INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
15–240/2016/18–2–330–336
DOI: 10.17957/IJAB/15.0090
http://www.fspublishers.org

Full Length Article

GA Mediated *OsZAT-12* Expression Improves Salt Resistance of Rice

Qari Muhammad Imran[1], Muhammad Kamran[2], Shafiq Ur Rehman[2], Abdul Ghafoor[4], Noreen Falak[1], Kyung-Min Kim[1], In-Jung Lee[1], Byung-Wook Yun[1*] and Muhammad Jamil[3]
[1]*School of Applied Biosciences, Kyungpook National University, Daegu, Republic of Korea*
[2]*Department of Plant Sciences, Kohat University of Science and Technology Pakistan*
[3]*Department of Biotechnology and Genetic Engineering, Kohat University of Science and Technology Pakistan*
[4]*Plant Genetic Resources Program, National Agricultural Research Centre, Islamabad, Pakistan*
[*]For correspondence: bwyun@knu.ac.kr

Abstract

Abiotic stress prevents plants from absorbing available nutrients resulting in yield loss and soil contamination. Growth regulators like gibberellic acid (GA) may improve plant growth under stress conditions. This study was conducted to evaluate the role of GA on the rice performance under saline conditions and to investigate its effects on regulatory gene expression by GA-mediated seed priming. Seeds of rice variety KSK 282 were primed in GA solution (0, 50, 100 and 150 ppm) and grown under different salt (NaCl) concentrations (0, 50, 100 and 150 mM). Increase in salt concentration led to decrease in rice growth. GA priming reversed the negative effects of salt stress and enhanced different growth attributes like germination, seedling growth and weight, while decreased the concentration of toxic ions such as Na^+ in some treatments. However, GA priming was not very effective on Chlorophyll a, b (Chl-a, Chl-b) and total carotene contents. Reverse transcription polymerase chain reaction (RT-PCR) approach was used to study the effect of GA-mediated seed priming on the expression of two stress responsive genes, *OsZat 12-1* (LOC_Os01g62130 also called ZOS1-14 - C2H2 zinc finger protein) and *OsZat 12*-9 (LOC_Os01g62190 also called ZOS1-15 - C2H2 zinc finger protein). There was no or very low expression of *OsZat 12-1* and *OsZat 12-9* in hydroprimed seeds under all salt stress conditions tested, while induction of gene expression was observed for plants raised from GA primed seeds under salt stress. In conclusion, GA regulated the growth at early stages of rice life cycle by inducing regulatory genes expression; therefore it is noteworthy that while studying salinity factor, the induction of genetic determinants (genes) by plant growth regulators should also be considered. © 2016 Friends Science Publishers

Keywords: Salinity; Gene expression; Ion analysis; Germination; Chlorophyll contents

Introduction

One of the major environmental causes for loss of crop production is salt stress all over the world in general and in Pakistan in particular. Among various other reasons, osmotic inhibition and the toxicity of salt ions are considered to be the main reasons of salinity (Hakim *et al.*, 2010). A detailed study on seed germination, seedling growth and other growth attributes under saline conditions suggested that germination, shoot and root length, tiller number, leaf area index and grain yield were decreased when exposed to high saline conditions (150 mM) in almost all varieties studied (Ashraf *et al.*, 1991; Khan *et al.*, 1995; Hasanuzzaman *et al.*, 2009).

Seed priming is a technique used for plants to give them good start and to enhance the productivity with increase in germination rate, germination percentage, early emergence and vigor (Basra *et al.*, 2005; Farooq *et al.*, 2005). Seed priming with a plant growth regulator resulted in improved seed performance as it is considered to play an important role in the activation of α-amylase, an enzyme necessary for germination (Farooq *et al.*, 2005).

Rice is considered sensitive (Maas and Hoffman, 1977; Shannon *et al.*, 1998) moderately sensitive (Mori and Kinoshita, 1987) or sensitive at particular stage of its life cycle (Moradi and Ismail, 2007) when exposed to salt stress. This leads to poor crop establishment and these effects may continue up to the reproductive stage where salinity can damage grain formation and yield depending on growth stage, concentration and duration of exposure to salinity (Flowers and Yeo, 1981; Lutts *et al.*, 1995). To address these problems, it is of paramount importance to revamp salt tolerance in crops despite the fact that stress resistance is a convoluted process in plants; however the biochemistry and genetic study of these processes will definitely help plant biologists to improve tolerance in plants in order to overcome the drastic effects of salinity. The genetic diversification for stress tolerance can be found in various genera of plants (Greenway and Munns, 1980). Reports suggest changes in expression of certain regulatory genes

To cite this paper: Imran, Q.M., M. Kamran, S.U. Rehman, A. Ghafoor, N. Falak, K.M. Kim, I.J. Lee, B.W. Yun and M. Jamil, 2016. GA mediated *OsZAT-12* expression improves salt resistance of rice. *Int. J. Agric. Biol*., 18: 330–336

due to salinity stress in rice and other plants (Pillai *et al.*, 2001; Yokoi *et al.*, 2002; Jamil *et al.*, 2010). Furthermore plants respond to different kind of environmental stresses by a complex network of regulatory genes and unlike other stress specific genes, the Zinc finger protein *Zat 12* responds to a large number of biotic and abiotic stresses and this property makes it an interesting subject for analysis (Davletova *et al.*, 2005).

Gibberellins are known phytohormones that play vital role in seed germination, shoot growth and development of reproductive parts (Ghaafar and Rawi, 2011). Reports suggested that GA has shielding effects against metal toxicity (Maggio *et al.*, 2010). Similarly exogenous GA_3 application increases salicylic acid (SA) biosynthesis (Halter *et al.*, 2000) that plays important role in plant defense. Gangwar *et al.* (2011) reported that exogenous application of GA_3 improves plant growth by regulating the endogenous levels of stress related phytohormones (ABA, ethylene). However, reports about GA-mediated seed priming effect on regulatory genes expression are lacking. Therefore, this study was conducted to explore the role of GA-mediated seed priming on rice growth under saline conditions and to investigate its effect on biochemical composition and expression of *OsZat 12-1* and *OsZat 12-9* genes.

Materials and Methods

Seeds of rice variety (KSK-282) used in this study were obtained from National Agricultural Research Center (NARC), Islamabad, Pakistan. The healthy and mature seeds were surface sterilized with 3.5% sodium hypochlorite solution for 5 min and then rinsed three times with distilled water for further use.

Plant Material and Physiological Attributes

GA-mediated seed priming was performed as described earlier (Ashraf *et al.*, 1991; Farooq *et al.*, 2006) with some modifications. GA dilutions were selected after screening a range of dilutions amongst, which three dilutions were selected on the basis of their optimum germination percentage. Seeds were soaked in DW (control), 50, 100 and 150 ppm aerated GA solution at 30±2ºC for 24 h. The ratio of seed weight to solution volume was 1:5 (g/mL) (Farooq *et al.*, 2006). The seeds were then rinsed with DW and dried to its original weight by putting in between two sterile filter papers and were grown on double layer of filter paper in 9 cm petri dishes (Three replicates for each treatment with 20 seeds in each replicate) irrigated with 20 mL DW for control and 50, 100 and 150 mM sodium chloride solutions as salt stress treatments and were kept in the dark at 30±2ºC. Germination counts were taken after every 12 h for 7 days. Emergence of radicle 2 mm in length was considered as germination criterion. Data on root length, shoot length, fresh weights of shoot and roots and dry weight of shoot and root were taken and evaluated after 7 days. Relative root elongation was measured according to the formula described by Tam and Tiquia (1994). The vigor index was calculated as mentioned in (Kamran *et al.*, 2013).

Seedling vigor index = [seedling length × germination percentage]

For biochemical and molecular studies the seeds were grown as mentioned earlier except that stress was given in nutrient solution (Hoagland and Arnon, 1950) after transferring from petri dishes to water culture (Styrofoam with holes having net cloth underneath floating on plastic boxes that were coloured black to prevent sunlight penetration and algal growth were used) for further growth. The nutrient solution was changed after 5 days with pH maintained between 5 and 6. Salt stress was given to plants after two weeks in water culture and further observation were recorded after 5 days of salt exposure. The control plants were grown on the same nutrient solution without salt.

Biochemical Study

Pigment analysis: The green pigments Chl-a, Chl-b and total carotenoids were analyzed as described earlier (Lichtenthaler and Buschmann, 2001). Briefly, twenty five milligram of dry leaf sample and equal amount of magnesium oxide with 5 mL of methanol was kept on shaker and centrifuged. The supernatant was used to measure optical density at 470, 653 and 666 nm and the values were calculated according to formulae given by (Türlerinde *et al.*, 1998).

Electrolytes study (Na, K, Ca)

Ion analysis was done by already described method (Awan and Salim, 1997) with slight modifications. The powdered leaf sample was digested with sulfuric acid (concentrated) in a beaker until the formation of black slurry after which hydrogen peroxide (H_2O_2) was added (2:1) and the extracts were digested and dissolved in 20 mL distilled water to analyze for sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) concentration using flame photometer.

Gene Expression Study

Total RNA was extracted from fresh leaves using trizol method and complementary DNA (cDNA) was synthesized using M-MLV Rtase enzyme and OG2 primer "(5′-GAGAGAGGATCCTCGACTTTTTTTTTTTT-3′)".
Actin was used as internal control and cDNA was used as template to amplify specifically designed primers for *OsZat 12-1* and *OsZat 12-9* using ABI thermo cycler. The PCR products were visualized on a 1.5% agarose gel in a gel documentation system (Table 1).

Statistical Analysis

The experiment was completely randomized block design with three replicates per treatment. All the data was

analyzed statistically by using the Analysis of variance (De Clercq *et al*., 2013) followed by standard error. The relationship between physiological and biochemical parameters were analyzed by Pearson correlation coefficient.

Results

Physiological Study

To assess the role of GA mediated seed priming on physiological growth of rice under saline condition; we studied different parameters like germination percentage, vigor index, relaltive root elongation percentage, fresh and dry weight of shoot and root for comparison between hydro and GA primed plants. It was observed that with an increase in the salt concentration, there was a decrease in all growth attributes studied compared to control; and that GA-mediated seed priming reversed the negative effect of salt stress. Seeds primed with 100 ppm GA showed the highest germination percentage (76.66–86.66%; Fig. 1A). Seeds primed with GA showed significant increase ($P<0.05$) in vigor index (Fig. 1B) The highest vigor index was recorded for seeds primed with 100 and 150 ppm GA solution under 50 mM salt stress condition while the lowest was recorded for hydro primed seeds under 150 mM salt stress (Fig. 1B). Seeds primed with 50 and 150 ppm GA showed significant increase in shoot fresh weight under different salt stress condition (50, 100 and 150 mM) (Fig. 2A). Significant increase was also recorded for shoot dry weight by GA priming under salt stress condition but at different dilutions. Seeds primed with 50 and 150 ppm GA showed significant increase in shoot fresh weight under 50 mM salt stress, while less effective on root fresh weight and has no significant effect under 50 and 150 mM salt stress condition respectively, (Fig. 2A, B). Seed priming with 100 and 150 ppm GA dilution showed significant increase in root dry weight under 50 and 100 mM salt stress (Fig. 2D).

Biochemical Study

To further investigate whether GA mediated seed priming has some effect on biochemical properties of rice, we studied some biochemical parameters. Results showed that an increase in salt stress leads to a decrease in chl-a, chl-b and total carotenoids both in GA primed and hydro-primed plants (Table 3). However 50 and 100 ppm GA primed plants showed increased chl-b concentration (Table 3). It was also observed that with an increase in salt stress there is a regular increase in sodium (Na^+) ion concentration while potassium (K^+) and calcium ion (Ca^{2+}) concentration decreased (Table 3). GA mediated seed priming decreased the concentration of Na^+ under 150 mM salt stress while increased it under 100mM. GA priming has no significant effect on K^+ and Ca^{2+}, however 100 and 150 mM salt stress decreased Ca^{2+} concentration (Table 3).

Gene Expression

To find out whether GA mediated seed priming has some effect on regulatory genes expression, we investigated the expression of *OsZat 12* gene that is considered to have regulatory function in plants. There was no detectable expression of *OsZat 12-1* in hydro-primed plants under salt stress conditions (50, 100 and 150 mM), while expression was observed for GA primed (all dilutions) plants under 100 and 150 mM salt stress condition. However, 50 ppm GA primed plants showed low expression of *OsZat 12-1* under 50 mM salt stress (Fig. 3). In case of *OsZat12-9* a high induction of expression was observed for 50 ppm GA primed plants under 50 mM salt stress, while expression with reduced kinetics was also observed for 0, 50 and 150 mM salt treatment, and GA primed plants (Fig. 3).

Pearson correlation coefficients were used to analyze the relationship between physiological and biochemical properties under control and treated conditions. There was strong positive correlation of vigor index with total relative root elongation percent, chl-a and chl-b (Table 2), however, correlations of chl-b and Na^+ concentration, K^+ and Na^+ concentration were negative (Table 2).

Discussion

Seed priming is an effective strategy for enhancing the plant growth and yield under optimal and stress conditions. Various reports described the role of priming on growth and development including its effect on the lag phase of germination, early DNA replication (Bray *et al.*, 1989) enhancement of protein synthesis (Fu *et al.*, 1988) and increased ATP production (Mazor *et al.*, 1984).

Results from our study shows a decrease in all growth attributes with increase in salinity. GA priming at different dilutions reversed the negative effect of salinity and increased different growth attributes under saline conditions compared to hydro primed seeds (Fig. 1, 2). The increased germination rate, percentage and seedling growth by GA may be considered as the possible effect of GA in enhancement of α-amylase activity for breakdown of starch to provide the energy required for germination as suggested by Farooq *et al*. (2005). This might be because of the myriad applications of GA in plant metabolism, protein and nitrogen contents and activity of certain enzymes. It is reported that GA increase the activity of glutathione reductase and dehydro reductase that are involved majorly in reactive oxygen species scavenging, and prevent oxidative stress (Noctor and Foyer, 1998). These results are in accordance with Cha-um *et al.* (2009) who reported that exposure of rice to salt stress decreased shoot height, fresh weight, dry weight, and leaf area of seedlings. Chl-a and chl-b are the primary pigments being the active site for photosynthesis along with other accessory pigments are very important to plants. Our results that show decreased chlorophyll contents under salt stress that might

Table 1: Sequences of different primers (forward and reverse)

Gene code	Primer sequence forward	Primer sequence Reverse
RAC-1	5′- TGCTATCCCTCGTCTCGACCT-3′	5′- CGCACTTCATGATGGAGTTGTAT-3′
OsZat 12-1	5′- ATGAGCAAGAGGAGCAGGAG-3′	5′- TCAAAGGAAACAATCCAACA -3′
OsZat 12-9	5′- ATGACAATCACGAGAGAAGA -3′	5′- CACGTCTTGCACTCGAACAC -3′

Table2: Correlation (Pearson) coefficients among different physiological and biochemical parameters of rice

Variables	Na	Ca	VI	RRE	K	Chl a
Ca	-0.2088					
VI	-0.8363**	0.1455				
RRE	-0.7672**	0.2490	0.9239**			
K	-0.8608**	0.1833	0.7398**	0.7065**		
Ch la	-0.8163**	0.2362	0.5538**	0.5487**	0.5235**	
Chl b	-0.8834**	0.3030	0.5536**	0.5254**	0.7305**	0.9144**

**= Significant at the P≤0.01; Na: Sodium ion, Ca: Calcium ion, VI: Vigor index, RRE: Relative root elongation, K: Potassium ion, Chla: Chlorophyll a, Chl b: Chlorophyll b

Table 2: GA priming effect on biochemical properties of rice (KSK-282) under salt (NaCl) stress

NaCl (mM)	GA (ppm)	Chl a	Chl b	Carotene	Na^+	K^+	Ca^+
0	0	10.398 a	12.350 ab	648.749 a	17.2 h	48.4 abc	24.4 ab
	50	9.560 a	13.605 a	706.985 a	17.7 g	55.7 bc	26.25 abc
	100	7.822 abc	14.315 a	617.079 ab	17.65 de	56.15 abc	26.9 ab
	150	8.533 ab	12.673 ab	605.914 ab	16.55 ab	54 c	26.25 a
50	0	9.672 a	11.726 ab	672.154 a	27.9 h	44.1 ab	23.95 ab
	50	9.696 a	10.478 abc	572.745 ab	33.95 h	47.6 a	27.5 ab
	100	8.269 ab	9.114 bcd	442.044 bc	26.65 h	47.65 abc	22.05 ab
	150	6.742 bcd	7.167 cde	288.938 cde	31.2 f	47.6 abc	18 ab
100	0	4.279 de	4.421 ef	260.432 cde	44 e	47 abc	26.7 ab
	50	2.894 e	3.157 ef	191.058 de	41.8 bc	47.95 c	26.3 ab
	100	2.748 e	2.837 f	149.645 e	50.1 g	46.75 abc	25.35 bc
	150	2.839 e	3.011 f	164.292 e	47.9 bc	47.4 abc	14.15 ab
150	0	5.165 cde	6.706 cdef	360.691 cd	54 a	42.65 abc	29.45 ab
	50	3.864 e	4.554 ef	227.547 de	50.2 fg	43.45 abc	25.3 cd
	100	4.963 de	5.870 def	359.709 cd	50 cd	44.75 abc	25.55 d
	150	5.447 cde	6.668 cdef	370.906 cd	49 c	44.95 abc	14.55 d

The data points are the means of three replicates. Values with different letters are significantly different (P≤0.05). The abbreviations should be read as mentioned above

be because of its sensitive nature, when there is accumulation of ions, the chlorophyll disassociates as suggested by Šiler *et al.* (2007) who reported that salinity leads to a reduction in absorption of light by leaves. The damage to different growth attributes by salinity is due to physiological drought, a condition that occurs mainly due to salt accumulation in root growing medium, leading to a reduction in cell division and enlargement in the growing regions of roots thus reducing root growth (Netondo *et al.*, 2004). GA priming seems to have some role in restoration of osmotic potential between plant roots and soil/water culture so that it could absorb water and minerals. The increase in shoot and root fresh weight might be due to increased cell division within the apical meristem of roots that leads to increase in plant growth (Farooq *et al.*, 2007). GA priming did not have a significant effect on Chl-a, Chl-b and total carotene contents. This could be due to the intervention of salt stress on action and biosynthesis of phytohormones. Another important property of plant metabolism and maintenance is the ionic balance that plays a vital role in the proper functioning of different kinds of enzymes that regulate biological process. Results from this study showed that with an increase in salt concentration, Na^+ was increased, while K^+ and Ca^{2+} ions were decreased. GA priming reduced the concentration of toxic ions like Na^+ in some treatments and also increased K^+ but the increase was not significant; the results correlate with Aldesuquy (1995) who described that priming with GA_3 reduced the accumulation of Na^+ and increased K^+/Na^+ ratio in flag leaves of wheat under salt treatments. Possibly it is due to antagonistic effect of ionic pumps located at membranes that allows certain level of a particular ion to enter; hence the Na^+ being cation will definitely remove some K^+ to compensate itself in the cell.

The zinc-finger protein *Zat 12* is considered to respond to a large number of biotic and abiotic stress conditions (Davletova *et al.*, 2005; Zhang *et al.*, 2010). We demonstrated GA priming effect on the expression of two stress responsive genes *OsZat 12-1* and *OsZat 12-9* (belonging to zinc finger gene family) under saline conditions and it was observed that in case of *OsZat 12-1* there was no expression in seedlings from hydroprimed seeds under salt stress, while expression was observed for

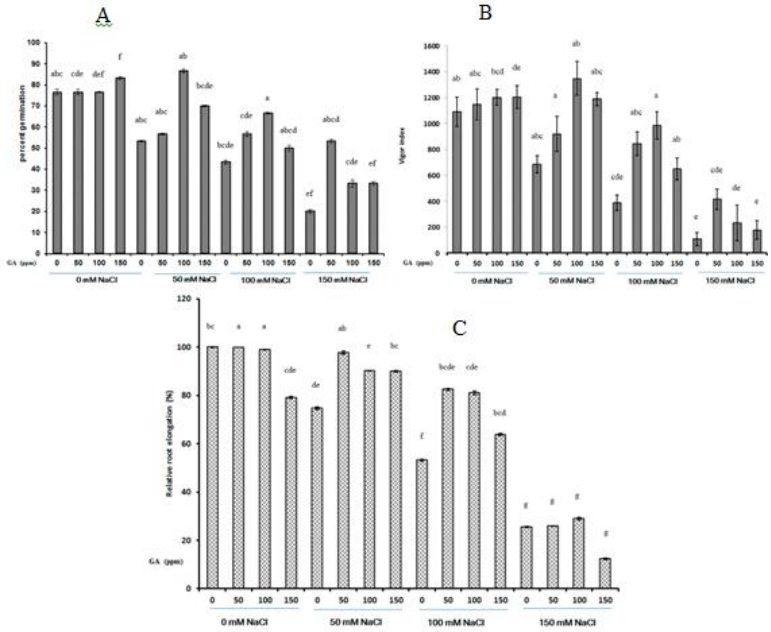

Fig. 1: GA mediated seed priming effect on different growth attributes of rice under salt (NaCl) stress: (A), percent germination: (B). vigor index: (c) relative root elongation percent. The data points are the means of three replicates Bars indicates mean ± SE, different letters indicates significant difference ($p \leq 0.5$)

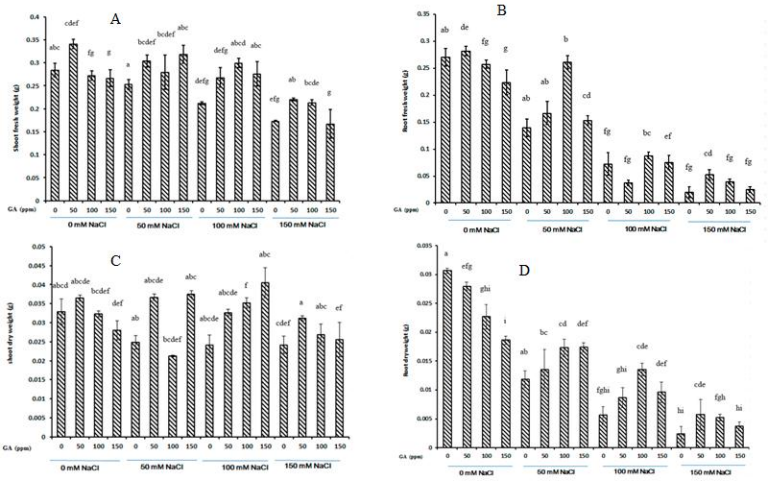

Fig. 2: GA mediated seed priming on (A) shoot fresh weight (B) root fresh weight (C) shoot dry weight (d) root dry weight. All the data points are mean of three replicates. Bars indicates mean ± SE, different letters indicates significant difference ($p \leq 0.5$)

GA primed plants grown under different salt conditions (Fig. 4). Similarly, in the case of *OsZat 12-9* the expression was very low in hydroprimed plants under 50 and 150mM salt stress while no expression for 100 mM treatment was detected, however the expression was clearly enhanced in GA primed plants grown under different saline conditions (Fig. 3). Plants produce certain reactive oxygen species (ROS) when subjected to stress conditions. One important ROS is H_2O_2 and according to Davletova *et al.* (2005) H_2O_2 induces "Heat Shock Factor" (HSF 21) that regulates the expression of the *Zat 12* gene. Alternatively, plants releases superoxide dismutase (SOD) that kills H_2O_2 to prevent plant tissues from being oxidized. There are two reasons for low or no expression of *OsZat 12-1* and *OsZat 12-9* in plants under salt stress. The first is the duration of the stress period, the plants were subjected to salt stress for a long time (4–5 days) as described earlier that caused a pathological effect on gene expression, and the second may be due to SOD function against H_2O_2 that regulates HSF 21. GA priming seems to play an important role in the expression of *Zat 12*. It suggests that GA along with H_2O_2 induce the HSF 21 to enhance the expression of *Zat 12*. This statement is

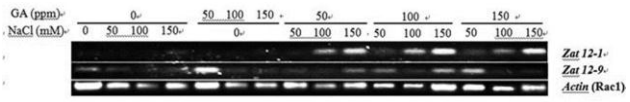

Fig. 3: GA priming enhanced expression of Zat 12-1 and Zat 12-9 under salt stress conditions. The first four treatments are hydroprimed plants, then GA primed grown in distelled water followed by 50, 100 and 150 ppm GA primed plants grown under salt stress (50, 100, 150 mM)

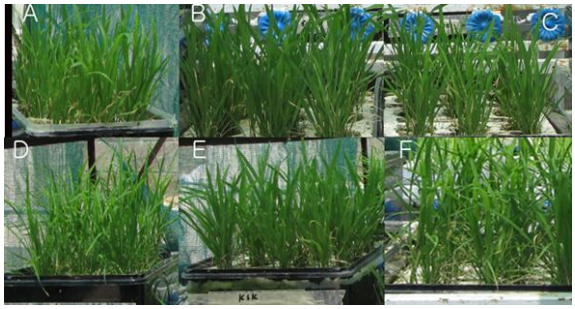

Fig. 4: Hydroprimed and GA primed plants in hydroponics under different saline conditions. A, Control plants; B, 50 ppm GA primed; C, Hydroprimed plants grown under 50 mM salt stress; D,E,F, 50 ppm GA primed plants grown under 50, 100 and 150 mM salt stress respectively

supported by Rizhsky *et al.* (2004) who described that although it is not known which signals are involved in enhancing *Zat12* expression, different signals such as ROS and some stress response hormones control the expression of *Zat 12* under stress condition.

Conclusion

It is concluded form the current study that GA mediated seed priming promotes growth and helps in resistance towards salinity. The biochemical studies shows that GA has no significant effect on biochemical properties; however it provides a base for studying biochemical composition of plant in detail. Expression of *Zat 12*-1and *Zat 12-9* confirms the role of these genes in regulatory functions and suggests that GA mediated seed priming can induce expression of regulatory genes; therefore genetic study should be included while studying stress physiology of crops.

Acknowledgments

Eesearch was supported by Higher Education Commission of Pakistan through Research grant number, 1348 and Kyungpook National University Research Fund, 2013.

References

Aldesuquy, H.S., 1995. Hormones-induced modifications in the response of wheat flag leaf to NaCl. *Biol. Plant.*, 37: 605–611

Ashraf, M., M.A. Khan and S.S.M. Naqvi, 1991. Effect of salinity on seedling growth and solutes accumulation in two wheat genotypes. *RACHIS (ICARDA)*, 10: 30–31

Awan, J.A. and U.R. Salim, 1997. Food Analysis Manual. *Pak. J. Bot*., 5: 2–17

Basra, S.M.A., M. Farooq, R. Tabassam and N. Ahmad, 2005. Physiological and biochemical aspects of pre-sowing seed treatments in fine rice (*Oryza sativa* L.). *Seed Sci. Technol.,* 33: 623–628

Bray, C.M., P.A. Davison, M. Ashraf and R.M. Taylor, 1989. Biochemical events during osmopriming of leek seed. *Ann. Appl. Biol*, 102: 185–193

Cha-um, S., T. Boriboonkaset, A. Pichakum and C. Kirdmanee, 2009. Multivariate physiological indices for salt tolerance classification in indica rice (*Oryza sativa* L. spp. indica). *Gen. App. Plant Physiol*., 35: 75–87

Davletova, S., K. Schlauch, J. Coutu and R. Mittler, 2005. The zinc-finger protein Zat12 plays a central role in reactive oxygen and abiotic stress signaling in Arabidopsis. *Plant Physiol*., 139: 847–856

De Clercq, I., V. Vermeirssen, O. Van Aken, K. Vandepoele, M.W. Murcha, S.R. Law, A. Inzé, S. Ng, A. Ivanova, D. Rombaut, B. van de Cotte, P. Japsers, Y. Van de Peer, J. Kangasjärvi, J. Whelan and F. Van Breusegem, 2013. The Membrane-Bound NAC Transcription Factor ANAC013 Functions in Mitochondrial Retrograde Regulation of the Oxidative Stress Response in Arabidopsis. *Plant Cell*, 25: 3472–3490

Farooq, M., S.M.A. Barsa and A. Wahid, 2006. Priming of field-sown rice seed enhances germination, seedling establishment, allometry and yield. *Plant Growth Regul*., 49: 285–294

Farooq, M., S.M.A. Basra, N. Ahmad and K. Hafeez, 2005. Thermal hardening: a new seed vigor enhancement tool in rice. *J. Integr. Plant Biol*., 47: 187–193

Farooq, M., S.M.A. Basra, H. Rehman, M. Hussain and Y. Amanat, 2007. Pre-sowing salicylicate seed treatments improve the germination and early seedling growth in fine rice. *Pak. J. Agric. Sci*., 44: 1–8

Flowers, T.J. and A.R. Yeo, 1981. Variability in the resistance of sodium chloride salinity within rice (*Oryza sativa* L.) varieties. *New Phytol*., 88: 363–373

Fu, Y.H., J.L. Young and G.A. Marzluf, 1988. Molecular cloning and characterization of a negative-acting nitrogen regulatory gene of Neurospora crassa. *Mol. Gen. Genetic.*, 214: 74–79

Gangwar, S., V.P. Singh, P.K. Srivastava and J.N. Maurya, 2011. Modification of chromium (VI) phytotoxicity by exogenous gibberellic acid application in *Pisum sativum* (L.) seedlings. *Acta Physiol. Plant*, 33: 1385–1397

Ghaafar, A.C. and I.M.T. Rawi, 2011. Response of mungbean (*Vigna radiata* L., Wilczek) to gibberellic acid (GA3) rates and varying irrigation frequencies. *Int. J. Biosci. (IJB),* 1: 85–92

Greenway, H. and R. Munns, 1980. Mechanisms of salt tolerance in nonhalophytes. *Annu. Rev. Plant Physiol.*, 31: 149–190

Hakim, O., M.H. Sung and G.L. Hager, 2010. 3D shortcuts to gene regulation. *Curr. Opin. Cell Biol*., 22: 305–313

Halter, L., R. Habegger and W.H. Schnitzler, 2000. *Gibberellic Acid on Artichokes (Cynara scolymus L.) Cultivated in Germany to Promote Earliness and to Increase Productivity*. Paper presented at the IV International Congress on Artichoke 681

Hasanuzzaman, M., M. Fujita, M. Islam, K. Ahamed and K. Nahar, 2009. Performance of four irrigated rice varieties under different levels of salinity stress. *Int. J. Integ. Biol.*, 6: 85–90

Hoagland, D. and D. Arnon, 1950. *The Water-Culture Method for Growing Plants without Soil*. California Experiment Station Circular 347. The College of Agriculture. University of California, Berkeley, California, USA

Jamil, M., W. Iqbal, A. Bangash, S.U. Rehman, Q.M. Imran and E.S. Rha, 2010. Constitutive expression of OSC3H33, OSC3H50 and OSC3H37 genes in rice under salt stress. *Pak. J. Bot.*, 42: 4003–4009

Kamran, M., Q.M. Imran, A. Khatoon, I.J. Lee and Shafiq-Ur-Rehman, 2013. Effect of plant extracted smoke and reversion of Abscisic acid stress on lettuce. *Pak. J. Bot.,* 45: 1541–1549

Khan, A., M. Ashraf, S. Naqvi, B. Khanzada and M. Ali, 1995. Growth, ion and solute contents of sorghum grown under NaCl and Na_2SO_4 salinity stress. *Acta Physiol. Plant.*, 17: 261–268

Lichtenthaler, H.K. and C. Buschmann, 2001. *Chlorophylls and Carotenoids: Measurement and Characterization by UV-VIS Spectroscopy*. Current protocols in food analytical chemistry

Lutts, S., J. Kinet and J. Bouharmont, 1995. Changes in plant response to NaCl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance. *J. Exp. Bot.*, 46: 1843–1852

Maas, E. and G. Hoffman, 1977. Crop salt tolerance\-current assessment. *J. Irrigation Drain. Division*, 103: 115–134

Maggio, A., G. Barbieri, G. Raimondi and S.D. Pascale, 2010. Contrasting effects of GA3 treatments on tomato plants exposed to increasing salinity. *J. Plant Growth Regul.*, 29: 63–72

Mazor, L., M. Perl and M. Negbi, 1984. Changes in some ATP-dependent activities in seeds during treatment with polyethyleneglycol and during the redrying process. *J. Exp. Bot.*, 35: 1119–1127

Moradi, F. and A.M. Ismail, 2007. Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice. *Ann. Bot.*, 99: 1161–1173

Mori, I.K. and T. Kinoshita, 1987. *Salt Tolerance of Rice Callus Clones*. Rice Genetics Newsletter

Netondo, G.W., J.C. Onyango and E. Beck, 2004. Sorghum and salinity. *Crop Sci.*, 44: 797–805

Noctor, G. and C.H. Foyer, 1998. Ascorbate and glutathione: keeping active oxygen under control. *Annu. Rev. Plant Biol.*, 49: 249–279

Pillai, M.A., S. Yanagihara and T. Akiyama, 2001. Molecular cloning and characterization of salt responsive genes in rice (*Oryza sativa*). *J. Plant Physiol.*, 158: 1189–1194

Rizhsky, L., S. Davletova, H. Liang and R. Mittler, 2004. The zinc finger protein Zat12 is required for cytosolic ascorbate peroxidase 1 expression during oxidative stress in Arabidopsis. *J. Biol. Chem.*, 279: 11736–11743

Shannon, M., J. Rhoades, J. Draper, S. Scardaci and M. Spyres, 1998. Assessment of salt tolerance in rice cultivars in response to salinity problems in California. *Crop Sci.*, 38: 394–398

Šiler, B., D. Mišić, B. Filipović, Z. Popović, T. Cvetić and A. Mijović, 2007. Effects of salinity on *in vitro* growth and photosynthesis of common centaury (*Centaurium erythraea* Rafn.). *Arch. Biol. Sci.*, 59: 129–134

Tam, N. and S. Tiquia, 1994. Assessing toxicity of spent pig litter using a seed germination technique. *Resour. Conserv. Recycl*, 11: 261–274

Türlerinde, F.Ç.K.B.A., B. Klorofil-A and İ.S. Saptanması, 1998. Spectrophotometric determination of chlorophyll-A, B and total carotenoid contents of some algae species using different solvents. *Truk. J. Bot.*, 22: 13–17

Yokoi, S., F.J. Quintero, B. Cubero, M.T. Ruiz, R.A. Bressan, P.M. Hasegawa and J.M. Pardo, 2002. Differential expression and nction of Arabidopsis thaliana NHX Na^+/H^+ antiporters in the salt stress response. *Plant J.*, 30: 529–539

Zhang, M., J.N. Kimatu, K. Xu and B. Liu, 2010. DNA cytosine methylation in plant development. *J. Genet. Genomics*, 37: 1–12

(Received 06 March 2015; Accepted 20 August 2015)