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Alleviation of adverse effects of salt stress on physiological, biochemical and yield attributes in wheat by foliar treatment with Ascobin

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Abstract
A pot experiment was conducted during rabi season, 2018 in the cage house at Department of Plant Physiology, S.K.N. College of Agriculture, Jobner, Rajasthan to study the effect of ascobin in alleviating the adverse effects of salinity on physiological, biochemical and yield attributes of wheat. Wheat cultivars namely Raj-4037 (salinity susceptible) and Raj-3077 (Salinity tolerant) were grown in ceramic pots under salinity conditions (0, 4 and 8 dSm^{-1}). Different concentrations of ascobin (0, 500,750 and 1000 ppm) were sprayed at 45 and 65 days after sowing. Control plants were provided normal water. Different growth and physio-biochemical observations were recorded at 55 and 75 days after sowing in pot conditions. Yield parameters were recorded at harvest. Result revealed a significant decrease were recorded in chlorophyll, protein, relative water content, cell membrane stability, plant height, leaf area, number of effective tillers per plant, number of spikes per plant, length of spike, number of seeds per spike, grain yield, biological yield per plant, harvest index and test weight whereas, proline content and reducing sugar increased with salt stress up to EC 8 dSm^{-1} in both the cultivars at 55 and 75 DAS. The foliar spray treatment with ascobin up to 1000 ppm significantly increased growth and physio-biochemical. The 1000 ppm concentration of ascobin was found most effective in increasing the parameters studied. Reduction in physiological-biochemical and yield contributing parameters on account of salt stress was more in cultivar Raj-4037. On the basis of the above findings genotype Raj-3077 observed most salt tolerant and the tolerance was mediated by physiological-biochemical and yield characteristics.

Keywords: salinity, ascobin, proline, harvest index, wheat

1. Introduction
Soil salinity is one of the most important abiotic stress and limiting factor for worldwide conventional agriculture. Salinity is one of the most brutal environmental factors limiting productivity of crop plants because most of crop plants are sensitive to salinity caused by high concentrations of salts in the soil. A considerable amount of land in the world is affected by salinity which is increasing day by day. More than 45 million hectares (M ha) of irrigated land which account to 20% of total land has been damaged by salt in the worldwide and 1.5 M ha are taken out of production each year due to high salinity levels in the soil (Munns and Tester, 2008) [7]. Salt stress is a serious problem in crop production in India. Wheat (Triticum aestivum L.) is an important staple cereal crop throughout the world. In India, it is the second staple food crop following the rice. It is eaten in various forms by more than thousands million human beings in the world. Its straw is used as the feed for large population of animals. Plant height, number of tillers, leaf area index, dry matter, grain number, grain weight and harvest index are the yield contributing characters of wheat and the salinity has found to affect the yield by adversely affecting one or more of these parameters (Munns *et al.*, 1995) [8]. Salinity reduced leaf area and number of tillers and increase sodium (Na^+) and chloride (Cl^-) concentration of leaves (Munns and Termaat, 1986) [9].

Ascobin (ascorbic acid and citric acid with ratio of 2:1) have auxinic and also synergistic effect on plant. Ascorbic acid has also synergistic effect on plant. Ascorbic acid is an important primary metabolite in plants that functions as antioxidant, an enzyme cofactor and a cell signaling modulator in a wide array of crucial physiological processes, including biosynthesis of the cell wall, secondary metabolites and phytohormones, stress tolerance, photoprotection, cell division and growth (Wolucka *et al.*, 2005) [14]. Antioxidant (such as ascorbic acid and citric acid) are designing chemicals when added in small quantities to plant, react rapidly with radical intermediates of an auto-oxidation chain and stop it from progressing (Khan *et al.*, 2006) [5].

2. Materials and Methods

The effect of ascobin in alleviating the adverse effects of salinity on physiological, biochemical and yield attributes of wheat cultivars namely Raj-3077 salinity tolerant and Raj-4037 salinity susceptible will be screened out in pot conditions. Seeds were raised in seventy two cemented pots filled with about 15 Kg of wellmixed FYM soil in each pot. The crop will be irrigated with saline irrigation water one liter to each pot of EC 0 (Tap water), 4 and 8 dS m-1 prepared by mixing of NaSO4, NaCl, CaCl2, and MgCl2 salts. The different concentrations of ascobin were sprayed at 45 DAS and 65 DAS. Observations were recorded at 55 and 75 DAS (10 days after spray of ascobin). The relative water content (RWC) was calculated by the formula. chlorophyll content as mg g-1 fresh weight of leaf were estimated according to the method of Arnon (1949) [2]. Free proline (mg g-1 fresh weight of leaf) was determined using the method of Bates *et al*. (1973) [3]. Membrane stability (%) was calculated by taking the electrical conductivity of leaf leachates in double distilled water at 40° and 100° C by following the method of Sai ram (1994). Protein (mg g-1 fresh weight of leaf) was measured by method of Lowry *et al*., (1951) at 55 and 75 DAS. Leaf area was measured with the help of leaf area meter (LICOR 3000 USA). After harvest, length of the main spike excluding awns was measured and plant height with the help of scale in cm, plants were air dried and the grain was taken as number of effective tillers per plant, number of spikes per plant, length of spike, number of seeds per spike, grain yield, biological yield per plant, harvest index and test weight were counted and their average was recorded. The experiment was laid out in factorial based on Completely Randomized Design with three replication

3. Results and Discussion

Table 1: Effect of salinity and ascobin on chlorophyll content, relative water content and cell membrane stability of wheat

Treatments	**Chlorophyll content (mg/g f.w.)**		**Relative water content (%)**		**Cell membrane stability (%)**	
Varieties	55 DAS	75 DAS	55 DAS	75 DAS	55 DAS	75 DAS
Raj-3077	2.23	2.72	71.21	73.12	71.50	74.13
Raj-4037	1.95	2.43	66.56	68.05	67.41	70.82
S.Em.±	0.03	0.04	1.11	1.29	0.85	0.76
C.D.(P=0.05)	0.07	0.09	3.30	3.69	2.55	2.21
Salinity levels (dSm^{-1})						
0	2.32	2.78	65.31	65.97	75.40	79.16
4	2.11	2.39	64.10	64.44	71.15	75.50
8	1.75	2.17	62.45	62.90	60.89	66.95
S.Em.±	0.03	0.04	1.38	1.69	1.09	0.94
C.D. (P=0.05)	0.08	0.10	3.90	4.63	3.28	2.66
Ascobin (ppm)						
0	1.75	2.29	74.10	75.11	62.11	66.12
500	1.94	2.42	75.78	76.84	66.57	71.44
750	2.20	2.63	76.06	77.22	71.54	75.10
1000	2.31	2.86	76.82	77.96	76.22	79.15
S.Em.±	0.04	0.05	1.60	1.91	1.26	1.98
C.D. (P=0.05)	0.11	0.13	4.40	4.95	3.61	3.96

Data presented in table 1 further revealed that salt stress caused significant reduction in chlorophyll content up to EC 8.0 dSm^{-1} at 55 and 75 DAS. The decrease in chlorophyll content at EC 4.0 and EC 8.0 dSm^{-1} was recorded 9.05,24.56 and 14.02,21.94 per cent over control at both the stages, respectively. Effect of ascobin indicated that spray treatment with ascobin up to 1000 ppm concentration significantly increased chlorophyll content over its preceding levels at 55 and 75 DAS. The increase in chlorophyll content in leaves due to application of 500,750 and 1000 ppm concentration of ascobin was 10.85, 25.71, 32.00 and 5.67, 14.84,24.89 per cent at 55 and 75 DAS, respectively over that of control under non stress and salt stress conditions. The highest chlorophyll content was obtained due to treatment with 1000 ppm concentration of ascobin at both the stages of investigation, respectively. The data in Table 1 revealed that the RWC was recorded significantly higher in Raj-3077 than Raj-4037 at 55 and 75 DAS under non stress and salt stress conditions. The increase in RWC of Raj-3077 was 6.98 and 7.45 per cent than Raj-4037 at both the stages. RWC increased significantly up to 1000 ppm concentration of ascobin at 55 and 75 DAS under both non stress and salt stress conditions over control. The increase in RWC due to application of 500, 750 and 1000 ppm concentration of ascobin was 2.26, 2.64, 3.13 and 2.30, 2.80,3.79 percent over control at 55 and 75 DAS, respectively. The 1000 ppm concentration of ascobin was found to increase the RWC maximum at 55 and 75 DAS. The data in Table 1 revealed that the cell membrane stability was recorded significantly higher in Raj-3077 than Raj-4037 at 55 and 75 DAS under non stress and salt stress conditions. The increase in membrane stability of Raj-3077 was 6.06 and 4.67 per cent than Raj-4037 at both the stages. Cell membrane stability increased significantly up to 1000 ppm concentration of ascobin at 55 and 75 DAS under both non stress and salt stress conditions over control. The increase in membrane stability due to application of 500, 750 and 1000 ppm concentration of ascobin was 7.18, 15.18, 23.43 and 8.04, 13.58, 16.46 per cent over control, respectively. The 1000 ppm concentration of ascobin was found to increase the cell membrane stability maximum at 55 and 75 DAS.

Table 2: Effect of salinity and ascobin on Proline, Protein and reducing sugar of wheat

Treatments	**Proline (µg/g fr.wt. of leaf)**		**Protein (mg/g f.w.)**		**Reducing sugar (mg/g f.w.)**	
Varieties	55 DAS	75 DAS	55 DAS	75 DAS	55 DAS	75 DAS
Raj-3077	36	57	19.91	22.75	17.17	17.54
Raj-4037	28	50	18.35	21.28	19.25	19.95
S.Em.±	0.09	0.11	0.28	0.39	0.22	0.24

C.D.(P=0.05)	0.22	0.31	0.76	1.13	0.62	0.65
Salinity levels (dSm^{-1})						
0	20	43	21.22	23.19	15.92	16.91
4	32	54	19.80	22.95	18.30	18.70
8	46	71	17.65	19.53	19.02	19.34
S.Em.±	0.11	0.15	0.33	0.50	0.26	0.27
C.D. (P=0.05)	0.28	0.44	0.94	1.43	0.75	0.76
Ascobin (ppm)						
0	20	36	17.17	19.59	16.90	17.05
500	25	51	18.15	20.11	18.05	18.52
750	31	61	19.85	23.35	18.52	19.41
1000	50	75	22.13	23.91	18.95	20.15
S.Em.±	0.13	0.18	0.40	0.56	0.30	0.31
C.D. (P=0.05)	0.34	0.52	1.09	1.64	0.87	0.88

A perusal of data in Table 2 revealed that the increase in proline content of Raj-3077 was found significantly more than Raj-4037 under both non stress and salt stress conditions. The per cent increase in proline content of Raj-3077 was recorded 28.57 and 14.00 over Raj-4037 at 55 and 75 DAS, respectively. Increase in proline content significantly up to 1000 ppm concentration of ascobin was recorded at 55 and 75 DAS over control. The increase in proline content due to application of ascobin at 500,750 and 1000 ppm was obtained 25, 55, 150 and 41,.66, 69.44, 108.33per cent over that of control at 55 and 75 DAS. The maximum increase in proline content was recorded due to use of 1000 ppm concentration of ascobin under non stress and salt stress conditions, respectively. The cultivar Raj-3077 registered significantly higher protein content over Raj-4037 under both non stress and salt stress conditions. The per cent increase in protein content of Raj-3077 was 8.50 and 6.90 than Raj-4037 at 55 and 75 DAS. A further study of the data indicated that spray treatment with ascobin up to 1000 ppm concentration significantly increased protein content over its preceding levels at 55 and 75 DAS. The increase in protein content due to use of 500,750 and 1000 ppm concentration of ascobin was 5.70, 15.60, 28.88 and 5.65, 19.19, 22.05 per cent over that of control at 55 and 75 DAS. The maximum increase in protein content was recorded due to use of 1000 ppm concentration of ascobin under both non stress and salt stress conditions at both the stages of investigation. The cultivar Raj-4037 registered significantly higher reducing sugar content over Raj-3077 under both non stress and salt stress conditions. The per cent increase in reducing sugar content of Raj-4037 was 12.11 and 13.74 than Raj-3077 at 55 and 75 DAS. data indicated that spray treatment with ascobin up to 1000 ppm concentration significantly increased reducing sugar content over its preceding levels at 55 and 75 DAS. The increase in raducing sugar content due to use of 500,750 and 1000 ppm concentration of ascobin was 6.80, 9.58, 12.13 and 8.62, 13.84, 18.18 per cent over that of control at 55 and 75 DAS. The maximum increase in reducing sugar content was recorded due to use of 1000 ppm concentration of ascobin under both non stress and salt stress conditions at both the stages of investigation.

Table 3: Effect of salinity and Ascobin on Plant height (cm) at harvest, Number of effective tillers/plant at harvest and Leaf area (cm^2 / plant) at anthesis stage of wheat

Treatments	Plant height (cm) at harvest	Number of effective tillers /plant at harvest	Leaf area (cm^2 / plant) at anthesis stage
Varieties			
Raj-3077	58.32	2.32	96.51
Raj-4037	51.19	2.02	85.45
S.Em.±	0.48	0.02	0.70
C.D.(P=0.05)	1.33	0.07	1.97
Salinity levels (dSm^{-1})			
0	60.80	2.91	105.15
4	55.65	2.65	95.38
8	50.43	2.23	83.42
S.Em.±	0.59	0.04	0.88
C.D. (P=0.05)	1.62	0.11	2.30
Ascobin (ppm)			
0	49.15	2.04	83.15
500	54.44	2.42	90.65
750	58.85	2.57	101.25
1000	65.07	3.02	114.20
S.Em.±	0.68	0.03	1.02
C.D. (P=0.05)	1.87	0.08	2.85

Data in Table 3 depicted that Raj-3077 significantly performing better than Raj-4037 under both non stress and salt stress conditions. The increase in plant height of Raj-3077 was found 13.92 per cent over Raj-4037 at harvest, respectively. That application of ascobin enhanced plant height significantly up to 1000 ppm concentration over its preceding levels. The plant height increase due to use of 500, 750 and 1000 ppm concentration of ascobin were recorded 10.76,19.73 and 32.39 per cent higher over that of control at harvest. The highest plant height was recorded by application of 1000 ppm concentration of ascobin under non stress and salt stress conditions. A critical examination of data given in Table 3 revealed that a higher number of effective tillers per plant at harvest was recorded by variety Raj-3077 (14.85 per cent more than Raj-4037). Raj-3077 significantly performing better than Raj-4037 under both non stress and salt stress conditions. That application of ascobin enhanced significantly the number of effective tillers per plant at harvest up to 1000 ppm concentration under both non stress and salt stress conditions. The maximum number of effective tillers per plant at harvest was recorded due to the application of 1000 ppm concentration of ascobin which was higher by 48.03 per cent followed by 750 ppm (25.98 per cent), 500 ppm (18.62 per cent) concentration over control, respectively. It is evident from the data in Table 3revealed that at anthesis stage a significantly higher leaf area per plant was recorded by cultivar Raj-3077 which was higher by 12.94 per cent over Raj-4037 under both non stress and salt stress conditions. A further study of the data in above table revealed that the leaf area per plant increased significantly up to the 1000 ppm level of ascobin at anthesis. The leaf area increase due to use of 500, 750 and 1000 ppm concentration of ascobin were recorded 9.01,21.76and 37.34 per cent higher over that of control at anthesis stage. The highest leaf area per plant was recorded by application of 1000 ppm concentration of ascobin under non stress and salt stress conditions.

A critical examination of data given in Table 4 revealed that a higher number of spikes per plant was recorded by variety Raj-3077 (11.55 per cent more than Raj-4037). Raj-3077 significantly performing better than Raj-4037 under both non stress and salt stress conditions. A further study of the data in above table revealed that the leaf area per plant increased significantly up to the 1000 ppm level of ascobin at anthesis. The leaf area increase due to use of 500, 750 and 1000 ppm concentration of ascobin were recorded 9.01,21.76and 37.34 per cent higher over that of control at anthesis stage. The highest leaf area per plant was recorded by application of 1000 ppm concentration of ascobin under non stress and salt stress conditions. A critical examination data presented in Table 4 revealed that a higher number of seeds per spike was recorded by variety Raj-3077 (16.18 per cent more than Raj-4037). Raj-3077 significantly performing better than Raj-4037 under both non stress and salt stress conditions. Further reference to data in the above table showed that a significant increase in number of seeds per spike was recorded up to 1000 ppm concentration of ascobin over control under non stress and salt stress conditions.

Table 4: Effect of salinity and ascobin on number of seeds/spike, number of spikes/plant, length of spike /plant, test weight, grain yield, biological yield and harvest index s of wheat

Treatments	Number of Seeds /spike	Number of spikes /plant	Length of spike /plant	Test weight	Grain yield	Biological yield	Harvest index
Varieties	46.30	2.71	8.82	40.10	4.94	10.50	46.10
Raj-3077	39.85	2.43	8.04	37.56	3.83	9.05	42.34
Raj-4037	0.36	0.03	0.06	0.30	0.03	0.07	0.60
S.Em.±	1.07	0.08	0.17	0.85	0.08	0.20	1.70
C.D.(P=0.05)							
Salinity levels (dSm^{-1})	52.37	3.02	9.18	42.32	5.10	11.20	48.14
0	44.94	2.49	8.54	39.80	4.41	10.45	44.20
4	37.55	2.18	7.85	37.48	3.88	8.10	40.25
8	0.43	0.05	0.08	0.45	0.04	0.13	0.80
S.Em.±	1.28	0.12	0.22	1.10	0.12	0.32	2.35
C.D. (P=0.05)							
Ascobin (ppm)	37.42	2.24	7.50	35.20	3.72	7.90	41.05
0	40.85	2.52	7.96	36.72	4.27	8.10	43.34
500	47.27	2.80	8.65	39.42	4.45	9.60	47.13
750	50.15	3.05	9.10	40.54	5.10	10.25	48.92
1000	0.52	0.04	0.13	0.41	0.05	0.11	0.80
S.Em.±	1.50	0.11	0.35	1.21	0.13	0.30	2.38
C.D. (P=0.05)	46.30	2.71	8.82	40.10	4.94	10.50	46.10

The maximum increase in number of seeds per spike was obtained at 1000 ppm concentration of ascobin than its preceding levels. Number of seeds per spike was recorded 9.16, 26.32 and 34.01 per cent higher over control due to the application of 500,750 and 1000 ppm concentration of ascobin, respectively. Examination of data given in Table 4 revealed that a higher length of spike per plant was recorded by variety Raj-3077 (9.70 per cent more than Raj-4037). Raj-3077 significantly performing better than Raj-4037 under both non stress and salt stress conditions. Further reference to data from the above table showed that a significant increase in spike length per plant was recorded up to 1000 ppm concentration of ascobin over control. The maximum increase in spike length per plant was obtained due to the use of 1000 ppm concentration of ascobin than the others under both non stress and salt stress conditions. The increase in spike length was recorded 6.13, 15.33 and 21.33 per cent at 500,750 and 1000 ppm concentration of ascobin over control. A perusal of data given in Table 4 revealed that a higher test weight was recorded by variety Raj-3077 (6.76 per cent more then Raj-4037). Raj-3077 significantly performing better over Raj-4037 in salt stress as well as non-stress conditions. Further reference to data of the above Table 4 showed that test weight increased significantly with successive increase in level of ascobin up to 1000 ppm concentration over control. A maximum test weight was recorded due to the application of 1000 ppm concentration of ascobin under non stress and salt stress conditions which was higher by 15.17 per cent followed by 750 ppm (11.98 per cent), 500 ppm (4.31 per cent) concentration over control, respectively. It is clear from the data given in Table 4 that a higher grain yield per plant was recorded by variety Raj-3077 under both non stress and salt stress conditions which was higher by 28.98 per cent over Raj-4037. Further examination of data in the above Table 4 showed that application of ascobin up to 1000 ppm concentration brought significant increase in grain yield of wheat over control under non stress and salt stress conditions. The maximum increase in grain yield was recorded due to use of 1000 ppm concentration of ascobin which was higher by 37.09 per cent, followed by 750 ppm (19.62 per cent), 500 ppm (14.78 per cent) concentration over control, respectively. It is clear from the data given in Table 4 revealed that a significantly higher biological yield per plant was obtained by variety Raj-3077 under non stress and salt stress conditions. The increase in biological yield per plant of Raj-3077 was found 16.02 per cent over Raj-4037, respectively. Further reference to data in the above Table 4 showed that increasing level of ascobin up to 1000 ppm showed significant increase in biological yield per plant of wheat under non stress and salt stress conditions, respectively. The maximum increase in biological yield per plant was recorded under the application of 1000 ppm concentration of ascobin which was higher by 29.74 per cent followed by 750 ppm (21.51 per cent), 500 ppm (2.53 per cent) concentration over control, respectively. It is clear from the data revealed that a significantly higher Harvest index per plant was obtained by variety Raj-3077 under non stress and salt stress conditions. The increase in Harvest index per plant of Raj-3077 was found 8.89 per cent over Raj-4037, respectively. Further reference to data showed that increasing level of ascobin up to 1000 ppm showed significant increase in Harvest index per plant of wheat under non stress and salt stress conditions, respectively. The maximum increase in Harvest index per plant was recorded under the application of 1000 ppm concentration of ascobin which was higher by 19.17 per cent followed by 750 ppm (14.81 per cent), 500 ppm (5.57 per cent) concentration over control, respectively.

4. Conclusion
It is concluding that ascobin increases plant adaptation to salt stress by stimulating the physiological, biochemical process which would help to minimize yield reduction in wheat plants. Further, the results concluded that cultivar Raj3077 (Salinity tolerant) withstands more effectively than cultivar Raj4037 (Salinity susceptible) under salinity. We believe that cultivar Raj-3077 may be very promising to farmers for cultivation in saline areas up to EC 8 dSm-1.

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