Improving Growth and Yield of Salt-stressed Cowpea Plants by Exogenous Application of Ascobin

Zinab A. Abdelgawad

Botany Department, Women's College for Arts, Science and Education, Ain Shams University, Cairo, Egypt
zinab_abdelgawad@hotmail.com

Abstract: Among the abiotic stresses, salinity is one of the most destructive factors which limit crop production considerably. Greenhouse experiments were conducted in Faculty of Women for Arts, Science and Education, Botany Department. Ain shams University, Cairo, Egypt during two successive summer seasons 2011 and 2012 to study the differential responses of cowpea (*Vigna sinensis*) to salinity stress (0, 50 and 75 mM) and foliar treatment with Ascobin (compound composed of ascorbic acid and citric acid with ratio of 2:1). Irrigation with different salinity levels caused significant reduction in fresh and dry weight of cowpea plants. Meanwhile total soluble sugars content was increased under salt stress conditions. Salinity stress with different levels caused higher reduction in yield and yield components (Number of pods/plant, yield/plant and weight of 100seeds) Application of Ascobin not only mitigated the inhibitory effect of salt stress in cawpea, but also in some cases induced a stimulatory effect greater than that estimated in the control plants on growth parameters which were accompanied by marked increases in photosynthetic pigments. On the other hand, yield and yield components showed progressive increases with increasing Ascobin treatments. Ascobin improved salt tolerance in cowpea by enhancing the accumulation of nontoxic metabolites such as total soluble sugars, proline and glycine betaine as well as N, P and K as protective adaptation.

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1. Introduction

Salinity is one of the most important a biotic factors limiting plant growth and productivity. During the onset and development of salinity stress within a plant, major processes, such as protein synthesis, energy, lipid metabolism, and photosynthesis are disrupted, ultimately resulting in the loss of plant productivity (Evelin *et al*., 2009). The deleterious effects of salinity on plant growth are associated with low osmotic potential of soil solution, nutritional imbalance, specific ion effect, hormonal imbalance and induction of oxidative stress, or a combination of these factors (Rahnama *et al*., 2010). Plant biomass production depends on the accumulation of carbon products through photosynthesis, but elevated salinity can adversely affect photosynthesis (Ashraf and Harris, 2004). Salinity reduces the ability of plants to take up water, causing a reduction in growth along with a suite of metabolic changes. Plants can avoid the damage caused by salinity through several mechanisms such as compatible solutes accumulation (Hajiboland *et al*., 2010; Dudhane *et al*., 2011; Talaat and Shawky, 2013, 2014). These mediate osmotic adjustment and therefore protect sub-cellular structures and reduce oxidative damage caused by free radicals, produced in response to high salinity (Ashraf and Foolad, 2007).

An alternative strategy for overcoming the negative effects of salinity on the plant growth and yield could be to attempt to use supplement materials which mitigate detrimental effects of salinity. In recent year, a few of materials are in used to alleviate salinity stress in plants. Citric acid is an important substrate in Krebs cycle (series of chemical reactions used by all aerobic organisms to generate energy (ATP) through the oxidization of acetate to carbon dioxide). In addition, the cycle provides precursors including certain amino acids as well as reducing agent NADH that is used in numerous biochemical reactions. So, it plays an important role in stimulating biosynthesis processes (Abd El-Al and Faten 2009). Citric acid is considered as one of non-enzymatic antioxidants which act to eliminate free radicals produced in plants under stress (Yan-Lin and Soon 2001). Citric acid also induced defense mechanisms by increasing the activities of antioxidant enzymes (Sun avd Hong, 2011).

Another antioxidant which has been shown by Müller-Moulé *et al*. (2004) is ascorbic acid. Ascorbic acid is one of the most important antioxidants abundantly occurring in plants (Smirnoff 2000).Thus, high endogenous ascorbic acid in plants is necessary to counteract oxidative stress in addition to regulating other processes of plant metabolism. Endogenous ascorbic acid can be increased by exogenous application of ascorbic acid through the rooting medium, as a foliar spray or as seed priming. Despite its role in scavenging reactive oxygen species, ascorbic acid is also involved in regulating photosynthetic capacity by controlling stomatal movement (Chen and Gallie 2004). Ascorbate is also an important co-factor of some enzymes or protein complexes that are involved in the regulation of photosynthesis (Davey *et al*. 2000). Furthermore, such positive effects of ascorbic acid in overcoming the adverse effects of salt stress were attributed to the stabilization and protection of photosynthetic pigments and the photosynthetic apparatus from oxidative damage (Hamada 1998). Several investigations reported that ascorbic acid plays important roles in enhancing the salt tolerance of different plants (Athar, *et al*. 2008 and Paital and Chainy, 2010).

Foliar antioxidants nutrient is applied at times when demand is particularly high and rapid response may be desired. Similarly the new Ascobin foliar nutrient, (ascorbic acid and citric acid with ratio of 2:1), had a promotion effect on growth and active constituents compounds on various plants (Sheteawi, 2007). Foliar treatment with different concentrations of Ascobin could stimulate the growth parameters, endogenous growth hormones, carbohydrate constituents and yield parameters.under normal conditions and different salinity levels (sadak *et al* 2013).

Current study aimed to investigate the possible ameliorative effect of foliar application of Ascobin (ascorbic + citric acid, 2:1) on salt stressed *Vigna sinensis*.

2. Material and Methods

Greenhouse experiments were conducted in Faculty of Women for Arts, Science and Education, Botany Department. Ain shams University, Cairo, Egypt. during two successive summer seasons 2011 and 2012 to study the differential responses of cowpea (*Vigna sinensis*) to salinity stress and foliar treatment with Ascobin (compound composed of ascorbic acid and citric acid).

Cowpea (*Vigna sinensis*) and the commercial product Ascobin were obtained from Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. Cowpea seeds which were previously sterilized 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 to achieve soil water field capacity level. Once every 15 days pots were rinsed with water to avoid salt accumulation. Five plants after seedling thinning were let to grow in each pot.

Treatment sets were as follows:

I - Control and salt treatments:

The first set was subjected to 0, 50 and 75 mM NaCl

II - Salt treatment and Ascobin foliar application :

The second set was subjected to 0, 50 and 75 mM NaCl and after two weeks from sowing cowpea seedling were sprayed with 1.3g L^{-1} Ascobin and then sprayed again after 45 days from sowing

Plants were harvested at 30 d (vegetative growth stage), 60 d (flowering stage) and 90 d (pod setting stage) after sowing. Fresh and dry weight was determined. Yield components (number of pods per plant, yield per plant, and weight of 100seeds) were determined at the end of the experiment (150 of sowing date).

Shoot analysis

Photosynthetic pigments were determined as described by Metzner *et al*. (1965). Total soluble carbohydrates were analyzed according to Dubois *et al*. (1956). Free praline content was determined according to Bates *et al*. (1973). Glycine betaine content was estimated as described by Grieve and Grattan (1983). Ascorbic acid was measured as described by Jagota and Dani (1982). N, P and K were determined according to AOAC (1995).

SDS-polyacrylamide gel electrophoresis

SDS-PAGE was performed using 10% acryl amide slab gel according to Lammli (1970). Gels were photographed, scanned and analyzed using Gel Doc 2000 Bio Rad system.

Statistical analysis

The data were statistically analyzed using one way analysis of variance as described by Snedecor & Cockran (1969). The means were compared by LSD using SPSS (Version 10).

3. Results and Discussion

Growth Parameters

Shoot Fresh and dry weights at all stages of development were reduced progressively with increasing NaCl concentrations while reversibly, use of Ascobin had stimulated plant growth at three stages of development studied (Fig. 1). Control plants treated with Ascobin recorded 124.6% and 140.3% fresh and dry weights, respectively, compared to the control at 90 days old. The foliar Application of Ascobin on plants treated with 75 mM NaCl recorded 120.4% and 131.5% fresh and dry weights, respectively, compared to the non-sprayed plants at 90 days old. Treatment with 75mM NaCl caused reduction in growth more than that were obtained with 50mM NaCl treated plants. Silveira *et al*. (2001)found that addition of high level of NaCl (100 mol m-3) induced a decrease in nitrate uptake and assimilation parallel to a reduction in the shoot growth of cowpea plants while 50 mol m-3 NaCl resulted in a slight reduction in the shoot dry mass compared to the control. Ghoulam *et al* (2002) stated that salinity treatment results in a progressive decline in growth among the four cowpea cultivars. The results obtained indicate that foliar treatment with Ascobin increased fresh and dry weight in normal and saline conditions. These results were similar to that obtained by Fayed (2010), Khan *et al* (2003) and sadak *et al*. (2013). The ameliorative effect of Ascobin (ascorbic acid + citric acid) on growth improvement comes from the fact that they act as an antioxidant under salinity and thus enhanced salt tolerance on various crop plants.

Yield and Yield Components

Number of pods/plant, yield/plant and weight of 100seeds attained the highest values for control plants and were declined progressively by increasing NaCl concentration (Fig. 2). Ascobin sprayed plants gave significantly higher yield and yield components than non-sprayed plants.

These results agree with Ahmad *et al*.(2005) who stated that pod fresh weight, seed yield and weight of 100 seeds of Vigna radiata L. showed a reduction as the salinity levels increased. Moreover, Taffouo *et al*. (2009) reported that, the significant decrease of yield components observed under salt stress in cowpea would be partly related to a significant reduction of chlorophyll contents and K concentration in saline media. They added that metabolic toxicity of Na+ is largely a result of its stability to compete with K+ for binding sites essential for cellular function. With respect to Ascobin foliar treatment, data in Fig 2 clearly indicated that foliar treatment with Ascobin increased significantly all yield parameters of cawpea plants at normal and salinity stress conditions. These obtained results are in good agreements with those obtained by Fayed (2001) on grapevine, Sheteawi (2007) on Soybean and (sadak *et al*., 2013) on wheat.

Photosynthetic Pigments

Salinity stress (50 and 75mM NaCl) resulted in significantly progressive decline in the photosynthetic pigments (chlorophyll a, b, a+b and carotenoids) at 30, 60and 90 days old (Table 1). Ascobin treated plants gave the highest amount of photosynthetic pigments than non-treated plants. These results are in agreement with those obtained by Ghassemi-Golezaniet *et al*. (2012), Hellal *et al*. (2012) and Bahari *et al*. (2013). These reductions due to salinity might be attributed to the inhibitory effects of salinity on many metabolic processes including, activity of mitochondria and chloroplasts (Singh and Dubey, 1995). Because of chlorophyll importance as one of necessary factors in plant photosynthesis, it is possible that salt stress has limited photosynthetic capacity and finally plant yield in cawpea. However, spraying with Ascobin mitigated salinity induced effect on chlorophyll reduction. These obtained results are in agreement with those obtained by Sheteawi (2007) on soybean and Fayed (2010) on grapevine. The beneficial effect of Ascobin as antioxidant on photosynthetic pigments may be due to its role in decreasing the rate of photochemical reduction, chloroplast structure, photosynthetic electron transfer as well as photosynthesis (Kumar *et al*., 1988).

Total Soluble Carbohydrates: Total soluble carbohydrates increased in salinized plants compared with control (Fig. 3). Plants treated with Ascobin showed higher total soluble carbohydrate content than the non-treated plants.

To prevent this water loss from the cell and protect the cellular proteins, plants accumulate many metabolites such as sugars, mainly fructose and sucrose; these solutes do not inhibit the normal metabolic reactions. Hajar *et al*. (1996) also showed that carbohydrate accumulation in *Nigella Sativa* increased the ability for water absorption under salt stress. With concern with Ascobin treatment, foliar treatment of Ascobin increased significantly TSS of cowpea plants. These obtained results are confirmed with those of Fayed (2010), Sheteawi (2007) and sadak *et al*. (2013). The significant increases in TSS concomitantly with the increased growth rate led to the conclusion that the photosynthetic efficiency was increased in response to Ascobin treatments and thus led to enhance biosynthesis of carbohydrates which are utilized in growth of cowpea plants.

Proline and Glycine Betaine (GB)

Proline and GB contents of cowpea leaves increased with increasing NaCl levels and increased further more in plants treated with Ascobin (Fig. 3). These results agree with those obtained by Silveira *et al*. (2001) and Tawfik(2008) who stated that the salt treatment caused an increase in the concentration of proline and GB in cowpea plants. Proline and GB accumulation is a general response to salinity stress. Silveira *et al*. (1999) showed that in case of cowpea, proline is accumulated largely in leaves only under a drastic salt stress. Tawfik (2008) and Mahajan and Tuteja (2005) proposed that proline and GB accumulation may contribute to osmotic adjustment at the cellular level, may acts as osmoprotectants and stabilizing the structure of macro-molecules. Proline also acts as a major reservoir of energy and nitrogen for utilization upon exposure to salinity. Ascobin increased significantly the content of free proline this results are in agreement with (Sheteawi 2007) who reported that foliar treatment of Ascobin increased free proline total soluble amino acids and carbohydrates in presence of two salt levels.

Mineral Content

Salinity stress caused significant decreases in nitrogen, phosphorous and potassium contents (Fig.4). It is noticed that Ascobin application resulted in increasing the level of NPK under salt stress. The present findings agree with that obtained by Sheteaw (2007). While Na+ is deleterious for plant growth, K+ is a macronutrient required in quite large quantities for maintaining the osmotic balance, as activator for many enzymes as well as for its role in regulating opening and closing of stomata. The mineral nutrient P is a main constituent of energy currencies (ADP, ATP, NADP, etc.), genetic materials (DNA and RNA), and cell membrane component (phospholipids). It plays a very important role in photosynthesis, respiration, in the development of root, flower, and seed (Mishra *et al.*, 2014). Accumulation of K+, N, and P in the leaves of both rice cultivars was significantly reduced due to salt stress. Mishra and Choudhuri (1999) found that antioxidant application increased N, P and K in rice. The effect of Ascobin in inducing N, P and K content was previously reported by Sheteaw (2007).The increase in these elements may contribute to the mitigative effect of Ascobin obtained under salt stress.

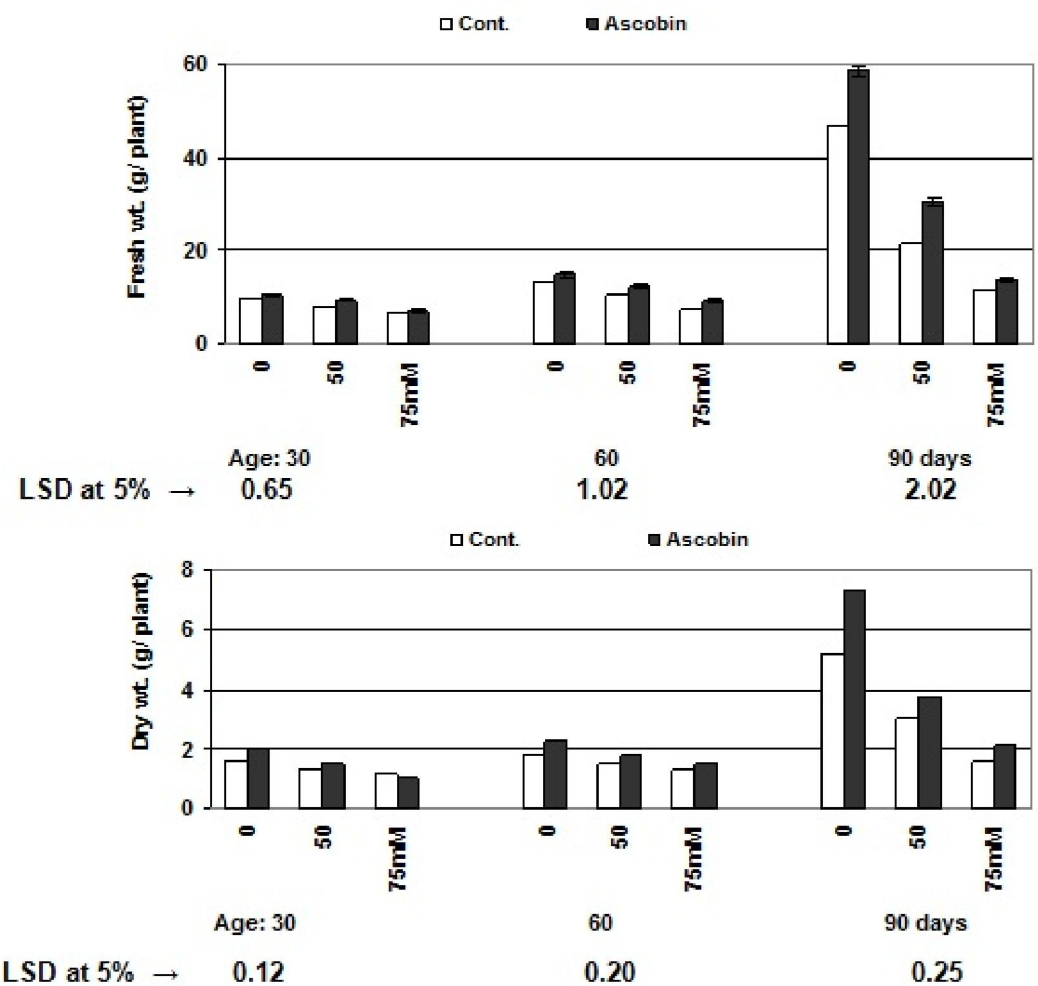

Figure 1. Effect of Ascobin foliar treatment on fresh and dry weights of cowpea plants grown under different concentrations of NaCl (Average of two seasons)

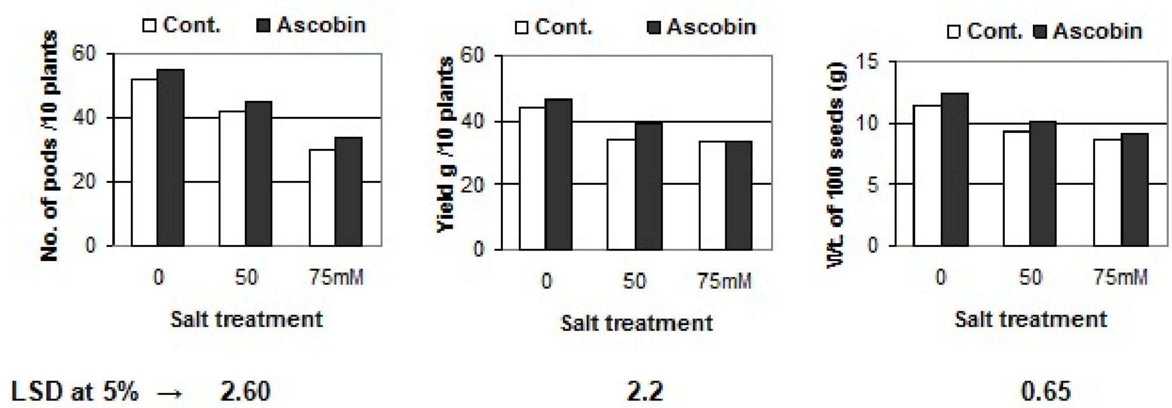

Figure 2. Effect of Ascobin foliar treatment on yield and yield components of cowpea plants grown under different concentrations of NaCl (Average of two seasons)

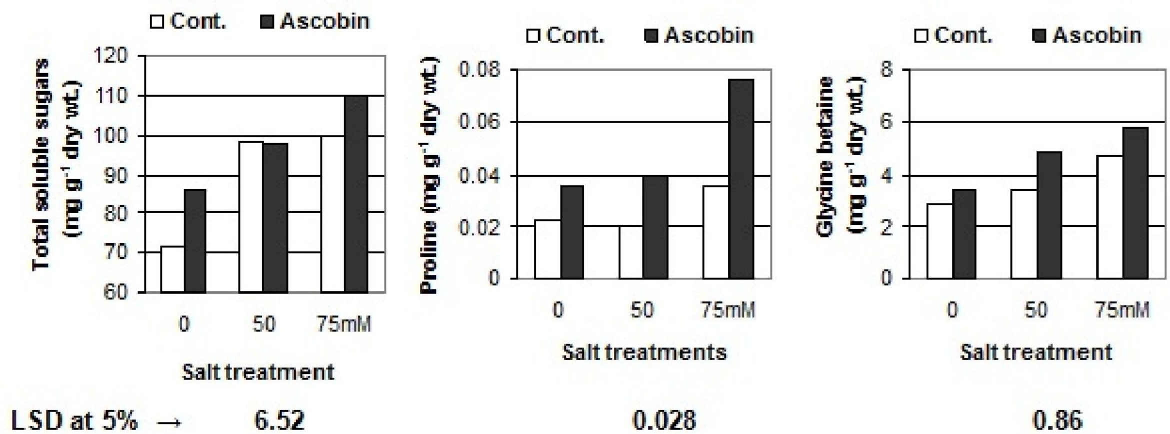

Figure 3. Effect of Ascobin foliar treatment on metabolic products of cowpea plants grown under different concentrations of NaCl

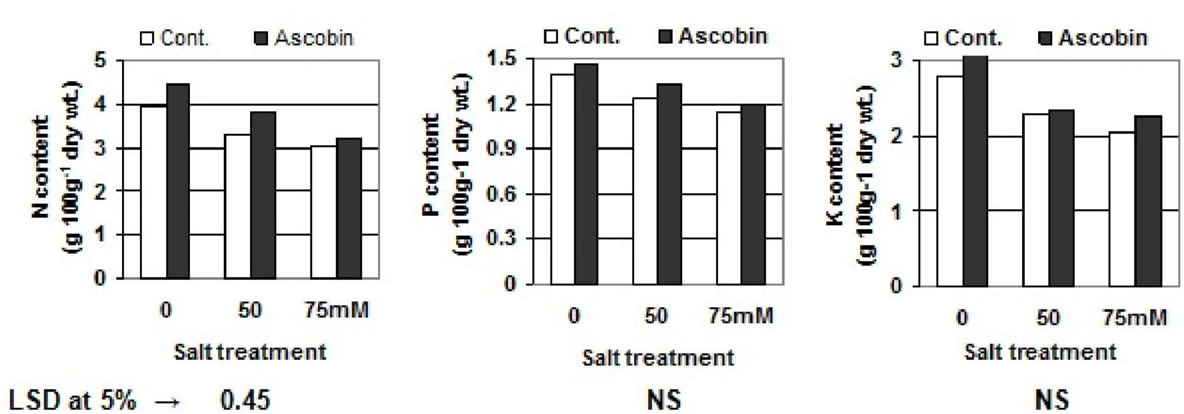

Figure 4. Effect of Ascobin foliar treatment on NPK of cowpea plants grown under different concentrations of NaCl

Taple 1. Effect of Ascobin foliar treatment on photosynthetic pigments of cowpea plants grown under different concentrations of NaCl

Treatment	Photosynthetic pigments (mg/g frish wt.)											
	30 day				60 day				90 day			
	Chl a	Chl b	T. chl	Carot.	Chl a	Chl b	T. chl	Carot.	Chl a	Chl b	T. chl	Carot.
0mM NaCl	50.6	46.2	96.8	10.4	57.6	49.7	107.3	14.3	69.1	59.7	128.8	19.3
50mM NaCl	44.5	44	88.5	10.3	53	48.2	101.2	15.7	63.6	57.8	121.4	17.1
75mM NaCl	43.8	39.3	83.1	9.5	51.2	39.3	90.5	9.1	61.4	47.2	108.6	16.5
0mM NaCl + Ascobin	46.6	42.3	88.9	12.6	57.2	50.7	107.9	9.2	68.6	60.9	129.5	26.7
50mM NaCl +Ascobin	51.2	48.4	99.6	11.1	75	67.7	145.7	13.2	93.6	81.2	174.8	25.7
75mm NaCl + Ascobin	45.2	43.2	88.4	10.6	50.9	43.6	94.5	11.9	61.1	52.3	113.4	23.6
LSD at 5%	1.22	1.47	2.52	1.76	1.22	1.98	3.46	1.32	3.52	3.3	4.58	1.69

Ascorbic acid Content

The concentration of ascorbic acid was determined in leaves of cowpea plants in order to confirm the effectiveness of exogenous Ascobin treatment in the level of this antioxidant on plant. It is evident that salinity stimulated the accumulation of ascorbic acid as compared with that of the unsalinized control (Fig. 5). These results are in harmony with those obtained by Sarwat and El-Sherif (2007) who reported that ascorbic acid content tends to increase with increasing salinity level in all cultivares of barley plant. Generally, application of Ascobin significantly increased the accumulation of Ascorbic acid in leaves of cowpea plants exposed to all concentrations of NaCl. Many compounds are being used to cope with the toxic effects of salinity including ascorbic acid (Khafagy *et al*. 2009). Ascorbic acid has been shown to play multiple roles in plant growth, such as in cell division, cell wall expansion, and other developmental processes (Pignocchi and Foyer, 2003). Also Ascorbic acid can directly scavenge superoxide, hydroxyl radicals and singlet oxygen and reduce H_2O_2 to water via ascorbate peroxidase reaction (Noctor and Foyer 1998).

SDS-PAGE Protein

Figure (6) demonstrates the SDS protein profiles of all treatments while Table 2 reveals their computer analysis and represents the occurrence of bands as (1) and absence as (0). A maximum number of 14 bands were detected at approximately molecular weights ranging between 15.13 KDa to 175.75 KDa. The minimum number of bands was 12 and recorded in (lane 3) of plants under severe salinity stress by absence of bands No. 1 and 5 of 175.75 and 99.77 KDa. The total number of bands in leaves of cowpea treated with 75mMNaCl was decreased as being compared with the respective controls. These results are in agreement with (El-Mashad and Mohamed, 2012) who indicated that the decrease in the protein level in salt-stressed plants might be attributed to a decrease in protein synthesis, the decrease availability of amino acids and the denaturation of enzymes involved in amino acid and protein synthesis. Sayed (2004) reported that the results of water non-soluble protein fractions of alfalfa under different stresses did not show clear-cut markers tolerance of stresses.

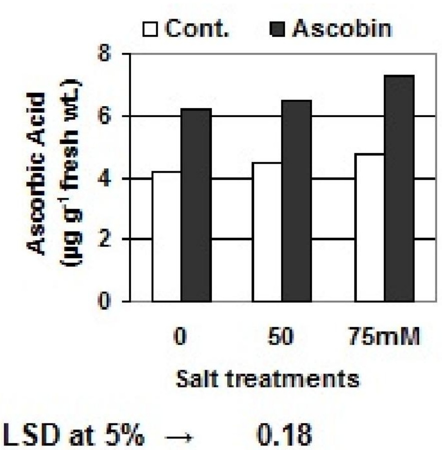

Figure 5. Effect of Ascobin foliar treatment on Ascorbic Acid of cowpea plants grown under different concentrations of NaCl

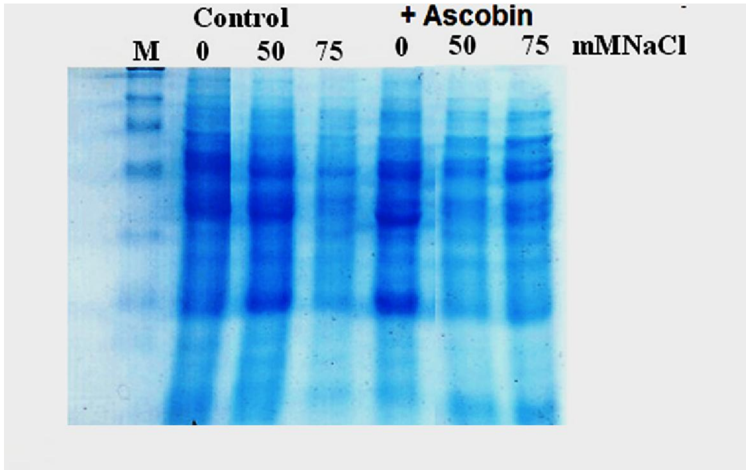

Figure 6. Protein banding pattern of cowpea plants grown under different concentrations of NaCl in presence or absence of Ascobin

Table 2. Presence (1) absence (0) of protein banding pattern of cowpea plants grown under different concentrations of NaCl in presence or absence of Ascobin

M.W.	Control			+ Ascobin		
	0	50	75	0	50	75
175.75	1	1	0	1	1	1
155.08	1	1	1	1	1	1
141.19	1	1	1	1	1	1
122.26	1	1	1	1	1	1
99.77	1	1	0	1	1	1
87.48	1	1	1	1	1	1
71.61	1	1	1	1	1	1
63.39	1	1	1	1	1	1
56.11	1	1	1	1	1	1
46.21	1	1	1	1	1	1
33.38	1	1	1	1	1	1
26.32	1	1	1	1	1	1
21.54	1	1	1	1	1	1
15.13	1	1	1	1	1	1
Total	14	14	12	14	14	14

4. Conclusion

From the aforementioned results, it can be concluded that, salinity stress reduced frish and dry weight, photosynthetic pigments, and yield parameters. While, foliar treatment with Ascobin (ascorbic + citric acid, 2:1) could stimulate all the above mentioned parameters under normal conditions and different salinity levels. Ascobin increased salt tolerance by enhancing the accumulation of nontoxic metabolites (sugars, proline and glycine betaine) and improving the levels of NPK and ascorbic acid. The conclusion of the present study is that Ascobin can be used to alleviate the harmful effect of salt stress. Therefore, the use of Ascobin can help to solve the production problems caused by salinity.

Corresponding Author:
Dr. Zinab A. Abdelgawad
Botany Department, Women's College
Ain Shams University, Cairo, Egypt
E-mail: zinab_abdelgawad@hotmail.com

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