



**Full Length Article**

## Seed Invigoration with Water, Ascorbic and Salicylic Acid Stimulates Development and Biochemical Characters of Okra (*Ablemoschus esculentus*) under Normal and Saline Conditions

Syed Hammad Raza<sup>1\*</sup>, Fahad Shafiq<sup>1</sup>, Mahwish Chaudhary<sup>1</sup> and Imran Khan<sup>1</sup>

<sup>1</sup>Environmental Botany Lab, Department of Botany, Govt. College University, Faisalabad, Pakistan

\*For correspondence: hamad\_shah@yahoo.com; shraza@gcuf.edu.pk

### Abstract

Role of seed priming in okra (*Ablemoschus esculentus* L.) in saline soil under field environment was investigated. A split plot design with two main factors including six priming treatments (control, hydropriming, ascorbic acid 50 mg L<sup>-1</sup>, 100 mg L<sup>-1</sup> and salicylic acid 50 mg L<sup>-1</sup>, 75 mg L<sup>-1</sup>) and two stress levels (control and 125 mM NaCl) was implicated. Soil salinity hampered the growth of okra plants causing reduction in yield of un-primed plants. Maximum growth, pigments, and yield of okra was observed with priming concentration of ascorbic acid 100 mg L<sup>-1</sup> under stress and non-stress conditions. However, lower concentration of SA priming (50 mg L<sup>-1</sup>) had more prominent effect on growth, chlorophyll contents as compared to its higher dose (75 mg L<sup>-1</sup>). Seed soaking with ascorbic acid (100 mg L<sup>-1</sup>) and salicylic acid (75 mg L<sup>-1</sup>) enhanced antioxidative activities of CAT and POD in saline environment. Hydropriming was quite effective in improving growth, pigments and yield. Furthermore, optimization of time duration for water seed soaking can be done to draw out its role in modulating growth, physiological attributes and yield under normal and saline conditions. © 2013 Friends Science Publishers

**Keywords:** Okra; Priming; Salinity; CAT; POD; Yield

### Introduction

In the arid and semi arid regions of the globe, salinity is one of the major stresses to crops as high salt concentrations in the growing medium are the serious restrictive factors for plant development (Majeed *et al.*, 2010). Interferences with plant physiological functions (Yamaguchi and Blumwald, 2005), induction of nutritional disturbances, osmotic stress, ionic disturbances and generation of oxidative stress are the common physiological changes triggered by salinity. Osmotic stress results from excessive salt concentrations in the rhizosphere, lowering water availability (Lloyd *et al.*, 1989) to plants thus hampering root growth, foliage characters (Munns, 2002) and cellular metabolism (Murphy and Durako, 2003). Similarly enzymatic disturbances are very much obvious upsetting photosynthetic pigments (Munns, 2002). The most detrimental outcome is the enhanced reactive oxygen species (ROS) production rate (Ashraf, 2009) that ultimately disrupts chloroplast and mitochondria (Apel and Hirt, 2004).

Plants detoxify excessive ROS by turning on defensive mechanisms (Ashraf, 2009) consisting of enzymatic (Harinasut *et al.*, 2000) and non-enzymatic antioxidant system (Piotr and Klobus, 2005). Antioxidative defence system involves SOD, CAT, POD and APX (Foyer and Noctor, 2003) that work as ROS scavengers (Jaleel *et al.*, 2009; Farooq *et al.*, 2009) and neutralizers in order to

combat oxidative stress, thereby reduce oxidative damage to plants. However, it is reported that antioxidant activity is also vulnerable to noxious effect of salt stress (Shalata and Neumann, 2001). Recent reports have explored various antioxidant compounds including plant growth regulators being effective in minimising of salt stress toxicity in terms of plant growth and metabolism (Shalata and Neumann, 2001; Hasanuzzaman *et al.*, 2013). Likewise, Raza *et al.* (2006) reported that the exogenous use of nutrients recover crop performance under salinity. Similarly application of certain chemicals like GB, SA and kinetin counteract the stress-induced suppression of plant growth and improve yield in many crops (Gunes *et al.*, 2007; Elwana and El-Hamahmyb, 2009).

In recent years, seed priming is regarded as an effective technique that can be applied for improvement of growth and yield of crops. It is reported that variety of chemicals, plant hormones and antioxidants are used as priming agent to ensure maximum germination and crop establishment (Lee *et al.*, 2002). Salt tolerance in different crops seedlings is induced from soaking seeds in salicylic acid and ascorbic acid solution (Tari *et al.*, 2002). Similarly, exogenous application of salicylic acid (SA) can bring plant tolerance to abiotic stresses like salinity and drought (Stevens *et al.*, 2006). It is documented that SA mediates regulation of different aspects of plant life such as growth, photosynthesis and chloroplast structure (Sakhabutdinova *et*

*al.*, 2003). Moreover, it can regulate the antioxidant chemistry (Shi and Zhu 2008), physiological drought (Amin *et al.*, 2009), improves germination and seedling growth (Farooq *et al.*, 2006). Ascorbic acid (AsA) is the member of plant non-enzymatic antioxidant defence team that plays multiple roles in plant growth such as cell wall spreading, cell division and other plant advancement processes (Pignocchi and Foyer, 2003). Pretreatment of AsA improved vitality and emergence rate of germinating seeds (Farooq *et al.*, 2006).

Okra (*Ablemoschus esculentus* L.) is a nutritionally important summer vegetable and is a member of malvaceae family. Being an excellent source of potassium, calcium and unsaturated fatty acids for instance, linolenic and oleic acid (Savello *et al.*, 1980; Martin, 1982), it is quite essential for human nutrition. As low yields of okra are often associated with soil salinization, the focus of this study was to improve yield under naturally saline fields. So it was hypothesized that seed priming with organic acids will improve yield in okra by inducing salt tolerance. For this purpose salicylic acid and ascorbic acid (AsA) were used as priming agents in pursue to improve okra growth and physiology and to draw out their role in improving yield attributes keeping in view their role of plant defence system under saline environment.

## Materials and Methods

### Experimental Methodology

Seeds for the experiment were obtained from Vegetable Research Institute, Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. Field experiment was conducted in Darapur village near Faisalabad. The changes in morpho-physiological attributes by seed priming under stress were investigated at Environmental Botany Laboratory, Department of Botany, GC University, Faisalabad. Experimental field was prepared by dividing the main plot into two sub plots, with an area of 8.1 m<sup>2</sup> of each. Soil of sub plots were dug up to 60 cm depth and a polythene sheet was laid down beneath to isolate them. The sub plots were filled with soil (EC<sub>e</sub> 2.38 and pH 6.0). Split-split plot statistical design was used with each experimental unit containing three replications. Surface sterilized seeds (with 0.1% HgCl<sub>2</sub>) of okra were primed with different concentrations of ascorbic acid and salicylic acid (Control, AsA 50 mg L<sup>-1</sup>, AsA 100 mg L<sup>-1</sup>, SA 50 mg L<sup>-1</sup>, SA 75 mg L<sup>-1</sup>) for 10 h period before sowing. Two week after germination salinity was imposed using sodium chloride (Control 2.38 dS/m and NaCl 12.5 dS/m).

### Growth and Biochemical Analysis

The plants were harvested after two weeks of salinity treatment and their growth characters were recorded. Chlorophyll contents were determined as described by Arnon (1949), while total soluble proteins were quantified by the method of Bradford (1976).

For antioxidant enzyme activity fresh leaves 0.5 g were grounded in 5 mL of ice cold phosphate buffer (50 mM, pH 7.8). The homogenate was centrifuged at 15000 g for 20 min at 4°C and the supernatant was used for assay of enzyme activities. CAT activity was measured at 240 nm as described by Chandlee and Scandalios (1984) with slight modifications. Fernandez-Garcia *et al.* (2004) method was used to determine POD activity by measuring absorbance at 470 nm. Activities of these enzymes were expressed in units mg<sup>-1</sup> protein (U = 1 mM of H<sub>2</sub>O<sub>2</sub> reduction min<sup>-1</sup> mg<sup>-1</sup> protein).

### Yield Analysis

Yield attributes like number of pods per plant, pod weight, pod length and pod girth were recorded at maturity stage.

### Statistical Analysis

Data recorded for different attributes was analyzed using CoStat software. The statistical significance was assessed by Duncan's Multiple Range (DMR) test ( $P \leq 0.05$ ).

## Results

### Growth Attributes

Salt stress hampered the growth of okra plants as indicated by the plants morphological characters. Reduction ( $P \leq 0.05$ ) was visualized in the shoot length, shoot fresh weight (25%) and dry weight (22.1%) due to the presence of high salt concentrations in the soil. Simultaneous retardation in root growth characters by salinity (41.2%, 38.35% and 47.1%) in root length, root fresh weight and root dry weight was recorded. Priming of okra seeds with water, salicylic acid and ascorbic acid was found beneficial in improving plant growth attributes in both control and saline conditions (Table 1). Briefly, ascorbic acid priming at 100 mg L<sup>-1</sup> was found most effective in improving growth of okra plants in control and saline conditions. Increase in shoot fresh weight (31.2%), dry weight (28.5%) and an increment of 14.5% was observed in the case of shoot length by higher priming concentration of AsA. Root fresh and its dry weight was ( $P \leq 0.05$ ) improved in AsA priming (100 mg L<sup>-1</sup>), while root length enhancement (14.1%) was maximum in AsA (50 mg L<sup>-1</sup>) in control plants under normal soil conditions. In salt treated level, AsA priming (100 mg L<sup>-1</sup>) resulted in significant improvements in the growth attributes of okra plants as shoot length, fresh and dry weights of shoot were improved by 35, 39.8 and 21.7%, respectively. Root growth was also improved with AsA application at 100 mg L<sup>-1</sup>. Significantly ( $P \leq 0.05$ ) greater number of leaves (23.8%) and total plant leaf area (51.4%) was recorded in control plants. However, salt stress grown plants showed 12.1% more leaf area ratio (LAR) than control plants. AsA (100 mg L<sup>-1</sup>) primed seeds had more number of leaves and total plant leaf area ( $P \leq 0.05$ ) in comparison with non-primed seeds (control) seeds. Maximum improvement in salt stress

for these attributes was produced by AsA (100 mg L<sup>-1</sup>), while SA was least effective.

### Pigment Analysis

Photosynthetic pigment contents deviated significantly ( $P \leq 0.05$ ) between control and salinity level as control plants had 47.9% more chlorophyll *a* while 43.2% elevated chlorophyll *b*, 45.8% total chlorophyll content and 28.5% carotenoids values in normal soil conditions. The priming treatments used for this experiment increased the pigment entities of okra plants in salt stress (Table 2). Seed soaking with ascorbic acid (100 mg L<sup>-1</sup>) resulted 40.8 and 62.7% increment in chlorophyll *a*, 32.7 and 53.3% in chlorophyll *b*, 37.5 and 58.6% for total chlorophyll, while 21.5% and

30.5% of carotenoids values for control and salinity levels respectively. Chl *a/b* ratio was maximum due to SA priming (50 mg L<sup>-1</sup>) in control plants.

### Biochemical Attributes

**Total soluble protein:** The amount of total soluble protein in stressed plants was significantly decreased (22.4%) and higher value was noted in control. In this regard, SA primed (50 mg L<sup>-1</sup>) plants showed maximum values for both the levels (Fig. 1a). In contrast minimum values of total protein were associated with AsA priming for control and salt imposed level. Total soluble proteins were increased more in hydroprimed seeds than AsA seed soaking ( $P \leq 0.01$ ).

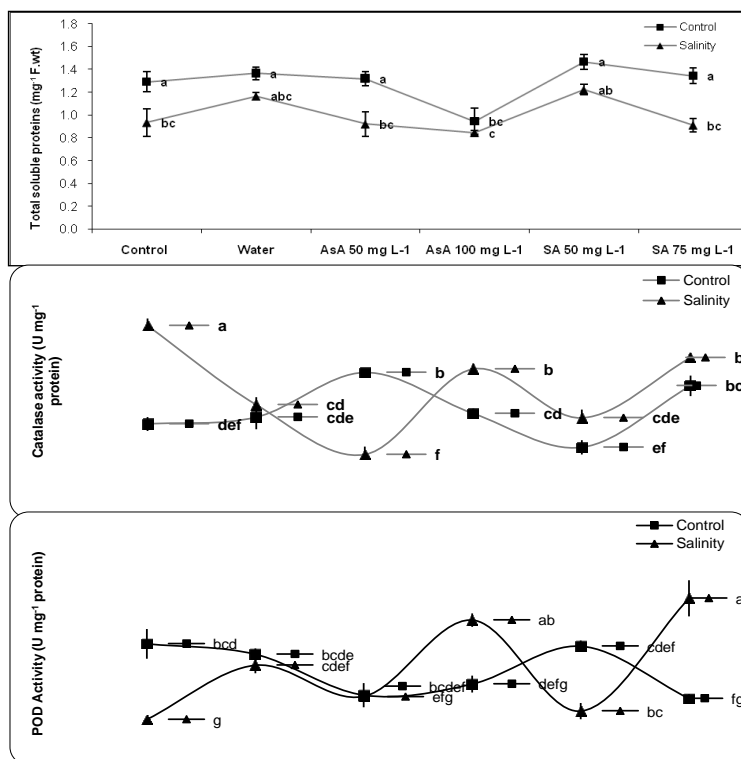
**Table 1:** F values from ANOVA and means from data for growth attributes of okra (*Ablemoschus esculentus*) as influenced by priming with varying levels of salicylic and ascorbic acid grown in salt stressed condition

Priming treatments	Shoot Length (cm)	Root Length (cm)	Fresh Weight (g)		Dry Weight (g)		No. of Leaves	Total Plant Leaf Area (m <sup>2</sup> )	LAR (cm <sup>2</sup> g <sup>-1</sup> )	Stem Diameter (cm)
			Shoot	Root	Shoot	Root				
<b>Control</b>										
Dry seeds	32.00 <sup>cd</sup> ±1.20	13.03 <sup>a</sup> ±0.50	22.33 <sup>d</sup> ±1.19	3.83 <sup>cd</sup> ±0.02	3.05 <sup>bcd</sup> ±0.03	0.35 <sup>d</sup> ±0.00	10.33 <sup>cd</sup> ±0.33	0.108 <sup>e</sup> ±0.002	107.54 <sup>d</sup> ±0.78	1.007 <sup>b</sup> ±0.015
Hydro priming	34.43 <sup>bc</sup> ±0.62	12.93 <sup>a</sup> ±1.89	28.90 <sup>b</sup> ±0.46	4.94 <sup>b</sup> ±0.03	3.67 <sup>ab</sup> ±0.13	0.41 <sup>b</sup> ±0.00	12.00 <sup>b</sup> ±0.57	0.179 <sup>bc</sup> ±0.002	137.40 <sup>bc</sup> ±1.18	1.140 <sup>a</sup> ±0.006
AsA 50 mg L <sup>-1</sup>	35.90 <sup>ab</sup> ±0.37	15.17 <sup>a</sup> ±0.49	26.20 <sup>c</sup> ±0.32	4.08 <sup>c</sup> ±0.03	3.65 <sup>ab</sup> ±0.07	0.39 <sup>bc</sup> ±0.00	12.33 <sup>ab</sup> ±0.33	0.184 <sup>b</sup> ±0.006	136.74 <sup>c</sup> ±0.82	1.137 <sup>a</sup> ±0.012
AsA 100 mg L <sup>-1</sup>	37.47 <sup>a</sup> ±0.27	14.43 <sup>a</sup> ±1.04	32.47 <sup>a</sup> ±1.08	5.20 <sup>a</sup> ±0.04	3.85 <sup>a</sup> ±0.08	0.49 <sup>a</sup> ±0.01	13.33 <sup>a</sup> ±0.33	0.220 <sup>a</sup> ±0.009	142.99 <sup>abc</sup> ±3.24	1.167 <sup>a</sup> ±0.038
SA 50 mg L <sup>-1</sup>	36.80 <sup>ab</sup> ±0.68	14.67 <sup>a</sup> ±0.14	29.80 <sup>b</sup> ±0.84	4.89 <sup>b</sup> ±0.01	3.56 <sup>abc</sup> ±0.24	0.40 <sup>bc</sup> ±0.00	11.33 <sup>bc</sup> ±0.33	0.166 <sup>c</sup> ±0.002	135.92 <sup>c</sup> ±0.31	1.143 <sup>a</sup> ±0.015
SA 100 mg L <sup>-1</sup>	27.43 <sup>e</sup> ±0.40	12.50 <sup>a</sup> ±1.03	26.00 <sup>c</sup> ±0.96	3.93 <sup>cd</sup> ±0.02	3.36 <sup>abcd</sup> ±0.14	0.39 <sup>c</sup> ±0.01	10.33 <sup>cd</sup> ±0.33	0.133 <sup>d</sup> ±0.007	118.71 <sup>d</sup> ±4.26	1.120 <sup>a</sup> ±0.042
<b>Salinity 125 mM</b>										
Dry seeds	20.53 <sup>e</sup> ±1.20	5.67 <sup>c</sup> ±0.23	15.20 <sup>e</sup> ±0.85	1.64 <sup>h</sup> ±0.24	2.37 <sup>e</sup> ±0.10	0.14 <sup>i</sup> ±0.00	7.33 <sup>f</sup> ±0.33	0.069 <sup>h</sup> ±0.006	158.51 <sup>a</sup> ±14.79	0.677 <sup>d</sup> ±0.030
Hydro priming	27.83 <sup>c</sup> ±0.36	8.40 <sup>bc</sup> ±0.24	21.60 <sup>d</sup> ±0.56	3.00 <sup>e</sup> ±0.06	2.92 <sup>cd</sup> ±0.24	0.24 <sup>f</sup> ±0.01	9.33 <sup>de</sup> ±0.33	0.083 <sup>gh</sup> ±0.002	142.59 <sup>bc</sup> ±0.29	0.960 <sup>b</sup> ±0.036
AsA 50 mg L <sup>-1</sup>	29.57 <sup>de</sup> ±0.37	8.90 <sup>b</sup> ±0.12	22.57 <sup>d</sup> ±0.45	2.91 <sup>ef</sup> ±0.07	2.83 <sup>de</sup> ±0.23	0.22 <sup>g</sup> ±0.04	9.33 <sup>de</sup> ±0.66	0.091 <sup>fg</sup> ±0.003	153.00 <sup>ab</sup> ±0.507	0.917 <sup>b</sup> ±0.038
AsA 100 mg L <sup>-1</sup>	31.63 <sup>d</sup> ±0.58	9.50 <sup>b</sup> ±0.17	25.27 <sup>c</sup> ±0.23	3.69 <sup>d</sup> ±0.04	3.03 <sup>bcd</sup> ±0.29	0.32 <sup>e</sup> ±0.01	10.00 <sup>d</sup> ±0.57	0.092 <sup>f</sup> ±0.005	149.03 <sup>abc</sup> ±4.912	0.990 <sup>a</sup> ±0.040
SA 50 mg L <sup>-1</sup>	24.17 <sup>f</sup> ±0.50	8.30 <sup>bc</sup> ±0.14	21.30 <sup>d</sup> ±0.56	2.73 <sup>fg</sup> ±0.11	2.69 <sup>de</sup> ±0.12	0.17 <sup>h</sup> ±0.02	8.66 <sup>e</sup> ±0.33	0.076 <sup>gh</sup> ±0.002	140.08 <sup>bc</sup> ±1.20	0.913 <sup>b</sup> ±0.037
SA 100 mg L <sup>-1</sup>	22.23 <sup>fg</sup> ±0.89	7.33 <sup>bc</sup> ±0.19	17.17 <sup>e</sup> ±0.61	2.51 <sup>g</sup> ±0.08	2.55 <sup>e</sup> ±0.10	0.20 <sup>g</sup> ±0.00	8.33 <sup>ef</sup> ±0.33	0.069 <sup>h</sup> ±0.006	143.57 <sup>abc</sup> ±2.291	0.783 <sup>c</sup> ±0.032
<b>Significance of variance sources</b>										
Stress levels	135.79**	140.32**	302.29**	885.57**	121.05**	2155.95***	192.30**	2776.76***	32.73*	579.71**
Priming	45.94***	3.04*	41.30***	108.07***	3.23*	18.20***	13.62***	43.72***	3.53*	13.63***
Prim × Stress	8.28***	0.70 <sup>NS</sup>	2.88*	13.47***	0.05 <sup>NS</sup>	0.84 <sup>NS</sup>	0.65 <sup>NS</sup>	16.44***	7.90***	2.30 <sup>NS</sup>

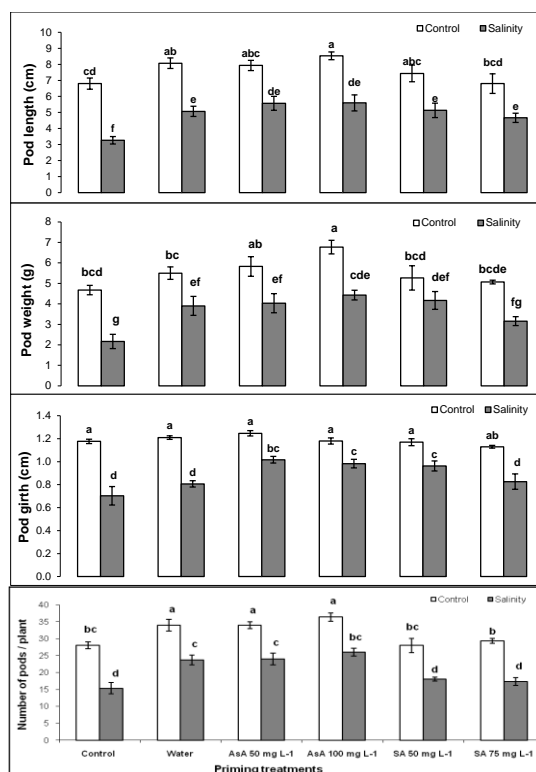
**Table 2:** F values from ANOVA and means from data for photosynthetic pigments in leaves of okra (*A. esculentus*) as influenced by priming with varying levels of salicylic and ascorbic acid grown in salt stress

Priming treatments	Chl <i>a</i> (mg/g FW)	Chl <i>b</i> (mg/g FW)	Car (mg/g FW)	Chl <i>a+b</i> (mg/g FW)	Chl <i>a/b</i> Ratio	Car/Chl Ratio
<b>Control</b>						
Dry seeds	2.008 <sup>d</sup> ± 0.026	1.609 <sup>d</sup> ± 0.063	0.135 <sup>d</sup> ± 0.010	3.617 <sup>de</sup> ± 0.065	1.252 <sup>a</sup> ± 0.052	0.037 <sup>d</sup> ± 0.002
Hydro priming	2.997 <sup>bc</sup> ± 0.20	2.121 <sup>ab</sup> ± 0.091	0.143 <sup>c</sup> ± 0.001	5.098 <sup>ab</sup> ± 0.122	1.417 <sup>a</sup> ± 0.158	0.028 <sup>e</sup> ± 0.000
AsA 50 mg L <sup>-1</sup>	2.777 <sup>c</sup> ± 0.167	1.987 <sup>bc</sup> ± 0.051	0.159 <sup>b</sup> ± 0.004	4.764 <sup>b</sup> ± 0.217	1.395 <sup>a</sup> ± 0.050	0.033 <sup>d</sup> ± 0.002
AsA 100 mg L <sup>-1</sup>	3.401 <sup>ab</sup> ± 0.40	2.394 <sup>a</sup> ± 0.072	0.172 <sup>a</sup> ± 0.005	5.795 <sup>a</sup> ± 0.465	1.415 <sup>a</sup> ± 0.135	0.030 <sup>d</sup> ± 0.002
SA 50 mg L <sup>-1</sup>	2.610 <sup>a</sup> ± 0.179	1.772 <sup>bcd</sup> ± 0.20	0.126 <sup>e</sup> ± 0.004	4.382 <sup>bc</sup> ± 0.378	1.487 <sup>a</sup> ± 0.061	0.029 <sup>e</sup> ± 0.003
SA 100 mg L <sup>-1</sup>	2.168 <sup>d</sup> ± 0.023	1.695 <sup>cd</sup> ± 0.043	0.120 <sup>f</sup> ± 0.001	3.863 <sup>cd</sup> ± 0.065	1.280 <sup>a</sup> ± 0.019	0.031 <sup>d</sup> ± 0.000
<b>Salinity 125 mM</b>						
Dry seeds	0.721 <sup>e</sup> ± 0.148	0.696 <sup>f</sup> ± 0.066	0.084 <sup>h</sup> ± 0.003	1.417 <sup>h</sup> ± 0.142	1.066 <sup>a</sup> ± 0.274	0.060 <sup>a</sup> ± 0.004
Hydro priming	1.819 <sup>de</sup> ± 0.145	1.143 <sup>e</sup> ± 0.210	0.119 <sup>f</sup> ± 0.002	2.962 <sup>ef</sup> ± 0.350	1.661 <sup>a</sup> ± 0.189	0.041 <sup>c</sup> ± 0.005
AsA 50 mg L <sup>-1</sup>	1.545 <sup>def</sup> ± 0.281	1.102 <sup>e</sup> ± 0.235	0.117 <sup>f</sup> ± 0.006	2.647 <sup>fg</sup> ± 0.210	1.028 <sup>a</sup> ± 0.114	0.044 <sup>c</sup> ± 0.001
AsA 100 mg L <sup>-1</sup>	1.935 <sup>de</sup> ± 0.048	1.491 <sup>d</sup> ± 0.018	0.121 <sup>e</sup> ± 0.009	3.426 <sup>de</sup> ± 0.065	1.297 <sup>a</sup> ± 0.020	0.035 <sup>d</sup> ± 0.003
SA 50 mg L <sup>-1</sup>	1.316 <sup>efg</sup> ± 0.28	1.067 <sup>e</sup> ± 0.022	0.101 <sup>g</sup> ± 0.002	2.383 <sup>fg</sup> ± 0.309	1.225 <sup>a</sup> ± 0.243	0.043 <sup>c</sup> ± 0.005
SA 100 mg L <sup>-1</sup>	0.992 <sup>fg</sup> ± 0.112	1.090 <sup>e</sup> ± 0.040	0.106 <sup>g</sup> ± 0.002	2.082 <sup>gh</sup> ± 0.147	0.907 <sup>a</sup> ± 0.077	0.051 <sup>b</sup> ± 0.003
<b>Significance of variance sources</b>						
Stress levels	133.24**	1038.89***	1087.11***	594.31**	0.211 <sup>NS</sup>	116.74**
Priming	11.98***	8.865***	16.73***	17.53***	1.096 <sup>NS</sup>	7.249***
Prim × Stress	0.154 <sup>NS</sup>	0.647 <sup>NS</sup>	4.304**	0.305 <sup>NS</sup>	0.592 <sup>NS</sup>	2.193 <sup>NS</sup>

NS non- significant, \* significant, \*\*\* highly significant



**Fig. 1:** Influence of varying levels of salicylic and ascorbic acid priming on a) total protein b) CAT activity c) POD activity of okra (*A. esculentus*) grown in salt stress.



**Fig. 2:** Influence of varying levels of salicylic and ascorbic acid priming on a) pod length b) pod weight c) pod girth d) number of pods per plant of okra (*A. esculentus*) grown in salt stress

### Antioxidant Enzymes Activities

Catalase activity in stress plants were 17.9% more than control plants. Salicylic acid ( $75 \text{ mg L}^{-1}$ ) primed okra plants under salinity had maximum activity; however AsA ( $50 \text{ mg L}^{-1}$ ) resulted in highest catalase activities (Fig. 1b) in normal soil condition. A significant shift in the activity of peroxidase (POD) in salt stressed plants was observed as the values were declined by 59.8% with respect to control (Fig. 1c). Improved peroxidase activities were recorded as a result of salicylic acid ( $75 \text{ mg L}^{-1}$ ) and AsA ( $100 \text{ mg L}^{-1}$ ) priming however, the effect of SA priming ( $75 \text{ mg L}^{-1}$ ) was much more prominent (75.9%) under salt stress. Hydropriming improved POD activity of okra plants greater than AsA  $50 \text{ mg L}^{-1}$ .

### Yield Attributes

Salt stress had negative impact on yield attributes like pod length, pod weight, pod girth and number of pods showing a reduction of 35.7, 33.9, 25.4 and 34.4%, respectively (Fig. 2). Seed priming had positive effects on these attributes of okra and a clear impact was observed with AsA ( $100 \text{ mg L}^{-1}$ ) as it increased all the yield attributes in both stress levels. Similarly, marked ( $P \leq 0.05$ ) increase in the pod length, weight, and numbers (41.7, 51 and 41% respectively) was associated with AsA primed plants ( $100 \text{ mg L}^{-1}$ ) under stress.

### Discussion

In the present investigation, salinity hampered growth and productivity of okra plants. Retarded growth rate is common under stress (Raza et al., 2007; Shahid et al., 2011; Ikram-ul-Haq et al., 2012) and can be attributed to disturbed nutrient uptake, plant water relations including decreased water availability causing lower water potential and ionic imbalance (Lloyd et al., 1989; Munns, 1993; Kao et al., 2003; Hayat et al., 2010) in salty soil. At cellular level, dehydration due to presence of salts in leaf apoplasm (Marschner, 1995), enhanced membrane permeability (Gupta et al., 2002) and membrane instability due to replacement of  $\text{Ca}^{2+}$  and  $\text{K}^{+}$  by  $\text{Na}^{+}$  (Grattan and Grieve, 1992) are intensively reported physiological effects of salt stress. Similarly, increased susceptibility of the chloroplast membrane is also observed under salt stress and is thereby associated with chloroplast content leakage. This overall salinity mediated disturbed plant physiology contributes to inhibited biomass production. The decrease in growth rates of okra in this study can be a result of a combination of above mentioned salinity mediated physiological dysfunction of okra plants.

Soaking of okra seed with SA and AsA significantly improved growth and yield characters, both in control and stress conditions. Remarkable improvement in biomass production and photosynthetic pigments were associated with high AsA ( $100 \text{ mg/L}$ ) and low SA ( $50 \text{ mg/L}$ )

treatments in the present study. Seed priming with  $100 \text{ mg L}^{-1}$  concentration was the best treatment that improved growth as reported before in *Secale montanum* under cold stress (Smirnoff, 1996; Ansari and Zadeh, 2012). In the present study ascorbic acid at  $100 \text{ mg L}^{-1}$  concentration was found to be the most impressive soaking treatment for both control and salinity grown plants. Activities of antioxidative enzymes (CAT and POD) were promoted by higher concentrations of both acidic compounds used in this experiment. The possible explanation for this positive effect mediated by ascorbic acid is its role as primary substrate in many reactive oxygen scavenging pathways (Khan et al., 2011). Beneficial effects of SA can be linked to improvement in nutrient uptake (Glass, 1974) and abiotic stress resistance through the enhancement in antioxidative potential as (Janda et al., 1999; Alvarez 2000) in *Brassica juncea* under salt stress (Yusuf et al., 2008).

Lower concentration of SA ( $50 \text{ mg L}^{-1}$ ) seed soaking imparted positive effect on growth and chlorophyll contents while higher concentration ( $100 \text{ mg L}^{-1}$ ) had a negative role on growth characters. The inhibitive effects of salicylic acid at higher concentration can be attributed to its role as anti-transpirant for the stomatal closure (Larque-Saavedra, 1978) and eventually its potentiality to suppress growth and yield under stress (El-Bahay, 2002; Liu et al., 2011).

Hydropriming of okra seeds impressively improved growth, pigments and yield characters under normal and stress environment in the present work. It is simpler than osmo-priming for rehydrating seeds (McDonald, 1999; 2000), that is beneficial under control and stress conditions due to initiation of early metabolic process and provides less mechanical restriction to seed emergence by softening the seed coat (McDonald, 2000; Dezfuli et al., 2008) It also improved antioxidant activity of POD under salt stress as observed in maize, rice, wheat and sunflower (Afzal et al., 2002; 2006a, b; Wahid et al., 2008; Farooq et al., 2010). A recent proteomic approach revealed the expression of a catalase isoform (Type 11 protein) whose abundance was increased by hydropriming treatment and this increase was continued through the passage of early developmental stages (Gallardo et al., 2001). Improvement in growth, pigments and yield characters of hydroprimed seeds in control and stress conditions in the present study can be attributed to hydration and imbibition. These contributed to improve germination and faster initial stages bypass without interfering normal seed metabolic stages and activities along with above mentioned benefits associated with water soaking.

In conclusion, higher level of AsA ( $100 \text{ mg L}^{-1}$ ) was found to be very impressive in both control and stress conditions. In future higher concentrations of AsA can be used as pretreatments to observe their effect on okra as well as on other crops under salinity. In contrast, SA was beneficial at  $50 \text{ mg L}^{-1}$  in normal and stressed conditions. Moreover, hydropriming mediated improvements in growth of okra were quite promising under control and salinity. In

future, hydropriming time duration can be optimized for okra to draw out its role under control and stress conditions.

## References

- Afzal, I., S.M.A. Basra, N. Ahmad, M.A. Cheema, E.A., Warraich and A. Khaliq, 2002. Effect of priming and growth regulator treatment on emergence and seedling growth of hybrid maize (*Zea mays*). *Int. J. Agric. Biol.*, 4: 303–306
- Alvarez, M.E., 2000. Salicylic acid and the machinery of hypersensitive cell death in disease resistance. *Plant Mol. Biol.*, 44: 429–442
- Amin, B., G. Mahlegah, H.M.R. Mahmood and M. Hossein, 2009. Evaluation of interaction effect of drought stress with ascorbate and salicylic acid on some of physiological and biochemical parameters in okra (*Hibiscus esculentus* L.). *Res. J. Biol. Sci.*, 4: 380–387
- Ansari, O. and F.S. Zadeh, 2011. Osmo and hydro priming mediated germination improvement under cold stress conditions in mountain rye (*Secale montanum*). *Cercetări Agron. Moldova*, 3: 53–62
- Apel, K. and H. Hirt, 2004. Reactive oxygen species: Metabolism, oxidative stress and signal transduction. *Annu. Rev. Plant Biol.*, 55: 373–399
- Arnon, D.T., 1949. Copper enzymes in isolated chloroplast polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.*, 24: 1–15
- Ashraf, M., 2009. Biotechnological approach of improving plant salt tolerance using antioxidants markers. *Biotechnol. Adv.*, 27: 84–97
- Basra, S.M.A., M. Farooq, A. Wahid and M.B. Khan, 2006a. Rice seed invigoration by hormonal and vitamin priming. *Seed Sci. Technol.*, 34: 753–758
- Basra, S.M.A., M. Farooq, R. Tabassum and N. Ahmed, 2006b. Evaluation of seed vigour enhancement techniques on physiological and biochemical basis in coarse rice (*Oryza sativa* L.). *Seed Sci. Technol.*, 34: 719–728
- Bradford, M.M., 1976. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72: 248–254
- Chandlee, J.M. and J.G. Scandalios, 1984. Analysis of variance affecting the catalase development programme in maize scutellum. *Theor. Appl. Genet.*, 69: 71–77
- Dezfuli, P.M., F. Sharif-Zadeh and M. Janmohammadi, 2008. Influence of priming techniques on seed germination behavior of maize inbred lines (*Zea mays* L.). *J. Agric. Biol. Sci.*, 3: 22–25
- El-Bahay, M.M., 2002. Metabolic changes, phytohormonal level and activities of certain related enzymes associated with growth of pre-soaked lupine seeds in salicylic acid and gallic acid. *Bull. Fac. Sci., Assiut Univ.*, 31: 259–270
- Elwana, M.W.M. and M.A.M. El-Hamamy, 2009. Improved productivity and quality associated with salicylic acid application in greenhouse pepper. *Sci. Hortic.*, 122: 521–526
- Farooq, M., S.M.A. Basra and K. Hafeez, K., 2006. Rice seed invigoration by osmo-hardening. *Seed Sci. Technol.*, 34: 181–187
- Farooq, M., A. Wahid, S.M.A. Basra and Islam-ud-Din, 2009. Improving water relations and gas exchange with brassinosteroids in rice under drought stress. *J. Agron. Crop. Sci.*, 195: 262–269
- Farooq, M., A. Wahid, N. Ahmad and S.A. Asad, 2010. Comparative efficacy of surface drying and re-drying seed priming in rice: changes in emergence, seedling growth and associated metabolic events. *Paddy Water Environ.*, 8: 15–22
- Fernandez-Garcia, N., M. Carvajal and E. Olmos, 2004. Graft union formation in tomato plants: peroxidase and catalase involvement. *Ann. Bot.*, 93: 53–60
- Foyer, C.H. and G. Noctor, 2003. Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiol. Plant.*, 119: 355–364
- Gallardo, K., C. Job, S.P.C. Groot, M. Puype, H. Demol, J. Vandekerckhove and D. Job, 2001. Proteomic analysis of arabidopsis seed germination and priming. *Plant Physiol.*, 126: 835–848
- Glass, A.D.M., 1974. Influence of phenolic acid on ion uptake. III. Inhibition of potassium absorption. *J. Exp. Bot.*, 25: 1104–1113
- Grattan, S.R. and C.M. Grieve, 1992. Mineral element acquisition and growth response of plant growth in saline environments. *Agric. Ecosys. Environ.*, 38: 275–300
- Gunes, A., A. Inal, M. Alpaslan, N. Cicek, F. Erslan and T. Guzelordu, 2005. Effect of exogenously applied salicylic acid on the induction of multiple stress tolerance and mineral nutrition in maize (*Zea mays* L.). *Arch. Agron. Soil Sci.*, 51: 687–695
- Gupta, N.K., S.K. Meena, S. Gupta and S.K. Khandelwal, 2002. Gas exchange, membrane permeability, and ion uptake in two species of Indian jujube differing in salt tolerance. *Photosynthetica*, 40: 535–539
- Harinasut, P., S. Srisunak, S. Pitukchaisopol and R. Charoensataporn, 2000. Mechanism of adaptation to increasing salinity of mulberry: Proline content and ascorbate peroxidase activity in leaves of multiplier shoots. *Sci. Asia*, 26: 207–211
- Hayat, Q., S. Hayat, M. Irfan and A. Ahmad, 2010. Effect of exogenous salicylic acid under changing environment: A review. *Environ. Exp. Bot.*, 68: 14–25
- Hasanuzzaman, M., K. Nahar and M. Fujita, 2013. Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. In: *Ecophysiology and Responses of Plants under Salt Stress*, pp: 25–87. Ahmad, P., M.M. Azooz and M.N.V. Prasad (eds.). Springer, New York, USA
- Ikram-ul-Haq, A.A. Khan, I.A. Khan and M.A. Azmat, 2012. Comprehensive screening and selection of okra (*Abelmoschus esculentus*) germplasm for salinity tolerance at the seedling stage and during plant ontogeny. *J. Zhejiang Univ.-Sci. B*, 13: 533–544
- Jaleel, C.A., P. Manivannan, A. Wahid, M. Farooq, H.J. Al-Juburi, R. Somasundaram and R.P. Vam, 2009. Drought stress in plants a review on morphological characteristics and pigments composition. *Int. J. Agric. Biol.*, 11: 100–105
- Janda, T., G. Szalai, I. Tari and E. Paldi, 1999. Hydroponic treatment with salicylic acid decreases the effects of chilling injury in maize (*Zea mays* L.) plants. *Planta*, 208: 175–180
- Kao, W.Y., T. Tsai and C.N. Shih, 2003. Photosynthetic gas exchange and chlorophyll *a* fluorescence of three wild soybean species in response to NaCl treatments. *Photosynthetica*, 41: 415–419
- Khan, T.A., M. Mazid and F. Mohammad, 2011. Ascorbic acid: An enigmatic molecule to developmental and environmental stress in plant. *Int. J. Appl. Biol. Pharm. Technol.*, 2: 468–483
- Larque-Saavedra, A., 1978. The antitranspirant effect of acetylsalicylic acid on *Phaseolus vulgaris*. *Physiol. Plant.*, 43: 126–128
- Lee, S.Y., J.H. Lee and T.O. Kwon, 2002. Varietal differences in seed germination and seedling vigor of Korean rice varieties following dry heat treatments. *Seed Sci. Technol.*, 30: 311–321
- Liu, C., J. Guo, Y. Cui, T. Lü, X. Zhang and G. Shi, 2011. Effects of cadmium and salicylic acid on growth, spectral reflectance and photosynthesis of castor bean seedlings. *Plant Soil*, 344: 131–141
- Lloyd, J., P.E. Kriedemann and D. Aspinall, 1989. Comparative sensitivity of prior Lisbon lemon and Valencia orange trees to foliar sodium and chloride concentrations. *Plant Cell Environ.*, 12: 529–540
- Majeed, A., M.F. Nisar and K. Hussain, 2010. Effect of saline culture on the concentration of Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> in *Agrostis tolonifera*. *Curr. Res. J. Biol. Sci.*, 2: 76–82
- Marschner, H., 1995. *Mineral Nutrition of Higher Plants*, 2<sup>nd</sup> edition. Academic Press, San Diego, USA
- Martin, F.W., 1982. Okra, potential multiple-purpose crop for the temperate zones and tropics. *Econ. Bot.*, 36: 340–345
- McDonald, M.B., 1999. Seed deterioration: physiology, repair and assessment. *Seed Sci. Technol.*, 27: 177–237
- McDonald, M.B., 2000. Seed Priming. In: *Seed Technology and its Biological Basis*, pp: 287–325. Black, M. and J.D. Bweley (eds.). Sheffield Academic Press Ltd., Sheffield, England
- Munns, R., 1993. Physiological processes limiting plant growth in saline soils: Some dogmas and hypotheses. *Plant Cell Environ.*, 16: 15–24
- Munns, R., 2002. Comparative physiology of salt and water stress. *Plant Cell Environ.*, 25: 239–250
- Murphy, K.S.T. and M.J. Durako, 2003. Physiological effects of short term salinity changes on *Ruppia maritima*. *Aquat. Bot.*, 75: 293–309
- Pignocchi, C., and C.H. Foyer, 2003. Apoplastic ascorbate metabolism and its role in the regulation of cell signaling. *Curr. Opin. Plant Biol.*, 6: 379–389

- Piotr, S. and G. Klobus, 2005. Antioxidant defense in the leaves of C<sub>3</sub> and C<sub>4</sub> plants under salinity stress. *Physiol. Plant.*, 125: 31–40
- Raza, S.H., H.R. Athar and M. Ashraf, 2006. Influence of exogenously applied glycine betaine on photosynthetic capacity of two differently adapted wheat cultivars under salt stress. *Pak. J. Bot.*, 38: 341–351
- Raza, S.H., H.R. Athar, M. Ashraf and A. Hameed, 2007. Glycine betaine-induced modulation of antioxidant enzymes activities and ion accumulation in two wheat cultivars differing in salt tolerance. *Environ. Exp. Bot.*, 3: 368–376
- 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. *J. Plant Physiol.*, 314–319
- Savello, P.A., F. Martins and W. Hull, 1980. Nutrition composition of okra seed meals. *J. Agric. Food Chem.*, 28: 1163–1166
- Shahid, M.A., M.A. Pervez, R.M. Balal, R. Ahmad, C.M. Ayyub, T. Abbas and N. Akhtar, 2011. Salt stress effects on some morphological and physiological characteristics of okra (*Abelmoschus esculentus* L.). *Soil Environ.*, 30: 66–73
- Shalata, A. and P.M. Neumann, 2001. Exogenous ascorbic acid (Vitamin C) increases resistance to salt tolerance and reduced lipid peroxidation. *J. Exp. Bot.*, 364: 2207–2211
- Shi, Q., and Z. Zhu, 2001. Effect of exogenous salicylic acid on manganese toxicity, element contents and antioxidative system in cucumber. *Environ. Exp. Bot.*, 63: 317–326
- Smimoff, N., 1996. The function and metabolism of ascorbic acid in plants. *Ann. Bot.*, 78: 661–669
- Stevens, P.W., S.L. Fox and C.L. Montague, 2006. The interplay between mangroves and salt marshes at the transition between temperate and subtropical climate in Florida. *Wetlands Ecol. Manage.*, 14: 435–444
- Tari, I., J. Csiszar, G. Szalai, F. Horvath, A. Pecsvaradi, G. Kiss, A. Szepesi, M. Szabo and L. Erdei, 2002. Acclimation of tomato plants to salinity stress after a salicylic acid pre-treatment. *Acta Biol.*, 46: 55–56
- Wahid, A., A. Noreen, S.M.A. Basra, S. Gelani and M. Farooq, 2008. Priming-induced metabolic changes in sunflower (*Helianthus annuus* L.) achenes improve germination and seedling growth. *Bot. Stud.*, 49: 343–350
- Yamaguchi, T., and E. Blumwald, 2005. Developing salt-tolerant crop plants: challenges and opportunities. *Trends Plant Sci.*, 12: 615–620
- Yusuf, M., S.A. Hasan, B. Ali, S. Hayat, Q. Fariduddin and A. Ahmad, 2008. Effect of salicylic acid on salinity-induced changes in *Brassica juncea*. *J. Integr. Plant Biol.*, 50: 1–7

(Received 31 December 2012; Accepted 17 January 2013)