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Foliar treatment with ascobin reduces the adverse effects of salt stress on physiological and biochemical parameters in wheat

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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 and biochemical parameters of wheat. Wheat cultivars namely Raj-4037and Raj-3077 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. Result revealed a significant decrease were recorded in chlorophyll, protein, relative water content, cell membrane stability 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 physio-biochemical. The 1000 ppm concentration of ascobin was found most effective in increasing the parameters studied. Reduction in physiological-biochemical 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.

Keywords: Salinity, ascobin, proline, wheat

1. Introduction

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. 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) [13]. Salt stress is a serious problem in crop production in India. 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) [15]. Salinity reduced leaf area and number of tillers and increase sodium (Na^+) and chloride (Cl^-) concentration of leaves (Munns and Termaat, 1986) [14].

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) [23]. 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) [8].

2. Materials and Methods

The effect of ascobin in alleviating the adverse effects of salinity on physiological and biochemical 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 well mixed 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 $NaSO_4$, NaCl, $CaCl_2$, and $MgCl_2$ 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). Free proline (mg g^{-1} fresh weight of leaf) was determined using the method of Bates *et al*. (1973). 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). 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 4	2.11	2.39	64.10	64.44	71.15	75.50
8 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 4	32	54	19.80	22.95	18.30	18.70
8 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 reducing 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.

4. Discussion

A significant decrease in chlorophyll content was observed under salt stress conditions at 55 and 75 days after sowing. The Raj-3077, a salt tolerant genotype, showed lesser reduction in chlorophyll content than Raj-4037 (Table 1). Higher chlorophyll content of Raj-3077 reflects its relative tolerance to salt stress. In current study, the chlorophyll contents significantly decreased under elevated salt stress, as the chlorophyll contents are sensitive to salt exposure and a reduction in chlorophyll levels due to salt stress has been reported in several plants, such as pea (Ahmad and Jhon, 2005) [1], wheat (Ashraf *et al.*, 2002) [5] and rice (Anuradha and Rao, 2003) [3]. Sairam *et al.* (2002) [20] revealed that salinity induced decrease in chlorophyll and carotenoids were significantly higher in KRL 19 than more tolerant Kharchia 65. Similarly, Sairam *et al.* (2002) [20] reported that reduction of chlorophyll content in a tolerant wheat cultivar was lower than in a sensitive one.

Membrane stability decreased under salinity stress in both the genotypes at both the stages of sampling (Table 2,). Higher membrane stability was retained by tolerant cultivar Raj-3077 compared to susceptible Raj-4037 under salt stress and non-stress conditions at both stages of investigation. The results are in accordance with the findings of Sairam and Srivastava (2002) [20] they found that salinity caused to decrease membrane stability index in two wheat genotypes but the reduction was more pronounced in susceptible one (Raj-4037) than tolerant (K-65) genotype. It has also been reported that salinity stress decreased significantly membrane stability index of wheat (Sairam *et al.*, 2002) [20].

Soil salinity significantly reduced protein content in the grain while proline accumulation was stimulated in the leaves. The increase in leaf proline was relatively greater in the tolerant genotypes than the susceptible ones (Sharma *et al.* 2003) [22]. Our studies also noticed that salt stress significantly increased the proline content (Table 2) of both wheat genotypes at 55 and 75 days after sowing. The results showed that the increase in proline content was higher in salt tolerant cultivar Raj-3077 than in comparison to salt susceptible cultivar Raj-4037 at both the stages of investigation. Free proline is known to accumulate in response to biotic and abiotic stresses and has been shown to protect plants against free radical induced damage as reported by Matysik *et al.* (2002) [10]. This is because proline accumulation in salt stressed plants is a primary defense response to maintain the osmotic pressure in a cell, which is reported in salt tolerant and salt sensitive cultivars of many crops (Misra and Gupta, 2005) [12].

A significant decrease in protein content was observed under salt stress conditions at 55 and 75 days after sowing in both wheat genotypes. The Raj-3077 salt tolerant genotype, showed lesser reduction in protein content than Raj-4037 (Table 2) at both the stages of study. Nucleic acid, protein level in NaCl treated rice seedling decreased with increase in salt concentration in comparision to control (Bera *et al.* 2006) [7].

5. 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 Raj-3077 (Salinity tolerant) withstands more effectively than cultivar Raj-4037 (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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