiates the effects of salt and abiotic stresses by enhancing ROS generation during Arabidopsis germination (Omar *et al.*, 2001)

Keeping in view the harmful effects of salinity on plant growth and productivity, the present study is an attempt to evaluate whether SA could be a protectant to ameliorate the influence of salt stress on mung bean genotypes and thereby increasing its salt tolerance.

MATERIALS AND METHODS

Seeds of two mung bean (*Vigna radiata* L. Wilezek) genotypes NM 19-19 and NM 20-21 were obtained from Pakistan Agricultural Research Council Islamabad, (PARC) Pakistan. Experiment was conducted in the lab of Department of Genetics in the year 2008.

Uniformed sized seeds were imbibed in distilled water (d/w) and in solution of SA (50, 100 and 1000 μ M, respectively) for 13 h. Then washed with d/w and allowed to germinate on petri plates lined with filter paper moistened with d/w for 24 h at 30°C as it is an optimum temperature for mung bean (Mansoor and Naqvi, 2011). Seedlings were then treated with 0, 50 and 100 mM NaCl solutions and harvested 24 h after treatment for biochemical analysis.

Extraction of proteins and hydrolytic enzymes: 1.0 g seedlings were homogenized in 2.0 mL of extraction buffer [0.25 M Sucrose, 0.02 M β -Mercaptoethanol, 3.0 mM Sodium EDTA in 20 mM Tris HCl Buffer (PH 7.5)]. Homogenate was centrifuged at 10,000 rpm for 20 min. The resulting supernatant was collected for protein and enzymes estimation.

Protein was estimated by the method of (Lowry *et al.*, 1951). Absorbance was recorded at 750 nm against the reagent blank. μ g protein was estimated using Bovine Serum Albumin (BSA) standard curve.

Estimation of amylase: Activity of amylase (AMY) was estimated by the method of Chrispeels and Varner (1967) with minor modifications. 50 μ L of homogenate and 100 μ L of 1.0 % Starch were added in the test tube, incubated at room temperature for 8-10 min. In the end, 100 μ L of Iodine reagent (0.6g potassium Iodide in 9.0 mL of 1.0 N HCl mixed with 10 mL of 0.6% of Iodine) was added to stop the reaction. Absorbance was recorded at 620 nm against the reagent blank. Specific activity was calculated as μ g maltose/mg protein/5 min using maltose standard curve.

Estimation of acid phosphatase: Activity of acid phosphatase (AP) was estimated by Fields and Tyson (1983). Reaction mixture consist of 10 μ L of homogenate, 0.4 mL of 0.003 M para nitro phenyl phosphate and 0.2 mL of 0.1 M sodium acetate buffer was added and incubated at 37°C for 10 min, 1.0 mL of 2.0 M sodium hydroxide was then added. Absorbance was recorded at 410 nm against reagent blank. Specific activity was calculated as μ g Para Nitro Phenol (PNP)/mg protein/10 min using PNP standard curve.

Statistical analysis: Petri plates were arranged in Complete Randomized Design (CRD) as two factorial with two replicates. The data were subjected to Analysis of Variance (ANOVA) using a (SPSS, 11.0). Comparative analyses of means were performed by Duncan's Multiple Range test (DMR) with the level of significance at $p < 0.05$ (Steel and Torrie, 1980).

RESULTS

Effect of NaCl and SA on protein: The treatments, genotype, as well as the interaction between them showed a significant effect on protein synthesis (Table 1). Protein content estimated was found to be increased at low concentration of NaCl (50 mM) and reduced at high concentration (100 mM) as compared o control, for both genotypes. However the pretreatment of SA prior to salt treatment caused induction in protein (Table 2). It was further noticed that more protein was synthesized in NM 19-19 than NM 20-21 in most of the treatments.

Effect of NaCl and SA on α -amylase: Amylase activity was affected significantly for treatments, genotypes and interaction (Table 1). AMY activity was reduced significantly by the increase in salt concentration for both genotypes. Pretreatment of all concentrations of SA enhanced AMY activity when compared with the samples treated with NaCl alone however the highest concentration of SA (1000 μ M) showed antagonistic effect on AMY activity under salt stress (Table 2). AMY activity was more in NM 19-19 as compared to NM 20-21 for any treatment.

Table 1: Analysis of variance of treatments and mung bean genotypes for Protein, Amylase and Acid Phosphatase

Sources of variation	df	Protein content		Amylase		Acid phosphatase	
		MS	F-value	MS	F-value	MS	F-value
Treatments	8	1764.0	11.8*	8170.6	7427.8*	2769517.2	25.7*
Varieties	1	18645.6	124.8*	736.0	669.0*	767057.2	7.1*
Treatments \times varieties	8	2567.0	17.1*	57.7	52.45*	48809.4	0.45 ^{ns}
Error	18	149.3		1.1		107439.0	

*: Represent significant and ns showed non-significant differences at $p < 0.05$ level of significance

Table 2: Effect of pretreatment of SA on protein (μ g/g fresh wt), amylase (μ g maltose/mg protein/5 min) and acid phosphatase (μ g PNP/mg protein/10 min) in two salt stressed mung bean genotypes

Treatments	Protein		Amylase		Acid phosphatase	
	NM 19-19	NM 20-21	NM 19-19	NM 20-21	NM 19-19	NM 20-21
d/w	170.0 ^e \pm 14.14	119.0 ^f \pm 1.41	128.7 ^a \pm 8.75	88.1 ^a \pm 5.11	1482.6 ^b \pm 295.10	1204.9 ^{bc} \pm 103.30
50NaCl	205.0 ^{de} \pm 7.07	131.0 ^e \pm 12.7	89.8 ^b \pm 2.19	64.5 ^{bcd} \pm 7.56	1626.3 ^b \pm 278.69	1333.1 ^{ab} \pm 95.43
100NaCl	119.0 ^f \pm 0.00	110.0 ^f \pm 0.00	84.0 ^c \pm 0.01	50.8 ^d \pm 3.67	2617.6 ^a \pm 575.60	1672.7 ^a \pm 9.10
50SA+50NaCl	197.5 ^{de} \pm 17.67	142.5 ^e \pm 3.53	90.82 ^{bc} \pm 2.18	67.4 ^{bc} \pm 6.80	1542.3 ^b \pm 191.00	794.3 ^d \pm 277.09
50SA+100NaCl	170.5 ^e \pm 14.84	187.0 ^d \pm 7.07	85.6 ^c \pm 0.58	52.7 ^{cd} \pm 2.74	1572.5 ^b \pm 322.50	889.0 ^{cd} \pm 55.71
100SA+50NaCl	214.0 ^{cd} \pm 22.62	200.0 ^e \pm 0.00	107.0 ^b \pm 12.09	69.1 ^b \pm 5.85	1208.5 ^b \pm 147.95	787.5 ^d \pm 37.50
100SA+100NaCl	264.5 ^{ab} \pm 20.50	209.0 ^e \pm 0.00	89.3 ^{bc} \pm 3.33	55.9 ^{bcd} \pm 3.35	1091.8 ^b \pm 47.89	633.9 ^{de} \pm 88.50
1000SA+50NaCl	299.0 ^a \pm 26.87	377.5 ^a \pm 3.53	77.2 ^c \pm 4.90	60.9 ^{bcd} \pm 0.390	1056.3 ^b \pm 270.64	401.4 ^e \pm 251.75
1000SA+100NaCl	245.0 ^{bc} \pm 0.00	282.5 ^b \pm 3.53	80.0 ^c \pm 0.00	51.6 ^{cd} \pm 0.955	1051.0 ^b \pm 348.98	539.4 ^{de} \pm 39.47

Data represent the mean values; different letters in each column indicate significant differences according to DMR test at $p < 0.05$

Effect of NaCl and SA on acid phosphatase: AP was significantly affected by treatments and genotypes while interaction between genotypes and treatment was not significant (Table 1). Mean AP activity in NM 19-19 was induced non-significantly for 50 mM NaCl which increased further significantly at 100 mM NaCl concentration for both genotypes. Pretreatment of three different concentrations of SA reduced AP activity for both genotypes but the activity was higher in NM 19-19 with less percent inhibition than in NM 20-21 (Table 2).

DISCUSSION

Current study is an attempt to further understand the biochemical mechanisms that involve in salt tolerance and induction of salt tolerance by SA. It was observed in the present study that at high salt concentration, protein content was reduced while increased in low concentration as compared to control. These results are in agreement of Khedr *et al.* (2003) who reported an increase in soluble protein at low salinity and decrease at high salinity in *Pancreaticum maritimum*.

Similarly Dhingra and Sharma (1993) also reported that salt stress reduced soluble protein in mung bean plants. Pretreatment of 50, 100 and 1000 μ M SA, respectively increased protein content in both genotypes. This may be due to the fact that proteins which accumulate in plants under saline conditions may provide a storage form of nitrogen that is re-utilized later and may play a role in osmotic adjustment (Singh *et al.*, 1987). There are data about SA induced synthesis of heat shock proteins in tobacco plants (Burkhanova *et al.*, 1999) and accumulation of wheat lectins (Shakirova and Bezrukova, 1997), fast activation of 48-kD protein kinase in suspension cell culture of tobacco at osmotic stress (Mikolajczyk *et al.*, 2000).

In present study salt stress significantly decrease AMY activity which in turn decreases maltose. These findings are supported by Besma and Denden (2010), reported that activity of AMY showed a decreasing trend with the increase in NaCl concentration even at low concentration in Moench seeds. In mung bean it is also reported that salt stress reduced total soluble sugar (Zayed and Zeid, 1998). Pretreatment of 50 and 100 μ M salicylic acid increased maltose but non-significantly. 1000 μ M SA decrease maltose content but also remain non-significant. Ashraf and Tufail (1995) determined the total soluble sugar content in five sunflower accessions differing in salt tolerance; the salt tolerant lines had generally greater soluble sugars than the salt sensitive ones. We found more maltose in NM 19-19 that may considered as salt tolerant genotype and NM 20-21 as salt sensitive genotype.

Abiotic stresses like salt stress affects the physiological biochemistry of plant cells under *in vivo* and *in vitro* conditions. Salt stress is reported to enhance

AP activity in wheat (Barrett-Lennard *et al.*, 1982). These results are similar with present study where salinity caused increase in AP activity and AP increases with increase in concentration of salt. These findings are also supported by Ahmad and Ebrahimian (2011), reported that forty five days after salinity induction, NaCl caused a significant increase in AP activity in treated canola plants with 150 and 200 mM NaCl in comparison with control treatment. Our results showed that the pretreatment of SA caused decrease in the activity of AP as the concentration was increased for both genotypes with more percent inhibition in NM 20-21.

As it is observed that the highest concentration of SA reduces the activity of AMY and AP activity, it may be due to the fact that SA is present at very low concentration endogenously and its high level disturbs physiological functions of plant. Durner and Klessig (1995) provided the evidence that inhibition of ascorbate peroxidase with high concentration of SA blocks the H_2O_2 degrading pathway in the plant cell leading to the increased levels of endogenous H_2O_2 . Borsani *et al.* (2001) demonstrated that transgenic Arabidopsis plants with lower endogenous SA were better able to resist the oxidative damage caused by salt stress than were the wild type plants.

CONCLUSION

Overall, all SA pretreatments prior to NaCl, negatively affected AP with positive effect on protein however the AMY activity was positively affected at low concentrations and negatively affected at 1000 μ M in mung bean genotypes. Furthermore the biochemical response of NM 19-19 was better than NM 20-21, therefore NM 19-19 may be considered as salt tolerant and NM 20-21 as salt sensitive genotypes.

REFERENCES

- Ahmad, B. and E. Ebrahimian, 2011. Effect of salinity stress on activity of enzymes involved in nitrogen and phosphorus metabolism case study: Canola (*Brassica napus* L.) Asian J. Agric. Res., 5: 208-214.
- Amirjani, M.R., 2011. Effect of salinity stress on growth, sugar content, pigments and enzyme activity of rice. Int. J. Bot., 7: 73-81.
- Ashraf, M. and M. Tufail, 1995. Variation in salinity tolerance in sunflower (*Helianthus annuus* L.). J. Agron. Crop Sci., 174: 351-362.
- Barrett-Lennard, E.G., A.D. Robson and H. Greenway, 1982. Effect of phosphorus deficiency and water deficit on phosphatase activities from wheat leaves. J. Exp. Bot., 33: 682-693.

- Benavides, M.P., P.L. Marconi, S.M. Gallego, M.E. Coma and M.L. Tomara, 2002. Relationship between antioxidant defense system and salt tolerance in *Solanum tuberosum*. Aust. J. Plant Physiol., 27: 273-278.
- Besma, B.D and M. Denden, 2010. Salt stress induced changes in germination, sugar, starch and enzyme of carbohydrate metabolism in *Abelmoschus esculentus* L. (Moench) seeds. Afr. J. Agric. Res., 5: 1412-1418.
- Bohnert, H.J., D.E. Nelson and R.G. Jensen, 1995. Adaptations environmental stresses. Plant Cell. 7: 1099-1111.
- Borsani, O., V. Valpuesta and M.A. Botella, 2001. Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in Arabidopsis seedlings. Plant Physiol., 126: 1024-1034.
- Boyer, J.S., 1982. Plant productivity and environment. Science, 218: 443-448.
- Burkhanova, E.A., A.B. Fedina and O.N. Kulaeva, 1999. Effect of salicylic acid and (2'-5')-oligoadenylates on protein synthesis in tobacco leaves under heat shock conditions: A comparative study. Russ. J. Plant Physiol., 46: 16-22.
- Chrispeels, M.J. and J.E. Varner, 1967. Gibberellic acid enhanced synthesis and release of α -amylase and ribonuclease by isolated barley aleurone layers. Plant Physiol., 42(3): 398-408.
- Dhingra, H.R. and P.K. Sharma, 1993. Biochemical and mineral composition of young healthy and shrivelled mungbean (*Vigna radiata* L. Wilczek) seeds in response to salinity. Indian J. Plant Physiol., 36: 115-117.
- Durner, J. and D.F. Klessig, 1995. Inhibition of Ascorbate Peroxidase by Salicylic Acid and 2, 6-Dichloro Isonicotinic Acid, Two Inducers of Defense Responses. Proceedings of Natural Academy of Science, USA, 92: 11312-11316.
- Ehsanpour, A.A. and F. Amini, 2003. Effect of salt and drought stress on acid phosphatase activities in alfalfa (*Medicago sativa* L.) explants under *in vitro* culture. Afr. J. Biotechnol., 2: 133-135.
- Fields, M.A. and H. Tyson, 1983. Molecular weight differences in acid phosphatase of stem homogenates from L and S flax genotypes. Biochem. Genet., 21(3-4): 391-404.
- Greenway, J. and R. Munns, 1980. Mechanisms of salt tolerance in nonhalophytes. Ann. Rev. Plant Physiol., 31: 149-190.
- Khedr, A.H.A., M.A. Abbas, A.A.A. Wahid, W.P. Quick and G.M. Abogadallah, 2003. Proline induces the expression of salt stress responsive proteins and may improve the adaptation of *Pancreaticum maritimum* L. to salt stress. J. Exp. Bot., 54: 2553-2562.
- Lowry, O.H., J.J. Rosebrought, A.L. Farnand and R.J. Randal, 1951. Protein measurement with folin reagent. J. Biol. Chem., 193: 265-275.
- Mansoor, S., and F.N Naqvi, 2011. Heat stress and acquisition of thermotolerance in mung bean (*Vigna radiata* L.). Int. J. Biol. Biotechnol., 8: 77-84.
- Mikolajczyk, M., O.S. Awotunde, G. Muszynska, D.F. Klessig and G. Dobrowolska, 2000. Osmotic stress induces rapid activation of a salicylic acid-induced protein kinase and a homolog of protein kinase ASK1 in tobacco cell. Plant Cell, 12: 165-178.
- Omar, B., V. Valpuesta and M.A. Botella, 2001. Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in Arabidopsis seedlings. Plant Physiol., 126: 1024-1030.
- Sakhabutdinova, A.R., D.R. Fatkhutdinova, M.V. Bezrukova and F.M. Shakirova, 2003. Salicylic acid prevents the damaging action of stress factors on wheat plants. Bulgarian J. Plant Physiol., Special Issue: 314-319.
- Shakirova, F.M. and M.V. Bezrukova, 1997. Induction of wheat resistance against environmental salinization by salicylic acid. Biol. Bull., 24: 109-112.
- Shinozaki, K. and K. Shinozaki-Yamaguchi, 1996. Molecular responses to drought and cold stress. Curr. Opin. Biotechnol., 7: 161-167.
- Singh, N.K., C.A. Bracken, P.M. Hasegawa, A.K. Handa, S. Buckel, M.A. Hermodson, F. Pfankoch, F.E. Regnier and R.A. Bressan, 1987. Characterization of osmotin. A thaumatin-like protein associated with osmotic adjustment in plant cells. Plant Physiol., 85: 529-536.
- SPSS. SPSS Software for Windows Version 11.0. Inc., Chicago II.
- Steel, R.G.D and J.H. Torrie, 1980. Principles and Procedures of Statistics. Mc Graw Hill Book Co., New York.
- Yu, Q. and Z. Rengel, 1999. Drought and salinity differentially influence activities of superoxide dismutases in narrow-leafed lupins. Plant Sci., 142: 1-11.
- Zayed, M.A and I.M. Zeid, 1998. Effect of water and salt stresses on growth, chlorophyll, mineral ions and organic solutes contents and enzymes activity in mungbean seedlings. Biol. Plant., 40: 351-356.