

Salt Tolerance in *Grevillea robusta* Seedlings via Foliar Application of Ascorbic Acid

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Abstract: Two pot experiments were carried out at the greenhouse of Research and Production Station, National Research Centre at Nubaria district, Behaira Governorate, Egypt during 2011 and 2012 seasons, to study the effect of saline water on vegetative growth and chemical constituents of *Grevillea robusta* seedlings under foliar application of ascorbic acid. The experiment included 12 treatments which were the combinations of spraying three ascorbic acid levels: tap water, 100 and 200 ppm with four salinity levels: tap water, 3000, 6000 and 9000 ppm. Results showed that, foliar application of ascorbic acid increased plant height, stem diameter, number of leaves/plant, root length as well as fresh and dry weights of shoots and roots, pigments content, total carbohydrates and also nitrogen, phosphorus and potassium percentages of shoots and roots. A significant reduction in all growth parameters, pigments and total carbohydrates and also, nitrogen, phosphorus and potassium percentages were occurred by using high salinity level (9000 ppm). The highest values of phenols, indoles and proline in shoots and roots resulted from the highest salinity level (9000 ppm). The combined treatment of ascorbic acid at 200ppm with water salinity at 3000 ppm gave the highest values of plant height, leaves number/plant, fresh and dry weights of shoots. Spraying the plants with ascorbic acid at 100 ppm + salinity level at 3000 ppm recorded the highest values of amino acids in shoots and roots. The results suggested that *Grevillea robusta* seedlings benefited from application with ascorbic acid especially under salinity conditions under sandy soil conditions.

Key words: *Grevillea robusta* % Proteaceae % Silky oak % Ascorbic acid % Salinity % Growth % Chemical composition

INTRODUCTION

Grevillea robusta A. Cunn. ex R.Br. commonly known as Silky Oak or Silver Oak, has gained widespread popularity in warm temperate, subtropical and tropical highland regions of many countries. It is the largest species in the genus *Grevillea*, in the plant family Proteaceae [1]. Ascorbic acid (AA) plays an essential role in plant growth and development, have been implicated in many physiological responses [2]. As well as, it is a well-known antioxidant and cellular reluctant with an intimate and complex role in the response of plants to O₃ [3]. Ascorbic acid is a small water soluble antioxidant molecule which acts as a primary substrate in the cyclic pathway of enzymatic detoxification of hydrogen peroxide. Improved of ascorbate in plants will lead to the possibility of increasing ascorbate concentration in

plants by genetic manipulation. This will have benefits for tolerance of plants [4]. Salinity causes large effects on higher plants, both halophytes and non-halophytes. In the latter, growth rate is generally reduced by salinity even at low salt concentration. However, within non-halophytes there is still large variability among species, ranging from extremely sensitive to tolerant species overlapping with halophytes [5]. The reduction in growth is consequence of several physiological responses including modifications of ion balance, water status, mineral nutrition, stomatal behavior, photosynthetic efficiency, carbon allocation and utilization [5-7].

The main aims of the present study were to find out the effect of ascorbic acid and water salinity on irrigation regime on growth and chemical composition of *Grevillea robusta*.

Table 1: The physical and chemical properties of the experimental soil

Physical properties							Anion (meq/L)			Cation (meq/L)			
Clay %	Silt %	Coarse sand %	Fine sand %	Soil texture	EC dS/m	pH	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻	Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺
6.9	3.9	77.3	11.9	Sandy	2.6	7.3	3.1	22.9	--	5	3	13	5

MATERIALS AND METHODS

The experiments were conducted in Research and Production Station, of the National Research Centre at Nubaria District, Behaira Governorate, Egypt, during 2011 and 2012 seasons to study the effect of spraying ascorbic acid and salinity on growth and chemical composition of *Grevillea robusta* seedlings. The physical and chemical properties of the experimental soil are presented in Table 1. One year-old seedlings of *Grevillea robusta* were obtained from Nursery of Forestry Department, Horticulture Research Institute, Agriculture Research Centre, Giza Egypt. The plants were transplanted on 15th March in free draining plastic pots (25 and 18 cm top and bottom diameter, respectively and 20 cm height, with holes in the bottom) which filled with 10 Kg sandy soil (one plant/pot, the average height of seedlings were 12-15cm). The experiment included 12 treatments which were the combinations of spraying three ascorbic acid levels: tap water, 100 and 200 ppm with four salinity levels: tap water, 3000, 6000 and 9000 ppm. Saline water was prepared by using a mixture of sodium chloride (NaCl) and calcium chloride (CaCl₂) 1:1 w/w. Irrigation with saline water treatments were started after one month from planting. The experimental design was Completely Randomized with three replicates. The following data were recorded at the second week of October of 2011 and 2012: plant height (cm), stem diameter (mm), leaves number/ plant, root length (cm), fresh and dry weights of shoot and roots (g). The following chemical analyses were determined: total carbohydrates percentages were determined according to the method of Dubois *et al.* [8], photosynthetic pigments (Chlorophyll a, b and Carotenoids in mg/g fresh leaves) were determined according to the procedure achieved by Saric *et al.* [9]. Total indoles were determined in the methanolic extract, using P-dimethyl aminobenzaldehyde test "Erlic's reagent" according to Larsen *et al.* [10]. Total soluble phenols were determined calorimetrically by using Folin Ciocalte a reagent A.O.A.C. [11]. Free amino acid content was determined according to Rosen [12]. Proline concentration was determined according to Bates *et al.* [13]. Nitrogen, phosphorus, potassium and sodium were determined in shoots and roots according to the method described by Cottenie *et al.* [14].

Statistical Analysis: The data were statistically analyzed for each season and then a combined analysis of the two seasons was carried out according to the procedure outlined by Steel and Torrie [15].

RESULTS AND DISCUSSION

Plant Growth: The results presented in Table 2 showed that spraying the seedlings of *Grevillea robusta* with 100 or 200 ppm ascorbic acid significantly increased plant height, stem diameter, root length, leaves number/plant, fresh and dry weights of shoots and roots compared with untreated plants (control). The highest values of growth characters were obtained by spraying the seedlings with ascorbic acid at 200 ppm. Application of ascorbic acid at 200 ppm increased plant height and stem diameter by 19.5 and 30.0 %, respectively corresponding values of control plants. Studies of Hassanein *et al.* [16] and Abd-El Hamid [17] suggested that ascorbic acid increased IAA content, which stimulates cell division and/cell enlargement and this in turn, improved plant growth. A significant reduction in all growth characters were occurred by using high salinity level (9000 ppm) compared with control or other treatments. Under saline conditions, a reduced plant growth due to specific ion toxicities (e.g. Na⁺ and Cl⁻) and ionic imbalances acting on biophysical and/or metabolic components of plant growth occurs [18]. Irrigating the seedlings with low salinity level (3000 ppm) gave a significant increase in plant height, leaves number/ plant, fresh and dry weight of shoots compared with control or other treatments. In general, low salinity levels did not appear to have a deleterious effect on the growth of *Atriplex* spp. and may actually stimulate growth [19-20]. However, high salinity levels may cause a reduction in total growth of *Atriplex* spp., especially in leaf biomass [21-23]. The combined treatment of ascorbic acid at 200 ppm with saline water at 3000 ppm gave the highest values of plant height, leaves number/plant, fresh and dry weights of shoots. Foliar application of 200 mg/l ascorbic acid counteracted the adverse effect of salinity that accompanied by significant increase in plant growth of flux cultivars [2]. Application of 200 ppm ascorbic acid combined with irrigating the seedlings with tap water (control) gave the highest values of stem diameter, root

Table 2: Growth parameters of *Grevillea robusta* seedlings as affected by ascorbic acid (AA) and irrigation with saline water (average two seasons)

Characters	Treatments	Plant height (cm)	Stem diameter (mm)	Leaves number/ plant	Root length (cm)	Shoots fresh weight (g)	Shoots dry weight (g)	Roots fresh weight (g)	Roots dry weight (g)
Ascorbic acid (AA) (ppm)									
	0	85.6	10.0	45.8	27.8	97.7	36.8	46.4	20.3
	100	93.5	11.0	54.0	31.8	108.5	43.2	53.7	25.3
	200	102.3	13.0	61.0	36.0	119.1	49.1	59.4	30.5
	LSD 5%	3.3	0.03	2.2	1.3	2.7	2.1	2.2	1.7
Salinity (S) (ppm)									
	0	96.2	15.0	56.3	40.6	111.1	46.4	65.1	32.8
	3000	108.4	12.0	65.8	33.8	126.5	56.3	57.0	28.1
	6000	88.9	9.0	49.7	28.9	102.5	37.8	48.6	22.7
	9000	81.9	8.0	42.0	24.0	93.9	31.6	41.9	17.6
	LSD 5%	4.2	0.05	2.7	1.8	3.6	4.7	3.4	2.2
Interaction (ppm)									
	AA 0+ S 0	89.3	13.0	50.0	36.1	101.7	40.0	57.0	28.1
	AA 0+ S 3000	96.7	11.0	55.5	30.1	113.6	49.3	50.3	23.1
	AA 0+ S 6000	82.1	8.0	42.2	25.0	92.6	32.3	42.4	17.3
	AA 0+ S 9000	74.4	7.0	35.3	20.1	82.4	25.9	35.3	12.5
	AA 100+ S 0	95.5	15.0	57.1	40.2	110.4	46.4	65.7	32.5
	AA 100+ S 3000	108.9	12.0	67.6	33.6	125.3	56.7	57.4	28.3
	AA 100+ S 6000	88.4	9.0	49.6	28.9	102.5	37.9	49.1	22.5
	AA 100+ S 9000	81.2	8.0	41.7	24.3	95.9	31.9	42.5	17.7
	AA 200+ S 0	103.9	15.0	63.4	45.6	121.3	52.8	71.9	37.9
	AA 200+ S 3000	119.3	13.0	74.4	37.7	140.5	62.9	63.3	33.0
	AA 200+ S 6000	96.3	11.0	57.3	32.9	112.3	43.3	54.4	28.3
	AA 200+ S 9000	90.0	10.0	49.0	27.6	102.4	37.4	48.0	22.7
	LSD 5%	6.2	0.08	3.7	3.3	6.2	4.7	5.1	3.7

Table 3: Chlorophyll (a), (b), carotenoids (mg/g F.W) and total carbohydrate % of *Grevillea robusta* seedlings as affected by ascorbic acid (AA) and irrigation with saline water (average two seasons)

With Saline Water (Average 7th Season)				Total carbohydrate %	
Characters				-----	
Treatments	Chlorophyll (a)	Chlorophyll (b)	Carotenoids	Shoots	Roots
Ascorbic acid (AA) (ppm)					
0	1.645	0.708	1.320	26.62	21.76
100	1.917	0.777	1.371	30.29	23.73
200	2.173	0.812	1.463	32.63	25.04
LSD 5%	0.018	0.015	0.021	1.05	2.03
Salinity (S) (ppm)					
0	2.143	0.937	1.584	35.28	27.65
3000	2.113	0.931	1.513	32.89	25.34
6000	1.755	0.707	1.295	27.97	22.46
9000	1.518	0.528	1.148	23.25	18.58
LSD 5%	0.026	0.018	0.027	2.01	3.11
Interaction (ppm)					
AA 0+ S 0	1.913	0.912	1.513	32.11	26.12
AA 0+ S 3000	1.823	0.831	1.461	30.12	24.13
AA 0+ S 6000	1.517	0.653	1.213	24.65	20.13
AA 0+ S 9000	1.325	0.435	1.096	19.61	16.65
AA 100+ S 0	2.215	0.935	1.565	35.61	27.81
AA 100+S 3000	2.106	0.907	1.512	33.31	25.13
AA 100+S 6000	1.835	0.713	1.296	28.12	23.13
AA 100+S 9000	1.513	0.553	1.112	24.13	18.85
AA 200+S0	2.653	0.965	1.673	38.12	29.02
AA 200+S 3000	2.411	0.931	1.567	35.25	26.75
AA 200+S 6000	1.914	0.755	1.375	31.13	24.12
AA 200+S 9000	1.714	0.597	1.235	26.01	20.25
LSD 5%	0.031	0.024	0.036	3.27	3.40

length, fresh and dry weights of roots. Spraying the seedlings with tap water + 9000 ppm saline water gave the lowest values of growth characters.

Photosynthetic Pigments and Total Carbohydrates

Content: Data presented in Table 3 indicated that foliar application of 100 or 200 ppm ascorbic acid significantly increased chlorophyll a, b, carotenoids content and total carbohydrates % of shoots and roots compared with control plants. The highest values were recorded with 200 ppm ascorbic acid. The substantial increase in carbohydrate contents may be due to the activation of photosynthetic machinery, as a result of the stimulatory effects of the used plant growth bio stimulators on photosynthetic process. Chlorophyll a & b contents and total carbohydrates % of shoots and roots were reduced as external salinity in irrigation water increased. This effect was most severe at 9000 ppm salinity, where significant reductions about 29.2, 43.6, 27.5, 34.1 and 32.8% in Chl (a), Chl (b), carotenoids and total carbohydrates of shoots and roots, respectively, were observed. Increasing sodium concentration in plant tissue can increase oxidative stress, which causes deterioration in chloroplast structure and an associate lose in chlorophyll. This leads to a decrease in chlorophyll, while increasing carotenoids content [24]. EL-Hariri *et al.* [2] suggested the effect of salinity on decreasing chlorophyll synthesis which ultimately reduces the biosynthesis of carbohydrates. The combination between foliar application of ascorbic acid at 200 ppm + irrigating the plants with tap water produced the highest values of chlorophyll a, b, carotenoids content and total carbohydrates % of shoots and roots, the lowest values resulted from the combination between foliar applications of ascorbic acid at 0 ppm (control) + salinity water at 9000 ppm.

Phenols, Indoles, Amino Acids and Proline Content:

Data in Table 4 recorded that the contents of phenols and proline in shoots and roots were decreased by using 100 or 200 ppm ascorbic acid. However, foliar application of ascorbic acid at 100 and 200 ppm gave the highest values of amino acids and total indoles, respectively in shoots and roots. The present data are in agreement with the finding of Reda and Gamal El-Din [25] on chamomile and Abdel-Aziz *et al.* [26] on gladiolus, they reported that foliar application of ascorbic acid increased total indoles content. The highest values of phenols, indoles and proline in shoots and roots resulted from the highest salinity level (9000 ppm), whereas salinity level at 3000 ppm resulted in the highest values of amino acids in

shoots and roots. The increase of proline concentrations in *Ceriops tagal* with increasing Na concentration indicates that higher proline accumulation may help alleviate NaCl stress in *C. tagal*, although the increase may be too small to influence osmotic balance significantly [27]. Rivero *et al.* [28] suggested that an accumulation of phenolic compounds in response to abiotic stress would be attributed to activation of phenyl alanine ammonialyase (PAL). This would be beneficial to achieve acclimatization and tolerance to water- deficit stress, since many kinds of plant phenolics have been considered to the main lines of cell acclimatization against stress in plant. Saline water at the highest level (9000 ppm) + foliar the plants with tap water (control) resulted in the highest values of phenols, indoles and proline in shoots and roots. While, spraying the plants with ascorbic acid at 100 ppm + salinity level at 3000 ppm recorded the highest values of amino acids in shoots and roots.

Minerals Concentration:

Results in Table 5 indicated that nitrogen (N), phosphorus (P) and potassium (K) contents in shoots and roots were increased by gradually increasing ascorbic acid level. Ascorbic acid (AA) protect metabolic processes against H_2O_2 and other toxic derivatives of oxygen affected many enzyme activities, minimize the damage caused by oxidative processes through synergistic function with other antioxidants and stabilize membranes [29]. Also, ascorbic acid is more effective at 400 mg/l and attributed to the increase in nutrient uptake and assimilation. Foliar application of ascorbic acid at 100 and 200 ppm caused the lowest values of sodium (Na) in shoots and roots, respectively. All salinity treatments reduced N, P and K % in shoots and roots (except N % in shoots) and the greatest reductions resulted from the highest salinity level (9000 ppm), while the maximum Na % in shoots and roots was recorded with the highest salinity level (9000 ppm). In the present study, the decrease of K^+ concentration with increasing irrigation salinity suggests that Na^+ inhibited the K^+ uptake. These results are in agreement with those obtained by Khan *et al.* [30], on *Atriplex griffithii* who found that the Na^+ and Cl^- content in both shoots and roots were increased with increasing salinity. Increasing NaCl induced decreases in K^+ and Ca^{++} , Mg^{++} in plants. Also, Patel *et al.* [27] on *Ceriops tagal* reported that concentrations of N, P and K decreased significantly in leaves, stems and roots in response to increasing salt concentration. Sodium content was increased significantly in leaves, stems and roots with increasing soil salinity. The highest N, P and K % in shoots and roots were recorded when foliar application of 200 ppm

Table 4: Phenols, indoles, free amino acid and proline ($\mu\text{mole/g F.W.}$) of *Grevillea robusta* seedlings as affected by ascorbic acid (AA) and irrigation with saline water (average of two seasons)

Characters Treatments	Phenols (mg/g F.W)		Indoles (mg/g F.W)		Free amino acid (%)		Proline	
	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
Ascorbic acid (AA)								
0	15.67	16.07	2.15	3.19	0.4 5	0.4 0	7.50	8.88
100	12.66	14.02	2.40	3.49	0.6 3	0.62	6.16	7.51
200	14.55	15.05	2.63	3.98	0.5 7	0.57	6.71	6.63
Salinity (S) (ppm)								
0	12.55	13.25	2.85	4.35	0.59	0.56	3.57	4.83
3000	13.00	13.74	2.35	3.69	0.64	0.59	6.16	6.83
6000	14.47	15.67	2.26	3.20	0.53	0.49	8.06	8.91
9000	17.14	17.58	2.10	2.97	0.43	0.47	9.36	10.12
Interaction (ppm)								
AA 0+ S 0	13.53	13.76	2.56	3.76	0.48	0.41	4.31	5.67
AA 0+ S 3000	14.12	14.25	2.14	3.27	0.54	0.48	6.96	7.76
AA 0+ S 6000	16.31	16.67	2.05	2.96	0.41	0.37	8.36	10.32
AA 0+ S 9000	18.71	19.61	1.83	2.78	0.39	0.36	10.37	11.75
AA 100+ S 0	11.25	12.75	2.87	4.15	0.57	0.55	3.05	4.85
AA 100+ S 3000	11.75	13.15	2.35	3.67	0.73	0.69	5.67	6.67
AA 100+ S 6000	12.56	14.63	2.26	3.12	0.61	0.58	7.65	8.67
AA 100+ S 9000	15.07	15.56	2.12	3.01	0.47	0.54	8.27	9.85
AA 200+ S 0	12.87	13.25	3.12	5.13	0.51	0.60	3.36	3.96
AA 200+ S 3000	13.13	13.81	2.56	4.12	0.64	0.61	5.85	6.65
AA 200+ S 6000	14.55	15.57	2.47	3.53	0.58	0.53	8.16	7.75
AA 200+ S 9000	17.65	17.56	2.35	3.12	0.44	0.51	9.45	8.76

Table 5: Nitrogen (N), phosphorus (P), potassium (K) and sodium (Na) % of *Grevillea robusta* seedlings as affected by ascorbic acid (AA) and irrigation with saline water (average of two seasons).

Characters Treatments	Shoots				Roots			
	N%	P%	K%	Na%	N%	P%	K%	Na%
Ascorbic acid (AA) (ppm)								
0	1.99	0.103	0.90	2.56	2.14	0.128	1.15	2.74
100	2.16	0.117	1.05	2.26	2.32	0.138	1.26	2.64
200	2.30	0.135	1.19	2.35	2.50	0.149	1.33	2.50
Salinity (S) (ppm)								
0	2.70	0.145	1.28	2.16	2.87	0.155	1.46	2.44
3000	2.76	0.128	1.12	2.28	2.70	0.150	1.36	2.55
6000	1.95	0.117	0.97	2.45	2.14	0.134	1.22	2.69
9000	1.22	0.083	0.81	2.67	1.55	0.113	0.95	2.82
Interaction (ppm)								
AA 0 + S 0	2.55	0.125	1.12	2.34	2.81	0.143	1.35	2.56
AA 0 + S 3000	2.63	0.116	0.95	2.45	2.63	0.140	1.30	2.63
AA 0 + S 6000	1.83	0.101	0.82	2.61	1.96	0.126	1.15	2.82
AA 0 + S 9000	0.96	0.071	0.70	2.83	1.15	0.102	0.81	2.93
AA 100+ S 0	2.72	0.144	1.29	2.02	2.85	0.156	1.49	2.45
AA 100+ S 3000	2.77	0.123	1.13	2.17	2.67	0.149	1.36	2.60
AA 100+ S 6000	1.96	0.119	0.96	2.31	2.12	0.135	1.21	2.70
AA 100+ S 9000	1.25	0.083	0.82	2.53	1.63	0.112	0.96	2.81
AA 200+ S 0	2.84	0.167	1.43	2.11	2.96	0.166	1.53	2.31
AA 200+ S 3000	2.87	0.145	1.29	2.21	2.81	0.161	1.42	2.42
AA 200+ S 6000	2.05	0.130	1.12	2.43	2.35	0.142	1.29	2.55
AA 200+ S 9000	1.45	0.096	0.91	2.65	1.87	0.125	1.09	2.73

ascorbic acid combined with tap water, except N % in shoots resulted from ascorbic acid at 200 ppm+3000 ppm salinity level. Ascorbic acid has effects on many physiological processes including the regulation of growth and metabolism of plants under saline conditions and increasing physiological availability of water and nutrient [31]. While, the lowest N, P and K % in shoots and roots resulted from the highest salinity level (9000 ppm) under ascorbic acid at 0 ppm (control).

CONCLUSION

From the above mentioned results, it can be concluded that ascorbic acid application decreased the hazard effect of irrigation with saline water, in addition had favorable effect on growth and availability of chemical compositions to *Grevillea robusta* seedlings grown in sandy soil.

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