Improving Growth and Yield of Salt-stressed Soybean by Exogenous Application of Jasmonic Acid and Ascobin

SOAD A. SHETEAWI

Botany Department, Women's College, Ain Shams University, Cairo, Egypt Corresponding author's e-mail: ahmed200497@yahoo.com

ABSTRACT

The effect of phytohormone Jasmonic acid (JA) and antioxidant ascobin on growth, yield and metabolism of soybean (*Glycine max* L. cv. Giza 111) grown at 0, 50 and 100 mM NaCl was investigated. Growth, yield and metabolic products were most affected by 100 mM NaCl. Treatment with JA or ascobin mitigated the harmful effect of NaCl. The greatest yield (157% of control) was obtained from plants sprayed with JA, while those sprayed with ascobin and irrigated with water yielded 127% of control plants. Salinized plants (50 mM NaCl) sprayed with JA yielded 146%, while those sprayed with ascobin yielded 159% as compared with nonsprayed plants. The JA or ascobin reduced the salt effects on seed carbohydrates, lipids, proteins, N, P and K. To conclude, JA or ascobin improved salt tolerance in soybean by enhancing the accumulation of nontoxic metabolites (sugars, free proline and proteins) as well as N, P and K as a protective adaptation.

Key Words: Ascdoin; Jasmonic acid; Metabolites; Nutrients; Salt stress; Soybean

INTRODUCTION

Soybean (*Glycine max* L.) is one of the most economic and nutritious crop, as it contains high protein and oil (Yaklich *et al.*, 2002). The biochemical composition and physical appearance of soybean seeds affect the quality of various soy preparations (Liu *et al.*, 1995).

Most of the crop species are glycophytes, and generally show limited growth and development due to salinity. However, with increasing amounts of arable land undergoing salinization and increasing food demand from the growing human population, there is a need to ameliorate the harmful effect of salinity using various strategies (Szabolcs, 1994). Charparzadeh *et al.* (2004) reported that high salinity caused reduction in growth, lipid peroxidation and hydrogen peroxide accumulation in Calendula. Soluble sugars and proteins and total free amino acids including proline were progressively accumulated as NaCl level increased in alfalfa (Antoline & Sauchez-Dais, 1992).

Glycophytic species employ different strategies to withstand salinity stress. The increase in salt resistance may involve protection of cell and organelle membranes (Maslenkova *et al.*, 1993; Mansour, 1997; Mansour *et al.*, 2004), and accumulation of some protector components (Mansour 2000). Phytohormones such as jasmonate, may act as modulator by suppressing or enhancing the stress responses of plants (Popova *et al.*, 1995). Jasmonic acid [3-oxo-2-(2-cis-pentylcyclopentane 1-acetic acid)] and its methyl ester (JA-Me) are considered a new class of endogenous growth substances identified in many plant species. They influence a wide variety of physiological and developmental responses (Parthier *et al.*, 1992). Jasmonates antagonistically regulate the expression of salt stress-

inducible proteins, associated with salt stress in rice (Moons et al., 1997).

One of the biochemical changes occurring in plants subjected to environmental stress conditions is the production of reactive oxygen species (ROS) such as superoxide radicle, H₂O₂, singlet oxygen and hydroxyl radicals (Cho & Park, 2000). The ROS can damage essential membrane lipids proteins and nucleic acids (Inz & Van Montague, 1995; Garratt et al., 2002). Several studies, however, indicate that levels of ROS in plant cells are normally protective by antioxidant activity. Association between saline environment and endogenous level of water soluble antioxidant enzymes has been reported (Foyer et al., 1993; Gueta-Dahan et al., 1997; Tsugane et al., 1999). Reports indicate that antioxidant could be used as a potential growth regulator to improve salinity stress resistance in several plant species (Shalata & Peter, 2001; Gunes et al., 2005; Khan, 2006). Ascorbate peroxidase is one of the most important enzymes playing a crucial role in eliminating toxic H₂O₂ from plant cell (Foyer et al., 1993).

The aim of this study was to investigate the possible ameliorative effect of seed soaking and foliar application of Jasmanic acid and ascobin (ascorbic + citric acid, 2:1) on salt stressed soybean.

MATERIALS AND METHODS

Soybean (*Glycine max* L. cv. Giza 111) and the commercial product ascobin were obtained from Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. Jasmanic acid was purchased from Sigma Co, U.S.A. Soybean seeds were grown in pots (diameter 35 cm and depth 40 cm) containing 7 kg soil. Characteristics of the

soil were as follows: texture, sandy loam; pH, 7.7; ECe, 0.23 dS m^{-1} ; organic matter, 0.41%. Ten seeds per pot and five replicates were used for each treatment. Irrigation was applied on weekly basis to achieve soil water field capacity. Treatment sets were as follows:

I. Control and salt treatments:

- a. Control (irrigated weekly with water)
- b. 50 mM NaCl irrigated weekly after two weeks of seed sowing
- c. 100 mM NaCl irrigated weekly after two weeks of seed sowing
- II. Jasmenic acid and salt treatments:
 - d. Seeds soaked in 1µM JA for 12 h then air dried 24 h and after sowing irrigated weekly with water to the field capacity
 - Seeds soaked in 1µM JA, irrigated weekly with 50 mM NaCl to the field capacity
 - f. Seeds soaked in $1\mu M$ JA, irrigated weekly with 100mM NaCl to the field capacity
 - g. Seedling were sprayed with 1μ M JA after two weeks from sowing then sprayed again after 45 days from sowing until the solution ran off the leaves, irrigated weekly with water
 - h. Plants sprayed twice with $1\mu M$ JA as in no. 7, irrigated weekly with 50 mM NaCl
 - i. Plants sprayed twice with 1µM JA as in no.7, irrigated weekly with 100mM NaCl,
- III. Ascobin and salt treatments:
 - j. After two weeks from sowing seedling were sprayed with 1.3g L⁻¹. ascobin and then sprayed again after 45 days from sowing, irrigated weekly with water
 - k. Plants sprayed twice with 1.3 g L⁻¹ ascobin as in no. 10, irrigated weekly with 50mM NaCl
 - 1. Plants sprayed twice with 1.3 g L⁻¹ ascobin, as in j, irrigated weekly with 100 mM NaCl.

Plants were harvested at 30 d (vegetative growth stage), 60 d (flowering stage) and 90 d (pod setting stage) after sowing. Shoot length, fresh and dry masses and leaf area were determined. Yield components (number of pods plant⁻¹, no. of seeds plant⁻¹, yield plant⁻¹ and weight of 100 seeds) were determined at the end (120 d).

Tissue analysis. Photosynthetic pigments were determined as described by Metzner *et al.* (1965). Total soluble carbohydrates were analyzed according to Dubois *et al.* (1956) and total soluble amino acids were determined according to Sadasivam and Manickam (1996). Free proline content was determined according to Bates *et al.* (1973). N, P and K were determined according to AOAC (1975).

Seed metabolic products. Total carbohydrates were determined according to Dubois *et al.* (1956). Lipids content was estimated according to Sadasivam and Manickam (1996). The crude N of soy flour ground from dried seeds was determined by the micro-Kjeldahl method (Peach & Tracey, 1956) and converted to protein content using the conversion factor 5.71 (AOAC, 1975).

Protein electrophoresis. SDS-polyacrylamide gel electrophoresis was performed using 10% acryl amide slab gel following Lammli (1970). Gels were photographed, scanned and analyzed using GelDoc 2000 Bio Rad system.

Fig. 1. Shoot growth criteria (Shoot length, fresh weight, dry weight and leaf area) of soybean cv. Giza 111 under the effect of NaCl, seed soaking and foliar spraying of jasmonic acid (JA) and foliar spraying of ascobin.

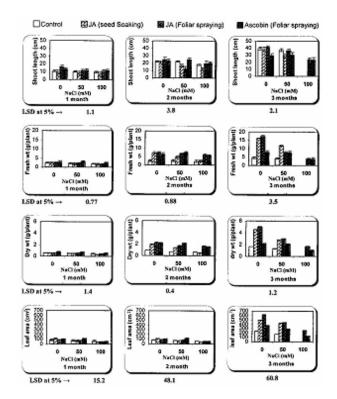
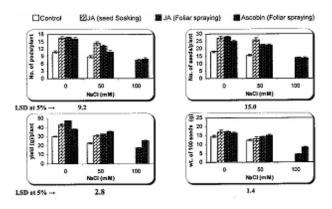


Fig. 2. Yield components and yield (No. of pods/plant, wt. of seeds/plant, yieldg/plant and wt. of 100seeds) of soybean cv. Giza 111 under the effect of NaCl, seed soaking and foliar spraying of jasmonic acid (JA) and foliar spraying of ascobin.



Statistical analysis. Standard error of means was calculated. The data were statistically analyzed using two way ANOVA (Snedecor & Cochran, 1969). The means were compared by LSD using SPSS (Version 10).

RESULTS AND DISCUSSION

Plant growth. Plant spraying or seed soaking with JA mitigated the inhibitory effect of NaCl on the growth parameters. A greatest effect was obtained when the plants

sprayed with JA. JA treated (foliar sprayed or seed soaked) plants irrigated with 50 mM NaCl resulted in better growth than 100 mM salinized plants (Fig. 1). Plants sprayed with ascobin and irrigated with water gave 174% fresh weight and 129% dry weight of the control (Fig. 1), which was similar to that obtained by Khan *et al.* (2003). Salinized plants (50 mM NaCl) sprayed with ascobin resulted in 186% fresh weight and 162% dry weight compared with 50 mM NaCl salinized plants. Salinized plants (100 mM NaCl) showed suppressed plant growth, and eventually death as

Table I. Photosynthetic pigments of soybean (cv. Giza 111) under the effect of NaCl, seed soaking and foliar spraying of jasmonic acid (JA) and foliar spraying of ascobin.

Treatments		Photosynthetic pigments (mg/g/L)											
		Chlorophyll a			Chlorophyll b			Clorophyll a + b				Carotenoids	
		1 month	2 months	3 months	1 month	2 months	3 months	1 month	2 months	3 months	1 month	2 months	3 months
0 mM NaCl	Control	43.3	51.0	68.9	27.0	24.9	31.2	70.3	75.9	100.1	17.6	20.9	23.0
	JA. (Soak)	47.2	59.3	72.6	27.5	28.8	38.1	74.7	88.1	110.7	22.8	23.2	26.7
	JA. (Foliar)	48.0	57.1	72.1	33.0	28.4	42.1	81.0	85.5	114.2	23.6	24.0	27.4
	Ascobin	55.0	65.9	72.3	33.0	29.7	40.1	88.0	95.6	112.4	20.0	25.3	21.9
	(Foliar)												
100 mM NaCl 50 mM NaCl	Control	30.8	44.7	61.9	20.2	21.9	35.3	51.0	66.6	97.2	13.7	18.0	18.2
	JA. (Soak)	38.2	55.7	64.3	21.0	24.7	37.6	59.2	80.4	102.9	16.9	21.8	24.4
	JA. (Foliar)	41.2	54.0	66.9	23.5	25.6	38.8	64.7	79.6	104.7	20.5	22.3	22.2
	Ascobin	47.1	61.4	66.0	31.6	29.7	37.2	78.7	91.1	103.2	18.4	21.0	20.7
	(Foliar)												
	Control	18.5	43.8	-	10.8	20.4	-	29.3	64.2	-	12.3	15.4	-
	JA. (Soak)	26.9	48.0	-	14.7	22.5	-	41.6	70.5	-	16.3	18.8	-
	JA. (Foliar)	27.8	50.1	62.2	16.2	20.1	33.3	44.0	70.2	95.5	14.2	17.0	18.2
	Ascobin	27.8	59.6	57.7	16.2	26.8	33.3	44.0	86.4	91.0	12.3	20.8	16.3
	(Foliar)												
LSD	at 0.05	3.0	2.1	3.4	3.3	2.4	1.5	5.7	6.2	5.4	2.0	2.3	2.7

Table II. Presence (+) and absence (-) of SDS-PAGE protein banding pattern in seeds of soybean (cv. Giza 111) under the effect of NaCl, seed soaking and foliar spraying of jasmonic acid (JA) and foliar spraying of ascobin.

Band no.	Treatment M.wt. K. Da	Control	50 mM NaCl	JA (Seed soaking)	JA (Foliar spray)	50 mM NaCl + JA (Seed soaking)	50 mM NaCl + JA (Foliarspraying)	ascobin (foliar spraying)	50 mM NaCl + ascobin (Foliar sprauing)	100 mM NaCl + ascobin (Foliar spraying
1	118.62	+	-	-	+	+	+	+	+	+
2	113.07	+	-	-	+	-	-	-	-	+
3	74.98	+	+	+	+	+	+	+	+	+
4	63.92	+	+	+	+	+	+	+	+	+
5	55.60	+	+	+	+	+	+	+	+	+
6	49.89	+	+	+	+	+	+	+	+	+
7	45.75	+	+	+	+	+	+	+	+	+
8	43.29	+	+	+	+	+	+	+	+	+
9	41.17	+	+	+	+	+	+	+	+	+
10	39.68	+	+	+	+	+	+	+	+	+
11	37.34	+	+	+	+	+	+	+	+	+
12	36.27	+	+	+	+	+	+	+	+	+
13	32.08	+	+	+	+	+	+	+	+	+
14	26.66	+	+	+	+	+	+	+	+	+
15	24.28	+	+	+	+	+	+	+	+	+
16	23.44	+	+	+	+	+	+	+	+	+
17	22.12	+	+	+	+	+	+	+	+	+
18	21.52	+	+	+	+	+	+	+	+	+
19	20.08	+	+	+	+	+	+	+	+	+
20	18.76	+	+	-	-	-	-	-	-	-
21	17.75	+	+	-	-	-	-	-	-	-
22	15.17	+	+	+	+	+	+	+	+	+
23	13.49	+	+	+	+	+	+	+	+	+
24	12.07	+	+	+	+	+	+	+	+	+
25	10.91	+	+	+	+	+	+	+	+	+
26	8.98	+	+	+	+	+	+	+	+	+
Total	26	26	24	22	24	23	23	23	23	24

Fig. 3. Total soluble carbohydrates, total soluble amino acids and proline of soybean cv. Giza 111 under the effect of NaCl, seed soaking and foliar spraying of jasmonic acid (JA) and foliar spraying of ascobin.

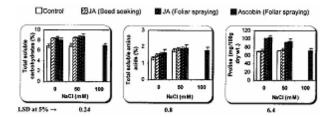
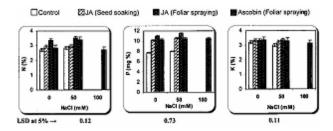


Fig. 4. Leaf N,P and K contents of soybean cv. Giza 111 under the effect of NaCl, seed soaking and foliar spraying of jasmonic acid (JA) and foliar spraying of ascobin.



they could not tolerate high salinity level. However, plants sprayed with JA or ascobin survived this salt level but with highly reduced growth (Fig. 1). Similar growth reduction in sugar beet was obtained under salt stress (Cherki *et al.*, 2002). These data agree with those of El Khallal (2001) who reports that pretreatment of pea seedling with JA alleviated the deleterious effect of NaCl on the plant growth. The mitigative effect of JA on the plant growth under salinity can be explained by its role in maintaining membrane stability in saline environment (Maslenkova *et al.*, 1993).

Ameliorative effect of ascobin (ascorbic acid + citric acid) on growth improvement comes from the fact that they act as an antioxidant under salinity. Our explanation is consistent with previous reports that different antioxidants mitigated salinity effects and thus enhanced salt tolerance on various crop plants (Foyer *et al.*, 1994; Shakirova *et al.*, 2003; Gunes *et al.*, 2005). These authors proposed that antioxidants ameliorate the damaging effect of salinity through interaction of antioxidant response and protection of membranes. In the current study ascorbic acid and citric acid appeared to act in a concert, which indicates a complete set of antioxidant under stressful conditions.

Yield and yield components. At all stages, 100 mM NaCl mortared the plants, but not those sprayed with JA or ascobin (Fig. 2). Plants sprayed with JA resulted in greater yield per plant (157% of the control) followed by JA seed soaking. Plants irrigated with 50 mM NaCl yielded 75% of that irrigated with water. Plants sprayed with ascobin and

irrigated with 50 mM NaCl yielded 159% of nonsprayed plants (Fig. 2). Plants sprayed or seed soaked with JA and irrigated with 50 mM NaCl yielded 146% and 138%, respectively, compared with 50 mM NaCl irrigated plants. This indicated stimulatory roles of JA as plant hormone and ascobin as antioxidant. Our results are in line with these of Sultana *et al.* (2001) who report that the yield contributing characters of rice plants were decreased by seawater. In their experiments, applying ascobin or JA to salinized plants equally ameliorated the harmful effect of salts.

Photosynthetic pigments. Salinity stress (50 or 100 mM NaCl) significantly declined the photosynthetic pigments (chlorophyll a, b and carotenoids) 30 and 60 days after sowing (Table I). The results are in concurrence with those of Brugnoli and Lauteir (1991) who found that salinity stressed cotton indicated reduced photosynthetic rate. This effect may result from stomatal closure due to osmotic stress, or salt-induced damage to photosynthetic apparatus. However, spraying with JA or ascobin mitigated NaClinduced effect on chlorophyll reduction; 114% of control and 118% of 50 mM NaCl irrigated plants (Table I). Plants sprayed with ascobin gave 112% of control and 116% of 50 mM NaCl irrigated plants. Khan et al. (2003) demonstrated that foliar application of salicylic acid increased the photosynthetic rate in corn and soybean. Salinized plants (50 mM NaCl) and sprayed with JA or ascobin gave 108% or 106% improvement in pigment levels respectively than that of 50 mM NaCl. Similar trend of chlorophyll content was obtained also for carotenoids (Table I).

Leaves metabolic products. All ameliorants increased the content of free proline, total soluble amino acids and carbohydrates in presence of two salt levels. The effect was more pronounced with ascobin (Fig. 3). Similar responses were reported in sugar beet, pea and black cumin (El Khallal, 2001; Cherki et al., 2002; Murakeozy, 2003). Siripornadulsil et al. (2002) and Mansour (2000) reported that proline accumulation plays an ameliorating role under environmental stresses. Naqvi et al. (1994) proposed that proline accumulation in salt-stressed seedlings may be related to the alteration of mitochondrial ultrastructure and decreasing protein oxidase activity or to an impaired incorporation of proline into protein. Hajar et al. (1996) suggested that carbohydrate accumulation in Nigella saliva may increase the ability for water absorption under salt stress, which was the case in our study (Fig. 3). Involvement of soluble sugars, total free amino acids and proline in osmotic adjustment have been proposed by Mansour (2000) in alleviating the adverse effect of salt stress.

Mineral contents. Jasmonic acid compared to ascobin greatly increased the level of NPK under salt stress. Greatest levels of N, P or K were obtained by spraying the plants with JA and irrigated with 50 mM NaCl as compared with those received only 50 mM NaCl (Fig. 4). Mishra and Choudhuri (1999) found that antioxidant application increased N, P and K in rice. The effect of JA and ascobin in inducing K and P content was previously reported by Abu-

Fig. 5. Total carbohydrates, lipids and protein percentage of soybean seeds cv. Giza 111 under the effect of NaCl, seed soaking and foliar spraying of jasmonic acid (JA) and foliar spraying of ascobin.

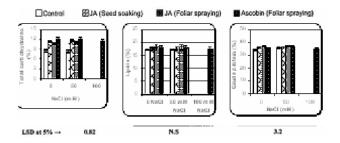
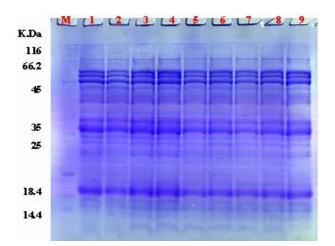


Fig. 6. SDS-PAGE protein banding pattern of soybean seeds (cv. Giza 111) under the effect of NaCl, seed soaking and foliar spraying of jasmonic acid (JA) and foliar spraying of ascobin. 1= Control, 2= 50 mM NaCl, 3= JA (seed soak), 4- JA (foliar spray), 5= 50 mM NaCl + JA (seed soak), 6= 50 mM NaCl + JA (foliar spray), 7= ascobin (foliar spray), 8= 50 mM NaCl + ascobin (Foliar spray) and 9=100 mM NaCl + ascobin (Foliar spray).



Ghalia and El Khallal (2001) and Cherki *et al.* (2002). The increase in these elements may contribute to the mitigative effect of JA and ascobin obtained under salt stress.

Seed metabolic products. Total carbohydrates were increased by JA and ascobin in absence and presence of salt stress, the effect was more pronounced with ascobin (Fig. 5). When the plants were sprayed with JA and ascobin, an increase in lipid content was obtained under salt stress (Fig. 5); although greatly with JA. Similar trend was obtained with the protein content in the absence and presence of salt, JA and ascobin (Fig. 5). These results could be explained by the stimulatory effect of the used antioxidant on metabolic activities of soybean.

SDS-PAGE protein analysis. Twenty-six bands were scored in the protein profile of soybean cultivar Giza-111 seeds under control condition (Table II, Fig. 6). Quantitative and qualitative differences were obtained when different

treatments of JA and ascobin were applied to soybean plants (Table II, Fig. 6). Consistent disappearance of two proteins (18.76 and 17.75 kDa) was observed under JA and ascobin treatments. It is interesting to mention that, banding pattern of JA (seed soaking and foliar spray) followed by 50 mM NaCl, as ascobin (foliar spraying) in absence and presence of 50 mM NaCl were similar (Table II, Fig. 6). Changes in the polypeptides pattern under antioxidant and hormonal application were previously reported. However, the function of such changed polypeptide is not clear yet.

CONCLUSION

Both JA and ascobin increased salt tolerance by enhancing the accumulation of nontoxic metabolites (sugars, amino acids, proline and protein) and improving the levels of NPK. Both JA or ascobin spray could be adopted as a potential growth regulator or antioxidant to improve growth, yield and nutrient utilization particularly under moderate NaCl salinity levels on soybean.

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