Journal of Plant Physiology and Breeding
2016, 6(2): 49-61 ISSN: 2008-5168

Role of Exogenous Application of Auxin on Antioxidant Enzyme Activities in Rice Under Salt Stress

Saeed Saedipour

Received: January 3 2016 Accepted: October 16 2016
Department of Agronomy, College of Agriculture, Shoushtar Branch, Islamic Azad University, Shoushtar, Iran
*Corresponding author; Email: saeeds79@gmail.com

Abstract
Phytohormones such as auxin are known to be involved in alleviating the detrimental effects of salinity by modulating the activity of enzymatic antioxidants and improving antioxidant system, which help in sustaining plant growth. The present study envisaged revealing the role of exogenous application of indole-3-acetic acid (IAA) in improving defense mechanisms in two genotypes (FL485 and IR29, salt tolerant and salt susceptible, respectively) of rice against NaCl stress. The results showed that salt stress led to an increase in the ascorbic acid (AsA) content of the tolerant cultivar suggesting that oxidative defense system was very operative in this cultivar. Exogenous application of IAA further increased the activities of AsA and ascorbate oxidase in the salt stressed FL485, whereas such effect was not observed in the susceptible cultivar (IR29). IAA was found to be more effective in enhancing the activities of α-tocopherol in salt stressed rice plants with more efficiently in the salt tolerant cultivar. In addition, IAA markedly increased hydrogen peroxide (H_2O_2) concentration in both cultivars as compared to untreated plants. Lipid peroxidation levels of both cultivars under salt treatment showed no change with foliar applications of IAA.

Keywords: Ascorbate oxidase (AAO); Ascorbic acid (AsA); Indole-3-acetic acid (IAA); Salinity; Rice

Introduction

Among abiotic stresses, salinity stress is a major contributor in decreasing the crop productivity. The mechanisms that enable plants to adapt to salinity stress and maintain growth will ultimately help in the selection of stress tolerant cultivars for exploiting saline soils. Salinity stress induces over production of reactive oxygen species (ROS) (Nazar *et al.* 2011; Khan *et al.* 2012) that triggers lipid peroxidation, DNA damage, inhibition of photosynthesis and disturbance in mineral nutrient status (Nazar *et al.* 2011; Turan and Tripathy 2012). However, plants possess a number of antioxidants, in varying degrees, that protect against the potentially cytotoxic species of activated oxygen. Ascorbate (AsA) is the most abundant, low molecular weight antioxidant that has a key role in defense against oxidative stress caused by enhanced level of ROS (Smirnoff 2005). AsA is considered powerful antioxidant because of its ability to donate electrons in a number of enzymatic and nonenzymatic reactions. Apoplastic AsA is believed to represent the first line of defense against potentially damaging external oxidants (Barnes *et al.* 2002). Tocopherols (α, β, γ, δ) represent a group of lipophilic antioxidants involved in scavenging of oxygen free radicals, lipid peroxy radicals and $^1O_2$ (Diplock *et al.* 1989). $^1O_2$ oxygen quenching by tocopherols is highly efficient, and it is estimated that a single α-tocopherol molecule can neutralize up to 220

$^1O_2$ molecules *in vitro* before being degraded (Fukuzawa *et al.* 1982). Accumulation of α-tocopherol has been shown to induce tolerance to chilling, water deficit and salinity in different plant species (Guo *et al.* 2006; Bafeel and Ibrahim 2008). Of the various strategies employed to improve crop growth under saline conditions, one is to use exogenous application of plant hormones which are active members of the signal cascade involved in the induction of plant stress responses (Pedranzani *et al.* 2003). One of these plant growth regulators which regulate growth under normal or stress conditions is indole acetic acid (IAA) which plays a vital role in maintaining plant growth under stress conditions including salt stress (Gulnaz *et al.* 1999; Iqbal and Ashraf 2007). Recently, while examining the ameliorative effect of IAA on salt stressed plants of blackgram (*Phaseolus mungo* L.), Guru Devi *et al.* (2012) found that foliar-applied IAA (15 mg l^{-1}) considerably ameliorated the adverse effects of salt on these plants. Similarly, Egamberdieva (2009) examined the effect of IAA producing bacteria on salt stressed wheat plants and reported considerable alleviation of salt-induced adverse effects on these plants. In the present study an attempt was made to assess the tolerance potential of rice cultivars with different sensitivity to salt stress based on exogenous application of IAA on some non-enzymatic antioxidants and lipid peroxidation. Comparison of these responses could be useful in identifying differences related to the ability of each cultivar to cope with the salinity effect.

Material and Methods

Plant materials, growth conditions and stress treatments

Two rice cultivars contrasting in salinity stress tolerance during reproductive stages (Moradi *et al.* 2003) were selected for this investigation. FL485 is a salt stress breeding line at both the seedling and reproductive stages, and IR29 is a cultivar sensitive to salt stress during both stages and is commonly used as a sensitive check in breeding nurseries. Salinity treatment was begun at around 10–7 d before panicle initiation and continued through harvest. The experiment was carried out in a greenhouse with air temperature in the range of about 25 to 35 ^{0}C and light intensity of about 600–1000 mmol m^{-2} $s^{-1}$and with 20 pots per cultivar in each replication. Pre-germinated seeds were sown in 1 L perforated plastic pots filled with fertilized (50 N, 25 P and 25 K mg kg^{-1}) Maahas clay soil (43% clay, 44% silt and 13% sand; pH 5.9; Tirol-Padre and Ladha 2004) and were kept in concrete tanks filled with tap water. The level of water was maintained at 3 cm below the soil surface for 2 d. Five seeds of each of the two cultivars were sown in each pot, thinned to one seedling two weeks later, and the water level was raised to about 1–2 cm above the soil surface. When the seedlings were 28 d old, water was siphoned

out and the pots were drained for 12 h, then flooded with tap water (control) or with a saline solution with EC of 3 dSm^{-1} using NaCl for 3 d, then increased progressively to 4 and 5 dSm^{-1} at 3 d intervals, and finally stabilized at 6±0.3 dSm^{-1} through harvesting. The pots were kept flooded thereafter for the duration of the experiment, and the EC of the water was monitored daily and adjusted when necessary using NaCl and tap water. From two days after anthesis, each pot was sprayed by IAA solution at a concentration of 50 µM. Deionized water was sprayed as a control. Tween-20 (0.5%) was used as a wetting agent for each treatment, including the water as control. We sprayed the plants with solution until the leaves were completely wet and the solution ran off the leaves.

Sampling

All traits were measured on flag leaves of the first two tillers that were tagged 25 d after sowing. Sampling of the flag leaves were removed from anthesis up to full grain maturity at seven-day interval [7, 14, 21 and 28 days after spraying (DASP)] for the various biochemical analyses. For the biochemical assays, samples were cut into small pieces after measuring their fresh weight, frozen in liquid nitrogen and stored at -80 ^{0}C. Three replicates were maintained for all measurements. The various plant parts were dried in oven at 80 ^{0}C for dry matter analyses and various estimations.

Determination of lipid peroxidation and H_2O_2

Lipid peroxidation was determined by the method of Stewart and Bewly (1980). Hydrogen peroxide content was determined according to Sergiev *et al*. (1997). Hydrogen peroxide was extracted from leaf tissues (0.5 g) on an ice bath with 5 mL of 0.1% (w/v) trichloroacetic acid. Crude extract was centrifuged at 12000g for 15 min and 0.5 mL of the supernatant was transferred to a 15 mL test tube. The supernatant was added with 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0), 1 mL of 1 M potassium iodide (KI) and mixed by vortexing briefly. The absorbance of mixture without the supernatant served as blank. Each variable was evaluated using four plants for each treatment.

Determination of α-tocopherol

Leaf tissues were homogenized in ethanol and the homogenate was centrifuged at 4500 ×g for 5 min. The supernatant was treated with n-hexane. α-tocopherol was extracted twice in hexane phase and the collected extract was dried in liquid nitrogen. Dried extract was dissolved in 0.5 ml methanol for HPLC. Quantification was according to Catignani (1983) and Miller *et al.* (1984) at absorption maxima of 296 nm for α-tocopherol. HPLC separations were done at room temperature

with a Perkin-Elmer liquid chromatography system (Series 1100) consisting of a sample injection valve (Cotati 7125) with a 20 l sample loop, UV spectrophotometric detector (Cecil 68174), integrator (HP 3395) and Techsphere ODS–2 packed (5 µM particle and 80 Å pore size) column (250×4.6 ID) with a methanol: acetonitrile: chloroform (47:42:11, v/v) mobile phase at 1 ml min^{-1} flow rate.

Determination of ascorbate

Leaf tissues were ground in chilled mortar with pestle in ice-cold 5% (w/v) metaphosphoric acid. The homogenate was centrifuged for 15 min at 25,000×g and the supernatant used for determinations. AsA was quantified spectrophotometrically at 525 nm according to Zhang and Kirkham (1996) using the α,ά-dipyridyl method.

Determination of ascorbate oxidase (AAO)

Leaf tissues were homogenized using a mortar and pestle at 4 ^{0}C with an ice-cold 50 mM Tris–HCl buffer pH 7.8, containing 1mM EDTA and 0.05% (w/v) cysteine. The homogenate was centrifuged for 20 min at 25000×g and the supernatant was collected and used to determine the enzyme activities. Spectrophotometric assay of AAO (EC 1.10.3.3) activity in the supernatant was carried out according to De Tullio *et al.* (2007), measuring the decrease in AsA absorbance at 265 nm.

Data were analyzed as factorial based on completely randomized design with four replications. Statistical analysis employed SPSS ver. 10.0. The significance of differences between factors was checked with the least significant difference (LSD) test. All values were means ±SE.

Results

Effect of salinity on lipid peroxidation and H_2O_2 content

The effect of salinity stress from anthesis onwards, on malondialdehyde (MDA) formation in the leaves of two rice varieties is shown in Figure 1C&F). In the salt-sensitive cultivar MDA pattern showed a strong initial increase until the day 14, reached to the value of 877.96 µM g^{-1} DW. From this point on, MDA content showed a progressive decrease until the end of the experiment. By contrast, in the salt-tolerant FL485 no significant difference was found until the day 21 among all stages of sampling. From this point on, a slight increment occurred up to 28 days, reaching a maximum value of 598.92 µM g^{-1} DW. IAA treatment failed to mitigate the adverse effect of salinity on MDA content in both cultivars. The pattern of H_2O_2 levels is shown in Figure1B&E. A very significant variation in H_2O_2 content was observed between salt-stressed and control plants in both cultivars. Salinity rose the H_2O_2 concentration in both cultivars; although this increase was

much higher in the susceptible cultivar. At the early days of the experiment, IAA application caused the accumulation of H_2O_2 in both cultivars, however, from day 21 onwards H_2O_2 levels dropped to values of salt-stressed plants in both genotypes (Figure 1B&E).

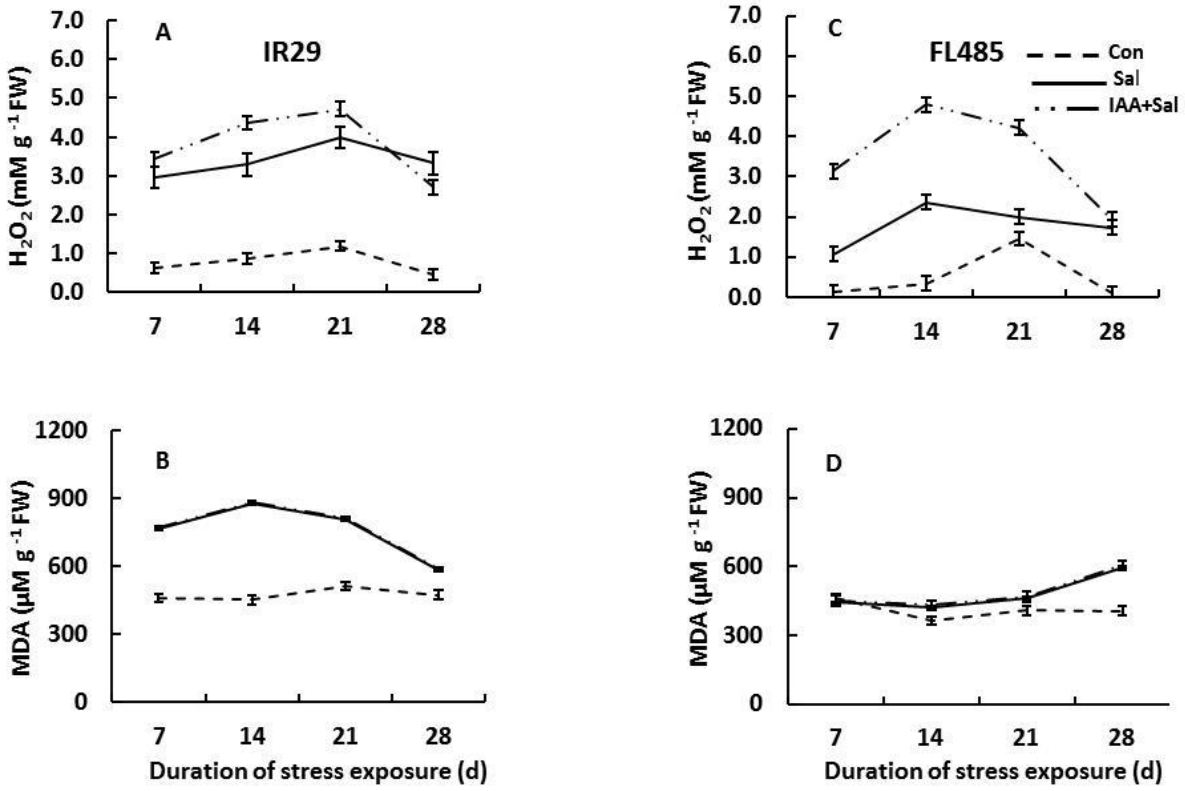

Figure 1 (A-D): Effects of salinity and exogenous IAA on carotenoids, H_2O_2 and malondialdehyde (MDA) in flag leaves during grain filling of two rice cultivars (Salt-Sensitive cv. IR29 and Salt-Tolerant cv. FL485). Vertical bars represent ± SE of the mean (n=4).

Effect of salinity on AsA, AAO and α-toc activity

Figure 2A&D describes the effect of salinity on ascorbic acid activities of the two rice varieties. Measurements of the ascorbic acid activity exposed to salinity showed different patterns in both cultivars when compared with their unstressed control counterparts. The total activity of AsA was higher in the salt-stressed FL485 than its control and the salt-stressed IR29. The AsA salt-stressed FL485 showed a rapid initial increase up to day 14 reaching a maximum of 233.38 µM g^{-1} FW (2.2 fold higher than its counterpart the stressed IR29). After that, the activity dropped sharply to a value of 89.96 µM g^{-1} FW up to the end of the experiment (Figure 2D). The pattern of AsA fluctuation in the salt-stressed IR29 was very similar to its control throughout of the experiment; however, salinity caused a

substantial reduction in AsA content during all sampling stages. In comparison, IAA foliar treatment caused higher AsA activity as compared to its control treatment (Figure 2D).

Measurements of the activity of ascorbate oxidase (ASO) in the susceptible cultivar revealed a substantial reduction in the stressed plants at all sampling stages, as the values from 120 µM g^{-1} FW in the nonstressed plants dropped to 40 µM g^{-1} FW in the stressed plants at the end of experiment (Figure 2B). No significant differences in ASO activity was observed between control and salinity treatments in the tolerant FL485 cultivar (Figure 2E). IAA treatment markedly elevated the ASO activity in FL485; the values from 88 in the nonstressed plants reached to a value 160 µM g^{-1} FW in the treated plants. In IR29, IAA application ameliorated this activity with values lower than the control plants (Figure 2B).

The α-tocopherol activity was significantly enhanced by salinity in both cultivars which was more extent in the tolerant cultivar than the sensitive one (Figure 2C&F). In the salt tolerant FL485, α-tocopherol activity between day 0 and day 21, had a gradual increase in slop, reaching a maximum value of 4.33 µg g^{-1} DW. From this point on, α-tocopherol activity showed a slow decrease and reached to value of 3.69 µg g^{-1} DW at the last sampling time. By contrast, in the salt sensitive IR29 gradual increase in α-tocopherol activity continued until day 14 (3.4 µg g^{-1} DW) and then, reduced activity occurred 7 days earlier than the tolerant cultivar. This decline continued and reached to 1.44 µg g^{-1} DW at the end of experiment (Figure 2C).

Effect of salinity on grain yield

In this study, we observed that seed is far more sensitive to salinity than other traits that contribute to yield. Grain yield was decreased by salinity in both cultivars and the reduction was 54 and 1.5% in IR29 and FL485, respectively as compared to those of the control treatment (Table 1). IAA proved to be the most effective in enhancing grain yield under salinity stress. However, grain yield of FL485 under IAA treatment was remarkably higher than that of IR29. Almost a similar pattern was obtained for the 1000-grain weight and number of panicles per plant. Similarly, number of filled and filled grain percentage was significantly increased by IAA in contrast to the salt stress regime. Higher grain yield under IAA treatment was mainly ascribed to the significant higher spikelet per panicle and grain filling percentage as compared to the salinity treatment.

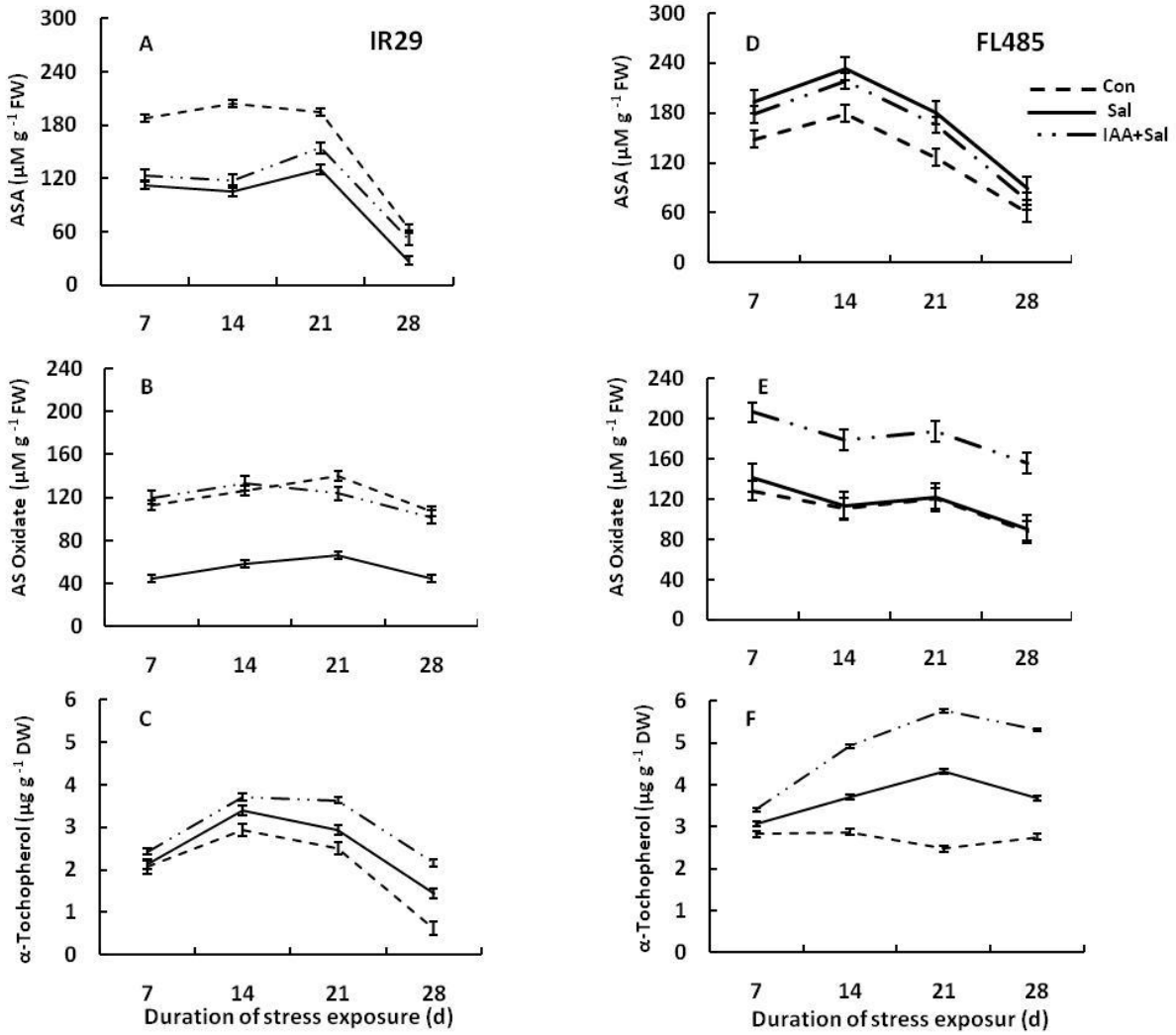

Figure 2 (A-E): Effects of salinity and exogenous IAA on ascorbate (AsA), ascorbate oxidase (ASO) and α-tocopherol in flag leaves during grain filling of two rice cultivars (Salt-Sensitive cv. IR29 and Salt-Tolerant cv. FL485). Vertical bars represent ± SE of the mean (n=4).

Table 1. Effects of foliar spray of IAA and salinity (control or salt stress of 6 dS m^{-1}) on grain yield, spikelet per panicle, panicle number and 1000 grain weight of two rice cultivars

Cultivars	Treatments	No. of panicle per plant	Spikelet per panicle	No. of filled spikelet	1000 grain weight (g)	Filled grain (%)	Grain yield (g $plant^{-1}$)
IR29	Con.	7.8	86.1	83.7	18.5	96.4	12.4
	Sal.	4.5	73.1	59.7	16.5	80.4	6.3
	Sal+IAA	6.1	77.3	74	18.7	95.7	8.8
FL485	Con.	7.3	88.3	87.3	22.7	98.5	14.9
	Sal.	5	78.3	70.3	20.5	90	10.2
	Sal+IAA	7	91	90.3	23.1	99.3	14.7
	LSD 5%	1.1	8.58	7.42	0.85	4.43	3.41
	CV	12.3	5.13	7.25	2.21	3.19	11.20

Discussion

A variety of strategies for counteracting the adverse effects of salt stress on plants are currently in practice. Of these, exogenous application of different types of plant growth regulators is contemplated to be an economical and shot-gun approach to alleviate the harmful effects of salinity on plant growth (Ashraf *et al.* 2008). Of various plant growth regulators, indoleacetic acid, one of the key auxins occurring naturally in plants, is known for its beneficial effects on growth of plants subjected to stress conditions including salt stress (Iqbal and Ashraf 2007; Guru Devi *et al.* 2012). It is already known that the alleviation of oxidative damage is often correlated with an efficient antioxidative system under NaCl stress (Smirnoff 1998). In the present study, salt stress increased the leaf ascorbic acid content of the tolerant cultivar suggesting that oxidative defense system in the salt stressed rice plants has been very operative to counteract various reactive oxygen species (ROS) produced due to salt stress (Ashraf 2009; Kaye *et al.* 2011). Exogenous application of IAA further increased the activities of ascorbic acid and ascorbate oxidase in the salt stressed FL485 in contrast to those in control plants, whereas such effect was not observed in the susceptible IR29 cultivar, as under salinity regime a marked reduction observed in the leaf AsA levels of IR29, however, a further slight elevation occurred as a result of IAA application as compared to the salt stressed plants receiving no exogenous hormone. AsA is a key component of the ascorbate-glutathione cycle and plays a protective role against ROS (Noctor and Foyer 1998). High level of endogenous ascorbate is essential to maintain the antioxidant system that protects plants from oxidative damage caused by different types of stresses including salt stress. It is well evident that plant cells are strongly redox-buffered due to very large quantities of the water soluble antioxidants including ascorbate (Athar *et al.* 2008, 2009; Hartmann *et al.* 2003). Thus, it is possible that enhanced endogenous AsA due to salinity might have protected salt tolerant plants from salt-induced oxidative damage by controlling cellular redox state. Under the salinity condition, IAA treatment strongly induced ASO activity in both cultivars, suggesting a novel mechanism of adaptation to salinity that to our knowledge never proposed or tested before. ASO is a plant-specific copper-containing enzyme of still unknown function (De Tullio *et al.* 2004). In comparison, salt stress led to a substantial reduction in ASO activity of the susceptible cultivar throughout the experiment whereas this was not seen in the tolerant cultivar as compared to the nonstressed plants. Indeed, removing excess oxygen in plants could be an

important mechanism to prevent formation of reactive oxygen species. Beside such prevention mechanism, it is tempting to speculate that ASO could have an additional role in salt stress by catalyzing intracellular production of water, which could somehow mitigate the stress (Liso *et al.* 2004; De Tullio *et al.* 2004, 2007). The significant correlation among yield and AsA (r= 0.605, p≤0.01), ASO (r= 0.645, $p \leq 0.05$) and α-Toc (r= 0.539, ≤ 0.05) suggests that improved growth under salinity conditions was associated with increased activities of antioxidant enzymes (Table 2). Plant growth regulators play vital roles in coordination of many growth and behavioral processes in rice, which regulate the amount, type and direction of plant growth (Bari and Jones 2009; Anjum *et al*. 2011). Salt stress caused more accumulation of α-tocopherol in both cultivars with higher increase in the salt tolerant FL485 cv. than the sensitive one as compared to their control treatments. Still higher α-tocopherol concentrations under salt stress conditions could contribute to improved photoprotection by maintaining an appropriate redox state in the chloroplasts (Falk and Munné-Bosch 2010; Tounekti *et al.* 2011).

IAA was found to be more effective in enhancing the activities of α-tocopherol in the salt stressed rice plants with more extent in the salt tolerant cultivar. This result shows that exogenously applied IAA had a promising effect in enhancing the activities of some key enzymes of the antioxidative defense system. This suggests that plant growth regulators including IAA that can regulate the activity/synthesis of key antioxidant enzymes, are involved in the metabolism of plant growth regulators (Synkova *et al.* 2004). The α-tocopherol levels change differentially in response to environmental constraints, depending on the magnitude of the stress and species-sensitivity to stress. Lucas *et al.* (1993) noticed that α-tocopherol concentrations were higher in the Acala cotton cultivars than the salt sensitive cultivars.

MDA is the decomposition product of polyunsaturated fatty acids of membranes under stress. The rate of lipid peroxidation level in terms of MDA can, therefore, be used as an indication to evaluate the tolerance of plants to oxidative stress as well as the sensitivity of plants to salt stress (Jain *et al.* 2001). It is also known that the formation of ROS enhances peroxidation at the cellular level and that the rate of such enhancement relates to plant species and the severity of stress (Navari-Izzo *et al.* 1996). Variation in MDA content was found in rice (Tijen and Ìsmail 2005) and cotton (Diego *et al.* 2003) cultivars differing in salt tolerance. Our research found significantly increased MDA content in both cultivars under salt stress and the results showed that the salt-tolerant FL485 had a lower MDA content than the salt-

sensitive IR29 under salt condition. This indicates that FL485 may be able to adopt improved antioxidant mechanisms under salt stress better than the IR29 cultivar. In addition, the IAA spray was found to be ineffective in reducing the adverse effect of salinity on MDA concentration in both cultivars.

Table 2. Pearson's correlation coefficients among physiological variables from two rice cultivars exposed to foliar spray of IAA and salinity (control or salt stress of 6 dS m^{-1})

	ASO	ASA	H_2O_2	MDA	α-Toc	PNO	SPP	FS	GW	FGP	Yield
ASO											
ASA	0.65**										
H_2O_2	-0.27	-0.41									
MDA	0.09	0.04	0.80**								
α-Toc	0.61**	0.53*	0.06	0.39							
PNO	0.24	0.28	-0.54*	-0.36	-0.09						
SPP	0.46	0.62**	-0.57*	-0.20	0.52*	0.50*					
FSP	0.69**	0.61**	-0.72**	-0.31	0.52*	0.55*	0.89**				
GW	0.61**	0.64**	-0.53*	-0.18	0.79**	0.26	0.76**	0.85**			
FGP	0.76**	0.50*	-0.67**	-0.35	0.33	0.41	0.42	0.78**	0.53**		
YIELD	0.65**	0.61**	-0.73*	-0.40	0.54*	0.57*	0.84**	0.96**	0.88**	0.75**	

∗ and ∗∗Significant at $p \leq 0.05$ and $p \leq 0.01$ probability levels, respectively.
PNO, SPP, FS, GW and FGP denote to panicle number, spikelet per panicle, filled spikelet, 1000 grain weight and filled grain percentage, respectively.

H_2O_2 is generally regarded as a toxic molecule that is produced as an undesirable side-product of various cellular reactions. In the present study, salt stress significantly enhanced H_2O_2, but it was lower in the tolerant genotype than the non-tolerant cultivar. From H_2O_2 values, it is depicted that salt stress disintegrated the membranes to a lesser extent in the tolerant genotype as compared to the sensitive cultivar. Low ratios of H_2O_2 and lipid peroxidation in the tolerant genotype (FL485) are also indication of high membrane stability index. Similarly, Noreen and Ashraf (2009) submitted various pea genotypes to different salinity levels and noted an elevation in H_2O_2 and lipid peroxidation level with the increasing salt stress. We observed strong positive correlation between MDA and H_2O_2 (r= 0.801, $p \leq 0.01$) activity (Table 2). Literature depicts that salt tolerance potential is highly linked with the maximum ratios of antioxidant enzymes (Shahid *et al.* 2011; Balal *et al.* 2012). Recent data have shown, however, that H_2O_2 may play a role in establishing resistance, as it can function as a stress signal for the induction of cellular defense responses (Xiao *et al.* 2009). Exogenously applied IAA is shown to have increased the H_2O_2 content in the leaves of both stressed rice cultivars, which in turn illustrates that altering the H_2O_2-scavenging capacity of the plant is not necessarily beneficial because H_2O_2 may have various cellular functions in plants. This would be in accordance with the observation that the

expression of AsA, AAO and α-tocopherol in the stressed plants receiving external treatment is constitutively maintained at elevated levels. This study illustrates that altering the H_2O_2-scavenging capacity of the plant is not necessarily beneficial, which is not unexpected, since, as outlined above, H_2O_2 may have various cellular functions in plants. If low H_2O_2 level acts as a stress signal in plants, diminishing the capacity to remove H_2O_2 would render the plant more vulnerable to oxidative damage, but at the same time, defense responses that are activated by H_2O_2 may be induced more rapidly.

Conclusions

From the results of this investigation, it can be concluded that the exogenous growth regulator IAA can be used as a potential tool to enhance the non-enzymatic antioxidants activity of rice cultivars. The use of IAA is becoming popular to ensure efficient production. We observed that spraying IAA with 50 µM at the anthesis stage could also increase number of spikelet per panicle, seed setting rate and grain yield in both cultivars of IR29 and FL485. There was more noticeable effect on yield in FL485 than in IR29 with foliar application of IAA.

References

Anjum SA, Wang LC, Farooq M, Hussain M, Xue LL and Zou CM, 2011. Brassinolide application improves the drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange. Journal of Agronomy and Crop Science 197: 177–185.

Ashraf M, 2009. The biotechnological approach of improving plant salt tolerance using antioxidants as markers. Biotechnology Advances 27**:** 84–93.

Ashraf M, Athar HR, Harris PJC and Kwon TR, 2008. Some prospective strategies for improving crop salt tolerance. Advances in Agronomy 97**:** 45–110.

Athar HR, Khan A and Ashraf M, 2008. Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. Environmental and Experimental Botany 63: 224-231.

Athar HR, Khan A and Ashraf M, 2009. Inducing salt tolerance in wheat by exogenously applied ascorbic acid through different modes. Journal of Plant Nutrition 32**:** 1799-1817.

Bafeel SO and Ibrahim MM, 2008. Antioxidants and accumulation of *α*-tocopherol induce chilling tolerance in *Medicago sativa*. International Journal of Agriculture and Biology 10 (6): 593–598.

Balal RM, Khan MM, Shahid MA, Mattson NS, Abbas T, Ashfaq M, Garcia-Sanchez F, Ghazanfer U, Gimeno V and Iqbal Z, 2012. Comparative studies on the physiobiochemical, enzymatic and ionic modifications in salt tolerant and salt sensitive Citrus rootstocks under NaCl stress. Journal of the American Society for Horticultural Science 137: 1-10.

Bari R and Jones JD, 2009. Role of plant hormones in plant defense responses. Plant Molecular Biology 69: 473–488.

Barnes JD, Zheng Y and Lyons TM, 2002. Plant resistance to ozone: the role of ascorbate. In: Omasa K, Saji H, Youssefian S and Kondo N (Eds). Air Pollution and Plant Biotechnology. Pp. 235–254. Springer.

Catignani GL, 1983. Simultaneous determination of retinol and tocopherol in serum or plasma by liquid chromatography. Clinical Chemistry 2914: 708–712.

De Tullio MC, Ciraci S, Liso R and Arrigoni O, 2007. Ascorbic acid oxidase is dynamically regulated by light, wounding, oxygen and hypoxia. A tool for oxygen management? Journal of Plant Physiology 164: 39–46.

De Tullio MC, Liso R and Arrigoni O, 2004. Ascorbic acid oxidase: an enzyme in search of a role. Biological Plant 48: 161–166.

Diego AM, Marco AO, Carlos AM and José C, 2003. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environmental and Experimental of Botany 49**:** 69-76.

Diplock, T., Machlin LJ, Packer L and Pryor WA, 1989. Vitamin E: biochemistry and health implications. Annual New York Academic Science 570: 372–378.

Egamberdieva D, 2009. Alleviation of salt stress by plant growth regulators and IAA- producing bacteria in wheat. Acta Physiologiae Plantarum 31: 861–864.

Falk J. and Munné-Bosch S, 2010. Tocochromanol functions in plants: antioxidation and beyond. Journal of Experimental Botany 61: 1549-1566.

Fukuzawa K, Tokumura A, Ouchi S. and Tsukatani H, 1982. Antioxidant activities of tocopherols on Fe^{2+}-ascorbate induced lipid peroxidation in lecithin liposomes. Lipids 17 (7): 511–514.

Gulnaz A, Iqbal J, Farooq S and Azam F, 1999. Seed treatment with growth regulators and crop productivity. I. 2,4-D as an inducer of salinity-tolerance in wheat (*Triticum aestivum* L.). Plant and Soil 210: 209–217.

Guo J, Liu X, Li X, Chen S, Jin Z and Liu G, 2006. Overexpression of VTE1 from *Arabidopsis* resulting in high vitamin E accumulation and salt stress tolerance increase in the tobacco plant. Chinese Journal of Applied and Environmental Biology 12 (4): 468–471.

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

Hartmann TN, Fricker MD, Rennenberg H and Meyer AJ, 2003. Cell-specific measurement of cytosolic glutathione in poplar leaves. Plant Cell and Environment 26: 965-975.

Iqbal M and Ashraf M, 2007. Seed treatment with auxins modulates growth and ion partitioning in salt-stressed wheat plants. Journal of Integrative Plant Biology 49: 1003–1015.

Jain M, Mathur G, Koul S and Sarin NB, 2001. Ameliorative effects of proline on salt stress-induced lipid peroxidation in cell lines of groundnut (*Arachis hypogea* L.). Plant Cell Reports 20: 463-468.

Kaya C, Kirnak H and Higgs D, 2001. Enhancement of growth and normal growth parameters by foliar application of potassium and phosphorus on tomato cultivars grown at high (NaCl) salinity. Journal of Plant Nutrition 24: 357–367.

Khan MIR, Igbal N, Masood A and Khan NA, 2012. Variation in salt tolerance of wheat cultivars: role of glycine betaine and ethylene. Pedosphere 22: 746–754.

Liso R, De Tullio MC, Ciraci S, Balestrini R, La Rocca N, Bruno L, Chiappetta A, Bitonti MB, Bonfante P and Arrigoni O, 2004. Localization of ascorbic acid, ascorbic acid oxidase and glutathione in roots of *Cucurbita maxima* L. Journal of Experimental and Botany 99: 726–736.

Lucas MC, Gosseti DR, Milihollon EP and Marney MM, 1993. Antioxidant scavengers and salt stress in cotton. Proceedings– Beltwide Cotton Conferences, 10-14 January, New Orleans, Louisiana, USA: 1259-1261.

Moradi F, Ismail AM, Gregorio GB. and Egdane JA, 2003. Salinity tolerance of rice during reproductive development and association with tolerance at the seedling stage. Indian Journal of Plant Physiology 8: 276–287.

Navari-Izzo F, Quatacci MF and Sgherri CLM, 1996. Superoxide generation in relation to dehydration and rehydration. Biochemical Society Transactions 24: 447-450.

Nazar R, Igbal N, Syeed S and Khan NA, 2011. Salicylic acid alleviates decreases in photosynthesis under salt stress by enhancing nitrogen and sulfur assimilation and antioxidant metabolism differentially in two mung bean cultivars. Journal of Plant Physiology 168: 807–815.

Noctor G and Foyer CH, 1998. Ascorbate and glutathione: keeping active oxygen under control. Annual Review of Plant Physiology 49: 249-279.

Pedranzani H, Racagni G, Alemano S, Miersch O, Ram´ırez I, Pe˜na-Cort´es H, Taleisnik E, Machado-Domenech E and Abdala G, 2003. Salt tolerant tomato plants show increased levels of jasmonic acid. Plant Growth Regulation 41**:** 149–158.

Sergiev I, Alexieva V and Karanov E, 1997. Effect of spermine, atrazine and combination between them on some endogenous protective systems and stress markers in plants. Comptes Rendus de l'Academie Bulgare des Sciences 51: 121-124.

Shahid MA, Pervez MA, Balal RM, Mattson NS, Rashid A, Ahmad R, Ayyub CM and Abbas T, 2011. Brassinosteroid (24-epibrassinolide) enhances growth and alleviates the deleterious effects induced by salt stress in pea (*Pisum sativum* L.). Australian Journal of Crop Science 5: 500-510.

Smirnoff N, 1998. Plant resistance to environmental stress. Current Opinion in Plant Biology 9: 214-219.

Smirnoff N, 2005. Ascorbate, tocopherol and carotenoids: metabolism, pathway engineering and functions. In: Smirnoff N (Ed). Antioxidants and Reactive Oxygen Species in Plants. Blackwell Publishing, Oxford, UK.

Stevart RRC and Bewley JD, 1980. Lipid peroxidation associated with accelerated aging of soybean axes. Plant Physiology 66: 245-248.

Synkova H, Semoradova S and Burketova L, 2004. The high content of endogenous cytokinins stimulates the activity of enzymes and proteins involved in stress response in *Nicotiana tabacum*. Plant Cell Tissue and Organ Culture 79: 169–179.

Tijen D and Ìsmail T, 2005. Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. Environmental and Experimental Botany 53: 247-257.

Tirol-Padre A and Ladha JK, 2004. Assessing the reliability of permanganate-oxidizable carbon as an index of soil labile carbon. Soil Science Society of America Journal 68: 969–978.

Tounekti T, Vadel AM, Onate M, Khemira H and Munné-Bosch S, 2011. Salt-induced oxidative stress in rosemary plants: damage or protection? Environmental and Experimental Botany 71: 298-305.

Turan S and Tripathy BC, 2012. Salt and genotype impact on antioxidative enzymes and lipid peroxidation in two rice cultivars during de-etiolation. Protoplasma 250: 209–222.

Xiao-Juan Z, Da-Peng L, Ling-Kun G, De-Quan L, Li-Xia L and Xiao-Li H, 2009. Abscisic acid and hydrogen peroxide induce a novel maize group C MAP kinase gene, *ZmMPK7*, which is responsible for the removal of reactive oxygen species. Planta 229: 485–495.

Zhang J and Kirkham B, 1996. Antioxidant responses to drought in sunflower and sorghum seedlings. New Phytologist 132: 361–373.