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Full Length Article

Benzyladenine Can Alleviate Saline injury of Two Roselle (*Hibiscus sabdariffa*) Cultivars via Equilibration of Cytosolutes Including Anthocyanins

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ABSTRACT

The changes in dry matter and the concentration of organic solutes in roots, shoots and sepals of two roselle (*Hibiscus sabdariffa* L.) cultivars (cvs. light red sepals & deep red sepals) under salt stress and spraying with 250 mg L^{-1} benzyladenine (BA) were assessed. The cv. light red sepals tolerated salinity up to 25 mM NaCl, while cv. deep red sepals showed least tolerance to salinity stress. This was accompanied with differences in accumulation of saccharides, nitrogen compounds and anthocyanin. The reaction to salt stress was expressed as proline accumulation in both roots and shoots of two roselle cultivars with higher response in deep red sepals. Anthocyanins content was also higher in cv. light red sepals (more salt tolerant) than cv. deep red sepals (more salt sensitive). These results suggest that differences in anthocyanins content in the two roselle cultivars under NaCl are a kind of response to this abiotic environmental stress. The results of the same experiment indicated that the application of BA could partially alleviate the salt stress symptoms in both roselle cultivars.

Key Words: Anthocyanins; Benzyladenine; Proline; Roselle cultivars; Salinity

INTRODUCTION

Roselle plant (*Hibiscus sabdariffa* L.) is one of the most important and popular medicinal and industrial plants. It is well known in Egypt with the name of "Karkadeh" (Chang *et al*., 2006). Salinity is one of the major environmental factors that limit crop production (Koyro, 2006). Egypt is one of the countries that suffer from severe salinity problems. For example, 33% of the cultivated land that comprises only 3% of total land area in Egypt is salinized due to low precipitation (<25 mM annual rainfall) and irrigation with saline water (El-Hendawy *et al*., 2004).

Osmotic adjustment is a plant adaptation mechanism found in both halophytes (Flowers *et al*., 1977) and glycophytes (Greenway & Munns, 1980) in order to maintain their water balance. It involves two processes: absorption of ions from root environment followed by their accumulation in the vacuoles and the synthesis of compatible organic solutes that are accumulated in the cytosol (Prisco, 1980; Almodares *et al*., 2008). Among the organic solutes found in higher plants acting as osmoregulators proline is conspicuous (Yoshiba *et al*., 1997; Heidari & Mesri, 2008). In addition to its function as an osmoregulator, proline may protect enzyme-proteins from ion inhibitory effect (Solomon *et al*., 1994). It also stabilizes cellular structures and functions as source of carbon and nitrogen for metabolism (Jager & Meyer, 1977) as well as a regulator of cytosolic pH (Venekamp, 1989). Beside proline, other N-containing compounds such as other amino acids (Karamanos, 1995; Morsy, 2008) and soluble proteins (Niu *et al*., 1997; Morsy, 2008) could protect plant tissues against osmotic stress. In addition to these compounds, soluble carbohydrates also accumulate in the cytoplasm of plants or cells subjected to salt stress (Garcia *et al*., 1997; Pattanaqul & Thitisakaskul, 2008).

Anthocyanins are one of the most important and ubiquitous compound, which acts both as osmotic adjusters (Chalker-Scott, 1999) and scavenger of active oxygen (Gould, 2000). Anthocyanins are water-soluble pigments derived from flavonoids via shikimic acid pathway. The best known function of these colorful pigments is that it does not only account for beautiful color in the petals of flowering plants, but also serve as factors in plant reproduction by recruiting pollinators and seed disperses (Winkel-Shirly, 2002). However, the role of anthocyanins in plant foliage has long been the subject of study and speculation.

Plant hormones are active members of the signal cascade involved in the induction of plant stress responses (Pedranzani *et al*., 2003). Abiotic stresses result in both altered levels of phytohormones and decreased plant growth (Morgan, 1990). An alternative strategy to ameliorate salt stress could be, therefore, to use exogenous application of

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plant growth regulators. Benzyladenine (BA) is one of the naturally occurring cytokinins (Nair *et al*., 2002) and its increasing concentration in crops could be a possible means of reversing the effects of salt stress.

The main purpose of the present work was to study the changes in growth and metabolic activities of two roselle cultivars (cv. light red sepals & cv. deep red sepals cv.) that are known to show differences in tolerance to salt stress. The evaluation of anthocyanin concentration in the sepals of two roselle cultivars under different levels of salt stress was also targeted for its possible role in plant protection from NaCl stress. We have also studied the role of BA application at 250 mg L^{-1} as a foliar spray in overcoming the stress effects.

MATERIALS AND METHODS

The Seeds of two roselle (*Hibiscus sabdariffa* L.) cultivars (cv. light red sepals & cv. deep red sepals) were obtained from the breeding program of Agriculture Research, Dokky, Cairo, Egypt. Seeds of two tested roselle cultivars were surface sterilized with mercuric chloride (0.01%) for 5 min and rinsed 3 times with distilled water. Seeds were then sown in weighed plastic pots (7 seeds per pot) containing 5 kg dry clay soil. The pots were daily irrigated with water and left until emergence of seedlings (15 d). Thereafter, the pots were watered with the salinization levels (0, 25, 50, 75, 100 & 125 mM NaCl) by adjusting the water content of soil to the field capacity. After one day from irrigation with NaCl, some plants were sprayed with an aqueous solution of 250 mg L^{-1} BA. After a week of spraying, the same plants were sprayed again with the same BA solution. There were two different groups of treatments; one group was salinity treatments, while in other group the salinity-treated plants sprayed with BA. Three replicates were prepared for each treatment. All plants were irrigated once every week with nitrogen-free 1/10 Hoagland's solution. At the end of the experimental period (160 days), the dry matter of roots and shoots was determined after drying the fresh roots and shoots in an aerated oven at 80°C. Fresh sepals were dried in the air to constant weight (El-Meleigy, 1989).

Carbohydrates were determined by the anthrone sulphuric acid method described by Badour (1959). The dried tissue of samples was extracted by distilled water (in case of soluble or HCl in case of total carbohydrates). One mL of the carbohydrate extract was mixed with nine mL of anthrone-sulphuric acid reagent in a test tube and heated for 7 min at 100°C. The absorbance was taken on spectrophotometer (Spectronic Genesys ZPC, Rochester, NY, USA) at 620 nm against blank containing only distilled water and anthrone reagent. The insoluble carbohydrates were calculated by subtract soluble from total carbohydrates.

Soluble and total proteins were determined according to Lowery *et al*. (1951). Free amino acids were extracted and estimated according to the method of Lee and Takahashi (1966). About 0.1 mL of the water extract containing free amino acids was mixed with 1.9 mL of ninhydrin-citrate glycerol mixture in a test tube for 20 min at 100°C. The absorbency was read at 570 nm against blank (only distilled water & the same reagent).

Free proline content was estimated according to Bates *et al*. (1973). A known weight of dried tissue was homogenized in 10 mL of 3% sulfosalicylic acid and filtered. Two mL of the filtrate was reacted with 2 mL glacial acetic acid and 2 mL of acid-ninhydrin reagent in a test tube and heated for 1 h at 100°C. The reaction mixture was extracted with 4 mL toluene. The chromophore was aspired from the aqueous phase and the absorbency was read at 520 nm using toluene as a blank. Anthocyanins content was determined according to the method adopted by Hoagland (1980).

Statistical analysis. The data of all experiments were subjected to analysis by the least significant differences test (L.S.D.) using SPSS program.

RESULTS

The results show that the two tested roselle cultivars were able to survive until reaching the fruiting stage when irrigated with salinity up to the level of 125 mM NaCl (Table I). Data revealed that NaCl salinity induced mostly a non-significant effect in the production of dry matter yield in different organs of cv. light red sepals up to the level of 25 mM NaCl (Table I). Thereafter, there was a gradual reduction in dry matter production with increasing of the salinity in the soil.

Supplementing the culture media with increased salinity resulted in a highly significant decrease in dry matter production in the different organs (roots, shoots & sepals) of cv. deep red sepals even at the lowest salinization level. This indicates that cv. deep red sepals was the more salt sensitive cv when compared to cv. light red sepals. Spraying with BA induced a considerable increase in dry matter yields in the different organs of the two tested cultivars as compared with the corresponding salinity treatments (Table I).

Salinity stress mostly induced a marked increase in the soluble and total carbohydrates of roots and sepals of cv. light red sepals (Table II) and in the content of total carbohydrates of shoots. On the other hand, there was an irregular decrease in soluble carbohydrates in shoots of this tested cultivar. There was a marked variation in the accumulation of carbohydrates content among the plant organs of cv. deep red sepals (Table III). All salt levels induced a marked reduction in soluble and total carbohydrates in the roots of cv. deep red sepals. In shoots of the same cultivar, soluble fraction markedly increased, while the total carbohydrates significantly decreased with the increase in salinity levels, in sepals, the soluble fraction increased gradually by increasing salinity and the total carbohydrates decreased significantly. In general, BA treatment resulted in a marked stimulation in soluble and total carbohydrates content of two tested roselle cultivars as compared with those of corresponding salinization levels (Table II & III).

Table I. Effect of salinity treatments or spraying with BA on dry matter (g plant^{-1}) of two roselle cultivars grown for 160 days

		cv. light red sepals			cv. deep red sepals		
Treatment	**NaCl (mM)**	**Roots**	**Shoots**	**Sepals**	**Roots**	**Shoots**	**Sepals**
	0	0.654	5.171	0.339	1.184	8.187	0.292
	25	0.574	4.547	0.330	0.529**	4.922**	0.180**
	50	0.543*	4.358*	0.276**	0.366**	4.595**	0.143**
Reference control	75	0.382**	3.505**	0.186**	0.325**	3.805**	0.133**
	100	0.324**	3.398**	0.130**	0.217**	3.093**	0.074**
	125	0.250**	2.345**	0.123**	0.223**	2.004**	0.060**
	0	0.985**	9.881**	0.651**	1.596**	12.106**	0.396**
	25	0.850**	5.875*	0.504**	0.598**	5.566**	0.347
NaCl + 250 mg L^{-1} BA	50	0.635	5.598	0.428**	0.583**	4.965**	0.291
	75	0.498**	4.885	0.330	0.459**	4.123**	0.252
	100	0.485**	4.182**	0.327	0.423**	3.987**	0.207*
	125	0.461**	4.013**	0.319	0.374**	3.849**	0.164**
	5%	0.084	0.685	0.024	0.246	0.668	0.077
L.S.D	1%	0.120	0.923	0.033	0.332	0.900	0.104

*Significant differences at ($p = 0.05$) level, ** Highly significant differences from control at ($p = 0.01$) level

Table II. Effect of salinity treatments or spraying with BA on soluble and total saccharides content (mg g^{-1} dry matter) of roselle cv. light red sepals grown for 160 days

		cv. light red sepals					
		Soluble carbohydrates			Total carbohydrates		
Treatment	**NaCl (mM)**	**Roots**	**Shoots**	**Sepals**	**Roots**	**Shoots**	**Sepals**
	0	35.302	103.094	54.621	111.416	126.312	171.038
	25	38.836*	98.914	55.229	111.910	127.452	171.000
Reference control	50	43.510**	85.348**	55.602	122.626**	123.120	177.612*
	75	48.412**	80.522**	55.011	121.486**	127.528	207.611**
	100	51.756**	90.402**	55.191	130.910**	130.112	213.978**
	125	38.190	94.202**	61.636	131.708**	140.752**	213.294**
	0	56.506**	105.111	64.898	177.688**	133.011*	166.917
	25	56.810**	99.880	64.779	172.254**	125.197	271.142**
NaCl + 250 mg L^{-1} BA	50	47.120**	96.558**	65.874*	150.290**	108.262**	267.620**
	75	46.512**	87.628**	66.147*	111.948	115.064**	226.340**
	100	45.904**	83.012**	66.850*	87.666**	155.192**	258.541**
	125	44.232**	70.794**	66.804*	81.358**	123.196	227.886**
L.S.D	5%	3.350	5.328	10.618	4.555	5.542	4.346
	1%	4.511	7.175	14.300	6.134	7.464	10.787

*Significant differences at ($p = 0.05$) level, ** Highly significant differences from control at ($p = 0.01$) level

Table III. Effect of salinity treatments or spraying with BA on soluble and total saccharides content (mg g^{-1} dry matter) of roselle cv. deep red sepals grown for 160 days

		cv.deep red sepals					
		Soluble carbohydrates			Total carbohydrates		
Treatment	**NaCl (mM)**	**Roots**	**Shoots**	**sepals**	**Roots**	**Shoots**	**Sepals**
	0	67.260	53.067	51.786	233.510	270.421	186.770
	25	57.646**	63.327**	54.659	199.405**	268.717	214.928**
Reference control	50	36.556**	70.053**	54.750	157.396**	237.975**	174.534**
	75	37.050**	73.568**	54.986	154.451**	227.088**	173.147**
	100	31.844**	68.058**	55.222	146.756**	207.898**	171.760**
	125	34.162**	60.667**	55.761	109.801**	236.417**	163.438**
	0	63.270*	83.182**	55.153	242.615	299.858**	178.220*
	25	59.052**	96.976**	55.693	241.543	262.960	178.486*
NaCl + 250 mg L^{-1} BA	50	58.558**	70.978**	55.738	209.448*	288.800**	196.004**
	75	58.216**	103.778**	56.278	190.182**	294.766**	214.153**
	100	53.941**	103.816**	56.525	176.354**	297.540**	232.009**
	125	49.666**	103.854**	56.772	162.526**	300.314**	232.783**
L.S.D	5%	3.706	3.728		18.750	11.170	7.053
	1%	4.991	5.021		25.250	15.043	9.499

*Significant differences at ($p = 0.05$) level, ** Highly significant differences from control at ($p = 0.01$) level.

From the data in Tables IV and V, it is evident that while salinity stress increased the soluble proteins, the total proteins content remained unchanged at the all salinity levels content in roots of cv. light red sepals and in the different organs of cv. deep red sepals. The used levels enhanced the soluble and total proteins content in both shoots and sepals of cv. light red sepals (Table IV). Spraying with BA induced a considerable increase in soluble and total proteins content in roots and sepals of both roselle cultivars and in total proteins content in shoots of cv. light red sepals when compared with those of corresponding salinized plants. On the other hand, the soluble proteins

content smoothly decreased in cv. light red sepals shoots due to BA treatment as compared with control. BA also induced a marked decrease in proteins content in shoots of cv. deep red sepals at the most salinity levels used as compared with the corresponding salinization levels (Tables IV & V).

Applied salinity levels induced a marked accumulation in total free amino acids content in roots, shoots and sepals of the two tested roselle cultivars (Table VI). In general, spraying with BA resulted in a significant reduction in total free amino acids content in the different organs of cv. light red sepals as compared with those of corresponding salinity

Table IV. Effect of salinity treatments or spraying with BA on soluble and total proteins content (mg g^{-1} dry matter) of roselle cv. Light red sepals grown for 160 days

		cv. Light red sepals					
		Soluble proteins			Total proteins		
Treatment	NaCl (mM)	Roots	Shoots	Sepals	Roots	Shoots	Sepals
	0	47.250	51.075	142.155	63.900	61.425	201.780
	25	45.450	53.325	167.175**	64.575	63.900	225.765**
Reference control	50	48.600	52.425	171.540**	60.075*	62.200	213.120**
	75	49.275	53.775	185.400**	60.300	61.875	221.490**
	100	50.175	58.050**	211.140**	60.750	64.800*	244.125**
	125	51.075*	61.875**	204.750**	58.500**	70.425**	234.225**
	0	41.513**	54.200*	165.870**	62.882	68.150**	224.505**
	25	49.950	47.800**	179.370**	62.775	62.875	235.260**
NaCl + 250 mg L^{-1} BA	50	51.975*	49.375	175.005**	64.575	63.550	234.450**
	75	58.725**	51.750	176.445**	70.875**	65.475*	224.910**
	100	55.013**	54.225*	191.205**	66.488	67.725**	233.685**
	125	54.675**	56.475**	197.460**	64.350	69.525**	242.010**
L.S.D	5%	3.519	3.123	4.343	3.618	4.021	5.319
	1%	4.740	4.206	5.849	4.872	5.415	7.163

*Significant differences at ($p = 0.05$) level, ** Highly significant differences from control at ($p = 0.01$) level

Table V. Effect of salinity treatments or spraying with BA on soluble and total proteins content (mg g^{-1} dry matter) of roselle cv. deep red sepals grown for 160 days

		cv. deep red sepals					
		Soluble proteins			Total proteins		
Treatment	NaCl (mM)	Roots	Shoots	Sepals	Roots	Shoots	Sepals
	0	31.725	49.725	139.005	67.500	81.225	216.450
	25	33.300	51.075	149.265**	69.075	73.350**	226.125**
Reference control	50	42.750**	58.275**	157.050**	69.300	79.425	220.230**
	75	41.850**	63.225**	158.535**	60.750	81.450	221.715**
	100	43.875**	68.625**	158.535**	62.775	87.300**	221.445**
	125	44.100**	66.600**	157.410**	61.200	86.400**	211.050**
	0	37.260**	53.190*	148.298**	69.323	87.390**	222.638**
	25	39.690**	53.190*	156.128**	69.360	78.390	230.198**
	50	41.580**	53.730*	176.175**	69.593	73.080**	248.715**
NaCl + 250	75	43.470**	54.270*	186.030**	69.120	74.520**	255.712**
mg L^{-1} BA	100	43.187**	50.082	204.660**	67.187	67.857**	271.485**
	125	42.930**	45.900*	223.290**	65.205	52.425**	290.115**
L.S.D	5%	3.307	3.380	4.574	9.521	3.671	5.634
	1%	4.454	4.551	6.160	12.822	4.944	7.588

*Significant differences at ($p = 0.05$) level, ** Highly significant differences from control at ($p = 0.01$) level

Table VI. Effect of salinity treatments or spraying with BA on total free amino acids content (mg g^{-1} dry matter) of two roselle (cv.light red sepals & cv. deep red sepals) grown for 160 days

		cv. light red sepals			cv. deep red sepals		
Treatment	NaCl (mM)	Roots	Shoots	Sepals	Roots	Shoots	Sepals
	25	12.900**	14.463*	16.593	8.925	9.363*	15.480**
Reference control	50	10.663**	17.350	16.635	10.600**	11.188**	16.173**
	75	14.163**	19.775**	17.463**	11.100**	11.768**	16.425**
	100	14.575**	20.388**	18.285**	11.375**	12.350**	16.675**
	125	9.388	17.813	19.093**	10.900**	15.300**	15.980**
	0	6.125**	8.375**	16.250	9.375*	11.013**	15.438**
	25	5.813**	6.988**	17.528**	9.325*	11.063**	15.693**
NaCl + 250 mg L^{-1} BA	50	5.713**	10.975**	16.490	9.550**	12.275**	16.023**
	75	8.338	14.125**	16.838	9.568**	12.230**	16.315**
	100	6.413**	15.025	16.785	9.588**	12.200**	16.121**
	125	10.600**	15.338	15.860	8.713	12.513**	15.795**
	5%	1.484	1.913	1.479	0.978	1.115	1.311
L.S.D	1%	1.999	2.577	1.992	1.318	1.501	1.765

*Significant differences at ($p = 0.05$) level, ** Highly significant differences from control at ($p = 0.01$) level.

Table VII. Effect of salinity treatments or spraying with BA on proline content (mg g^{-1} dry matter) of two roselle (cv. light red sepals & cv. deep red sepals) grown for 160 days

		cv. light red sepals			cv. deep red sepals		
Treatment	**NaCl (mM)**	**Roots**	**Shoots**	**Sepals**	**Roots**	**Shoots**	**Sepals**
Reference control	0	0.207	0.207	0.551	0.109	0.418	0.370
	25	0.229	0.205	0.562	0.151**	0.474**	0.476**
	50	0.247*	0.285**	0.662**	0.373**	0.553**	0.553**
	75	0.235	0.376**	0.682**	0.404**	0.539**	0.553**
	100	0.256**	0.445**	0.840**	0.499**	0.539**	0.554**
	125	0.260**	0.390**	1.206**	0.560**	0.543**	0.545**
NaCl + 250 mg L^{-1} BA	0	0.151**	0.140**	0.310**	0.104	0.429	0.359
	25	0.161**	0.135**	0.422**	0.210**	0.455	0.487**
	50	0.152**	0.215	0.425**	0.304**	0.518**	0.492**
	75	0.152**	0.221	0.434**	0.429**	0.518**	0.496**
	100	0.240	0.235	0.345**	0.441**	0.522**	0.520**
	125	0.221	0.249*	0.473**	0.641**	0.541**	0.545**
	5%	0.034	0.041	0.040	0.030	0.040	0.038
L.S.D	1%	0.046	0.055	0.053	0.040	0.054	0.051

*Significant differences at ($p = 0.05$) level, ** Highly significant differences from control at ($p = 0.01$) level

levels. A marked reduction was also evident in total free amino acids for both roots and sepals of cv. deep red sepals was recorded at most BA treated plants as compared with reference control. On the other hand, in shoots of cv. deep red sepals, phytohormonal treatment stimulated the accumulation of total free amino acids content as compared with salinized plants (Table VI).

The content of proline in roots, shoots and sepals of the cv. light red sepals was markedly accumulated especially at the higher salinization levels (Table VII).

All salinity levels induced a highly significant and progressive accumulation in proline content in the three organs of cv. deep red sepals. The accumulation of proline content in roots was much more obvious than shoots and sepals (Table VII). Phytohormonal treatments, in general, resulted in a pronounced decrease in free proline content of the three organs for both of the tested cultivars as compared with reference control (Table VII).

Salinity levels induced a significant reduction in anthocyanins content in most cases for both of roselle cultivars. This reduction was much more obvious in cv. deep red sepals rather than cv. light red sepals (Table VIII). Spraying with BA induced the accumulation of anthocyanins content in two tested roselle cultivars versus the salinized plants (Table VIII).

Table VIII. Effect of salinity treatments or spraying with BA on anthocyanin content ($A_{525-585}$ g^{-1} fresh matter in 10 mL) of two roselle (cv. light red sepals & cv. deep red sepals) grown for 160 days

Treatment	**NaCl (mM)**	**cv. light red sepals**	**cv. deep red sepals**
	0	10.045	9.455
	25	9.524	7.662**
Reference	50	9.213	6.540**
Control	75	8.607**	6.682**
	100	8.551**	6.760**
	125	7.950**	6.515**
	0	11.433**	9.682**
	25	11.451**	9.503
NaCl+ 250	50	10.860	9.894**
mg L^{-1} BA	75	10.862	9.433
	100	10.380	8.193**
	125	10.008	7.141**
	5%	0.909	0.220
L.S.D	1%	1.225	0.296

*Significant differences at ($p \leq 0.05$) level, ** highly significant differences from control at ($p \leq 0.01$) level

DISCUSSION

In the present study, the growth of roots, shoots and sepals showed marked differences between two roselle cultivars for their response to salinity and BA treatment. The cv. light red sepals seemed to be less salt sensitive than cv. deep red sepals. These differences were adjudged by progressive increase in carbohydrates content in cv. Light red sepals, while their level dramatically decreased in cv. deep red sepals. Sugar accumulation and its distribution in different parts of the plants could be a valid trait to discriminate genotypes of different tolerance to saline and osmotic stresses and it is important to know the sink-source relationships are affected in plants growing under stress conditions (Balibrea *et al*., 1997). Accordingly, from a physiological point of view, cvs light red sepals and deep red sepals might be considered two different genotypes.

The data of carbohydrates reported that, the soluble sugars increased in roots and sepals of cv light red sepals and in shoots and sepals of cv. deep red sepals that might have a role in osmoregulation of the two cultivars (Table II & III). Handa *et al*. (1983), using cultured tomato cells adapted to water stress, found that the concentration of reducing sugars in the cells increased with the degree of adaptation to salinity as high as 600 mM in the cells.

In the current study, the two roselle cultivars maintained their soluble and total proteins content mainly around the control level even at higher salinization levels (Table IV & V). Salinity stress also stimulated the biosynthesis of proteins in sepals of two cultivars even at the higher salinity levels; being more pronounced in cv. light red sepals. The accumulation of proteins and saccharides, especially at the higher doses of salt, was linked to the marked depression of growth. This might confirm the ability of these cultivars to synthesis of diverse proteins and saccharides from the state of active growth to state of

survival (osmoregulation) (Shaddad *et al*., 2005). Surprisingly, a high proteins content was not at the expense of other amino acids that were found to be increase also by salinity stress (Table VI). Thus, the mechanism of salt tolerance of two roselle cultivars could be operated by the observable increase in saccharides, proteins and even amino acids (Abd El-Samad *et al*., 2004).

Spraying with BA induced a considerable increase in dry matter yields of the tested plants and thus, partially alleviated the effect of salt stress. This increase in dry weight of salt stressed plants, after BA treatments, may be due to an increase of plant efficiency in water uptake, conservation and utilization (Hassanein *et al*., 2005). The increase in dry matter due to spraying with BA can also be attributed to rapid increase in cell division, cell enlargement and accumulation of building units that accompanied by greater saccharide content than those of unsprayed plants (Abdel-Latef, 2003). This accumulation of sugar due to BA treatment might be linked with the efficiency of photosynthetic apparatus, which leads to increase in plant productivity and dry matter production (Azooz *et al*., 2004).

Increasing proteins content by BA treatment (Table IV & V) may also increase the formation of rough endoplasmic reticulum that provides the appropriate medium for increasing polyribosomes and mRNA (Kaber, 1987). On the other hand, the total free amino acids decreased in both roselle cultivars as a result of BA treatment. This reduction in accumulation of amino acids by BA appears due to the role of the hormone in enhancing the incorporation of free amino acids into conjugated proteins or iso-enzyme in order to increase the salt tolerance (Kasim & Dowidar, 2004).

The data of proline content revealed that, there is a negative correlation between growth criteria and content of proline accumulation in different organs of the two roselle cultivars. However, there are some exceptions. Firstly, in the less salt sensitive cultivar (cv. light red sepals), both of growth criteria and proline content remained almost unchanged at the lower levels of the salt (Table VII). In accordance with this, in different plant species proline does not start to accumulate until the plants are subjected to moderate and severe levels of stress (Story & Wyn Jones, 1979; Weimberg *et al*., 1982). Secondly, while proline content was more than fivefold in roots of cv. deep red sepals at the level of 125 mM NaCl, growth of this plant organ decreased by more than 80% at the same salinization level. On the other hand, in roots of cv. light red sepals (the most salt tolerant), the increase in proline content was not exceeding than 26% over the control, while the reduction in growth was only about 40% at the same salinization level (Table I). Accordingly, free proline emerged as indicator of salt injury rather than salt tolerance (Balibera *et al*., 1997). In contrast, shoots grown at 125 mM NaCl, proline content of cv. light red sepals increased by 88% over control that corresponded to 55% reduction in growth at the same salinization level (Table I). In shoots of cv. deep red sepals, the increase in proline content was only 29% over the control that corresponds to 76% reduction in growth at the same salinization level. Under this condition, the opposite conclusion of physiological significance of proline as salt tolerance sensor rather than saline injury sensor might be accepted. In conformity to this, complication in physiological significance of proline, when the salt affected plants sprayed with BA, proline concentration significantly declined, while the amount of dry matter of both roselle cultivars increased as compared with the corresponding salinized plants. As per this controversy, the role of proline in osmoregulation seemed to be still complicated (Shaddad *et al*., 2005), especially when considering the decline in free proline with salinity and BA spray was accompanied by a pronounced accumulation of other organic solute (saccharides & proteins). This might play a major role in osmoregulation and consequently, the contribution of proline in osmoregulation could be neglected.

One most remarkable way of resistances, suggested here, is the induction of anthocyanin in leaves in response to salinity stress (Gueta-Dahan *et al*., 1997; Wahid & Ghazanfar, 2006). The present work showed that cv. light red sepals accumulated much more anthocyanins than cv. deep red sepals even at the level of control. Additionally, while the concentration of anthocyanin in cv. light red sepals remained around the control value up to the level of 50 mM NaCl, it was reduced by more than 30% at the same salinization level in cv. deep red sepals (Table VIII). This indicated better salt tolerance of cv. light red sepals than cv. deep red sepals. These findings are in conformity with those reported by Kaliamoorthy and Rao (1994) who recorded about 40% increase in anthocyanin accumulation in maize due to treatment with 1% and 2% KCl and NaCl.

The production of anthocyanin increased in BA treated plants, because anthocyanin serves as a hydroxyl radical scavenger (Cooper, 2001), as a solute that protects macromolecules against denaturation, inhibits lipid peroxidation (Chalker-Scott, 1999) and stimulates the activities of phenyalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL) (Hoagland & Duke, 1981; Hassanein *et al*., 2005). These enzymes are produced in plant tissues as a natural defense in response to biotic and abiotic events (Dorey *et al*., 1999; Redman *et al*., 1999).

CONCLUSION

The two roselle cultivars differed in their response to the salinity conditions and exogenous application of BA seemed to nullify either partially or completely the observed increments in the growth criteria of the two tested roselle cultivars under NaCl stress probably via equilibration of cytosolutes including anthocyanin.

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