The effects of treatment with polyamines on dry matter and some metabolites in salinity – stressed chamomile and sweet majoram seedlings

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ABSTRACT

The seeds of two medicinal plants, namely *Chamomilla recutita* (Babong) and *Origanum majorana* (Bardaqoush) were subjected to germination in different NaCl concentrations, polyamines (putrescine, spermidine, spermine) and to combination of both. The results revealed that the growth alterations induced by NaCl were alleviated by various levels of polyamines. The organic solutes of both plant seedlings exhibited somewhat variable responses to various salinity levels or polyamines treatments and in combination of both treatments. Putrescine in *Ch. recutita* seedlings was more effective in alleviating the stress effects of salinization than spermidine and spermine, while in *O. majorana* seedlings spermidine was more effective. Generally, the degree of stimulation differed according to the type, concentration of the additive used and the type of the plant tested.

Keywords: sodium chloride; antioxidants; polyamine-induced senescence; salt-induced oxidative stress; ionic toxicity; osmotic stress; signaling molecules; proline

Salinity is one of the most important problems in the agricultural areas of the world. Nearly 20% of the world's cultivated area and nearly half of the world's irrigated lands are affected by salinity (Zhu 2001). The salt-affected soils contain excess salts that affect plants by decreasing the osmotic potential of the soil solution (osmotic stress), interfering with normal nutrient uptake, inducing ionic toxicity, and associating nutrient imbalances (An et al. 2003). Processes such as seed germination and seedling growth are adversely affected by high salt concentration, ultimately causing diminished economic yield and also quality of production (Sairam and Tyagi 2004, Ali et al. 2007).

Polyamines (PAs) are small aliphatic amines that are ubiquitous in plants, animals and microorganisms. Putrescine (Put), spermidine (Spd) and spermine (Spm) are the major PAs in plants and they are involved in various processes such as cell proliferation, growth, morphogenesis, differentiation and programmed cell death (Serafini-Fracassini et al. 2002, Ali et al. 2007, Kusano et al. 2007). Because of their polycationic nature at physiological pH, PAs are able to interact with proteins, nucleic acids, membrane phospholipids and cell wall constituents, thereby activating or stabilizing these molecules (Martin-Tanguy 2001, Ali et al. 2007).

Recent studies focused on the involvement of PAs in the defense reaction of higher plants to various environmental stresses (Kusano et al. 2007). Among common polyamines (PAs), Put is diamine, Spd is triamine and Spm is tetramine; they are biologically active compounds that have been recognized as modulators of plant growth and development, involved in various physiological processes. They are cationic molecules, positively charged under intracellular pH, and are essential for plant growth and differentiation (Ali et al. 2007), related to aging and senescence, and are usually involved in plant responses to environmental stress (Ndayiragije and Lutts 2005). Polyamines are also potent reactive oxygen species scavengers and inhibitors of lipid peroxidation (Ali et al. 2007). To ameliorate or to alleviate the adverse effects of salinity on plant growth and metabolism, several seed pretreatments were used. Thus, the aim of the present work is conducted to study the response of antioxidants-treated seeds to various levels of salinity. The interactive effect of salinity and seed presoaking in antioxidants (Put, Spd, Spm) on seedling growth and the chemical composition of the test plants *Ch. recutita* and *O. majorana*

was also considered in the current studies. The most applicable method for testing the effect of these substances is seed presoaking.

MATERIALS AND METHODS

The plant materials used in this investigation were *Chamomilla recutita* L. as Asteraceae and *Origanum majorana* as aromatic Lamiaceae; both are important medicinal plants grown in sand area in Egypt. The present work was carried out to study the effect of various levels of salinity on seedling growth and some relevant metabolic activities in *Ch. recutita* and *O. majorana* plants. The interactive effects of seed presoaking in antioxidants (Put, Spd, Spm) and salinization treatments were also followed.

Seedling growth. The interactive effect of seeds presoaked in polyamines (Put, Spd, Spm) each with different concentrations (0.01, 0.1, 1.5mM) and salinization treatments was studied. Ten seeds were placed between folded paper towels, covered by plastic wrap, rolled up, and placed up right in 500 ml beakers. Eight ml of the saline experimental solution were used to saturate the towels in each treatment. Seedlings were left to grow in dark at about 25°C. Distilled water was added to compensate for evaporation loss. At the end of the experimental period (10 days) the seedlings dry matter yield was recorded. In addition, soluble carbohydrates, soluble protein, proline and other free amino acids contents were determined.

Determination of water-soluble carbohydrates. To estimate water-soluble carbohydrates, a known weight of the tissue powder was hydrolyzed by distilled water for 2 h in a boiling water bath. After cooling the hydrolysate was filtered and the filtrate was completed to a definite volume; then the water-soluble carbohydrates were determined with the method of anthrone sulphuric acid reagent according to Irigoyen et al. (1992).

Tissue powder sample (20 mg) was boiled in 10 ml distilled water for 2 h. After cooling the water extract was centrifuged and the supernatant was decanted and completed to a definite volume by distilled water, then the soluble proteins were determined according to the method applied by (Saenz et al. 1993).

Determination of free amino acids (FAA). For the determination of FAA, photometric ninhydrin method applied by Ahmed et al. (1989) was used.

Determination of free proline. Free proline was determined according to Bates et al. (1973). Approximately 50 mg of dry plant material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the homogenate was filtered through two filter papers (Whatman). The filtrate (2 ml) was left to react with 2 ml acid ninhydrin reagent and 2 ml of glacial acetic acid for 1 h at 100°C. After cooling, the coloured reaction product was extracted with 4 ml toluene and was consequently shaken vigorously with a test stirrer for 15–20 s. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature. The absorbency was read at 520 nm using toluene as a blank. The proline concentration was determined from a standard curve and was calculated as mg proline/g dry matter.

Statistical analysis. The experimental design was a random complete block, with three replications. The data were analyzed by the STATGRAPHICS (Statistical Graphics Corporation, Princeton USA) statistical package with the *t*-test and ANOVA functions to assess significant differences among means.

RESULTS AND DISCUSSION

Dry matter of *Ch. recutita* seedlings were markedly increased with the rise of salinization level up to (100mM NaCl) and above that decreased when compared with the control. On the other side, dry matter of *O. majorana* seedlings were increased with the salinization level rising up to 75mM NaCl. Relatively higher salinization level resulted in a progressive decrease of dry matter. Dry matter content of *Ch. recutita* seedlings exhibited more or less the same trend as those of the control with increased PAs (Put, Spd, Spm) concentrations. In case of *O. majorana* seedlings, dry matter exhibited similar trend to those of the control with the increasing PAs concentrations as well (Tables 1 and 2).

In the case of PAs-treated seeds, the adverse effects of low and moderate levels of NaCl on the growth of *Ch. recutita* and *O. majorana* seedlings were alleviated when compared with the corresponding treatments with NaCl; a more pronounced response appeared in *Ch. recutita* seedlings treated with Spd or Spm. The results indicate that Spd and Spm enhance salt tolerance in seedling growth in *Ch. recutita*.

Some metabolic changes in seedlings. The effects of seed presoaking in antioxidant metabolites (Put, Spd, Spm) on production of soluble carbohydrates, soluble proteins, proline and total FAA other than proline in salinized *Ch. recutita* and

Table 1. The effect of NaCl and Put, Spd, Spm on dry matter (μg/seedling) of *Chamomilla recutita* seedlings after 10 days of germination

Polyamine conc. (mM)	NaCl conc. (mM)	Put dry weight	%	Spd dry weight	%	Spm dry weight	%
	0.0	226.1 ± 6.4	100	226.1 ± 6.4	100	226.1 ± 6.4	100
	25	254.3 n.s. ± 10	112.47	254.3 n.s. ± 10	112.47	254.3 n.s. ± 10	112.47
	50	258.9* ± 10	114.51	258.9* ± 10	114.51	258.9* ± 10	114.51
0.0	75	239.7 n.s. ± 2.3	106.02	239.7 n.s. ± 2.3	106.02	239.7 n.s. ± 2.3	106.02
	100	232.0 n.s. ± 4.8	102.61	232.0 n.s. ± 4.8	102.61	232.0 n.s. ± 4.8	102.61
	125	216.1 n.s. ± 8.9	95.58	216.1 n.s. ± 8.9	95.58	216.1 n.s. ± 8.9	95.58
	150	186.1 n.s. ± 22	82.31	186.1 n.s. ± 22	82.31	186.1 n.s. ± 22	82.31
	0.0	229.4 n.s. ± 26	101.46	223.0 n.s. ± 13	98.63	243.0 n.s. ± 7	107.47
	25	255.0 n.s. ± 15	112.78	295.9* ± 6.3	130.87	292.2* ± 0.8	129.23
	50	242.1 n.s. ± 16	107.08	300.8* ± 6.3	133.04	295.0* ± 6.5	130.47
0.01	75	227.0* ± 2.8	100.40	250.1** ± 0.8	110.61	242.8 n.s. ± 6.9	107.39
	100	213.7 n.s. ± 27	94.52	241.0 n.s. ± 1.0	106.59	225.7 n.s. ± 0.7	99.82
	125	183.9 n.s. ± 16	81.34	239.9* ± 1.1	106.10	166.7** ± 2.1	73.73
	150	173.7 n.s. ± 7.7	76.82	223.8 ± 8.8	98.98	156.9 n.s. ± 3.6	69.39
	0.0	210.9 n.s. ± 12	93.28	218.4 n.s. ± 5	96.59	222.2 n.s. ± 6.1	98.28
	25	234.5 n.s. ± 9	103.72	250.3 n.s. ± 0.5	110.70	244.9 n.s. ± 13	108.31
	50	279.5 n.s. ± 12	123.62	261.4 n.s. ± 6.9	115.61	241.4 n.s. ± 11	106.77
0.1	75	255.6 n.s. ± 11	113.05	257.0 n.s. ± 7.7	113.67	209.6 n.s. ± 20	92.70
	100	240.9 n.s. ± 11	106.55	241.0 n.s. ± 1.0	106.59	188.7** ± 7.2	83.46
	125	225.8 n.s. ± 16	99.87	231.1 n.s. ± 9.5	102.21	159.5** ± 80	70.54
	150	228.2 n.s. ± 26	100.93	227.3 n.s. ± 7.7	100.53	157.1 n.s. ± 12	69.48
	0.0	214.3 n.s. ± 10	94.78	215.9 n.s. ± 1.0	95.49	211.8 n.s. ± 11	93.68
	25	236.8 n.s. ± 0.1	104.73	268.7 n.s. ± 6.8	118.84	267.7 n.s. ± 6.3	118.40
	50	250.2 n.s. ± 0.5	110.66	295.4* ± 6.7	130.65	249.8 n.s. ± 6.6	110.48
1.0	75	228.6** ± 0.6	101.11	278.1* ± 13	123.00	257.0 n.s. ± 7.7	113.67
	100	222.0 n.s. ± 1.8	98.19	273.1** ± 8.1	120.79	235.5 n.s. ± 6.6	104.16
	125	218.0 n.s. ± 4.5	96.42	249.9 n.s. ± 10	110.53	217.2 n.s. ± 1.5	96.06
	150	202.5 n.s. ± 0.6	89.56	236.0 n.s. ± 3.7	104.38	205.2 n.s. ± 14	90.76
	0.0	194.9* ± 5.7	86.20	222.6 n.s. ± 6.6	98.45	250.0* ± 0.6	110.57
	25	229.1 n.s. ± 11	101.33	257.3 n.s. ± 11	113.80	269.9 n.s. ± 6.2	119.37
	50	235.0 n.s. ± 0.6	103.94	235.1 n.s. ± 7.5	103.98	270.7 n.s. ± 8.1	119.73
5.0	75	215.1 n.s. ± 14	95.13	225.7 n.s. ± 7.7	99.82	249.9 n.s. ± 8.1	110.53
	100	189.8 n.s. ± 16	83.95	222.0 n.s. ± 1.8	98.19	242.8 n.s. ± 9.2	107.39
	125	181.4* ± 5.8	80.23	218.0 n.s. ± 12	96.42	226.1 n.s. ± 1.9	100.00
	150	148.3 n.s. ± 5	65.59	201.5 n.s. ± 25	89.12	186.5 n.s. ± 2.5	82.49
LSD 0.05		34.3		20.5		22.7	
LSD 0.01		45.7		27.4		30.3	
LSD 0.001		59.7		35.7		39.6	

n.s. non significant at $P > 0.05$; *significant at $P > 0.05$; **highly significant at $P > 0.01$; ***very highly significant at $P > 0.001$

Table 2. The effect of NaCl and Put, Spd, Spm on dry matter (μg/seedling) *Origanum majorana* seedlings after 10 days of germination

Polyamine conc. (mM)	NaCl conc. (mM)	Put dry weight	%	Spd dry weight	%	Spm dry weight	%
	0.0	210.9 ± 3	100	210.9 ± 3	100	210.9 ± 3	100
	25	235.7 n.s. ± 11	111.76	235.7 n.s. ± 11	111.76	235.7 n.s. ± 11	111.76
	50	216.8 n.s. ± 1.1	102.80	216.8 n.s. ± 1.1	102.80	216.8 n.s. ± 1.1	102.80
0.0	75	226.5 n.s. ± 9.2	107.40	226.5 n.s. ± 9.2	107.40	226.5 n.s. ± 9.2	107.40
	100	192.9 n.s. ± 21	91.47	192.9 n.s. ± 21	91.47	192.9 n.s. ± 21	91.47
	125	204.0 n.s. ± 19	96.73	204.0 n.s. ± 19	96.73	204.0 n.s. ± 19	96.73
	150	188.8 n.s. ± 15	89.52	188.8 n.s. ± 15	89.52	188.8 n.s. ± 15	89.52
	0.0	223.2 n.s. ± 9.9	105.83	214.6 n.s. ± 5.3	101.75	232.3 n.s. ± 31	110.15
	25	225.8 n.s. ± 13	107.06	214.5 n.s. ± 14	101.71	197.6 n.s. ± 14	93.69
	50	220.8 n.s. ± 9.2	104.69	200.8 n.s. ± 7.9	95.21	231.6 n.s. ± 6	109.82
0.01	75	194.7* ± 2.7	92.32	208.0 n.s. ± 21	98.62	220.5 n.s. ± 29	104.55
	100	166.6 n.s. ± 12	78.99	233.5 n.s. ± 29	110.72	214.3 n.s. ± 25	101.61
	125	195.0 n.s. ± 42	92.46	184.7 n.s. ± 37	87.58	202.3 n.s. ± 44	95.92
	150	157.2 n.s. ± 29	74.54	155.3 n.s. ± 13	73.64	208.0 n.s. ± 10	98.62
	0.0	206.5 n.s. ± 11	97.91	212.2 n.s. ± 29	100.62	247.4 n.s. ± 37	117.31
	25	226.2 n.s. ± 32	107.25	198.9 n.s. ± 11	94.31	237.2 n.s. ± 31	112.47
	50	183.9 n.s. ± 29	87.20	245.1 n.s. ± 13	116.22	229.0 n.s. ± 35	108.58
0.1	75	198.7 n.s. ± 32	94.22	215.9 n.s. ± 5.7	102.37	229.9 n.s. ± 22	109.01
	100	178.0 n.s. ± 27	84.40	225.4 n.s. ± 26	106.88	214.8 n.s. ± 37	101.85
	125	197.4 n.s. ± 4.4	93.60	237.5 n.s. ± 24	112.61	215.4 n.s. ± 18	102.13
	150	142.1 n.s. ± 12	67.38	198.7 n.s. ± 17	94.22	182.7 n.s. ± 38	86.63
	0.0	216.3 n.s. ± 13	102.56	216.4 n.s. ± 5.8	102.61	248.8** ± 6.7	117.97
	25	219.2 n.s. ± 11	103.94	224.9 n.s. ± 11	106.64	226.8 n.s. ± 24	107.54
	50	223.6 n.s. ± 13	106.02	161.1* ± 16	76.39	258.9 n.s. ± 18	122.76
1.0	75	214.7 n.s. ± 5.7	101.80	165.8** ± 9.7	78.62	265.5* ± 10	125.89
	100	137.3 n.s. ± 8.1	65.10	155.4 n.s. ± 20	73.68	224.5 n.s. ± 9.5	106.45
	125	102.9** ± 18	48.79	137.3* ± 14	65.10	220.8 n.s. ± 18	104.69
	150	147.4 n.s. ± 38	69.89	132.0* ± 1.0	62.59	227.2 n.s. ± 19	107.73
	0.0	215.4 n.s. ± 15	102.13	231.3 n.s. ± 23	109.67	204.6 n.s. ± 10	97.01
	25	180.0 n.s. ± 40	85.35	122.0** ± 13	57.85	198.2 n.s. ± 18	93.98
	50	151.4** ± 12	71.79	159.9 n.s. ± 29	75.82	175.8 n.s. ± 23	83.36
5.0	75	185.3* ± 9.4	87.86	168.6* ± 20	79.94	191.3* ± 5.3	90.71
	100	124.5 n.s. ± 17	59.03	184.5 n.s. ± 28	87.48	199.4 n.s. ± 41	94.55
	125	131.8 n.s. ± 20	62.49	161.7 n.s. ± 26	76.67	207.7 n.s. ± 25	98.48
	150	98.5** ± 12	46.70	121.2** ± 0.3	57.47	192.3 n.s. ± 16	91.18
LSD 0.05		62.2		53.6		n.s.	
LSD 0.01		83.0		71.5		n.s.	
LSD 0.001		108.5		93.4		n.s.	

n.s. non significant at $P > 0.05$; *significant at $P > 0.05$; **highly significant at $P > 0.01$; ***very highly significant at $P > 0.001$

O. majorana seedlings were also followed in the current studies. Seeds of *Ch. recutita* presoaked in different concentrations (0.01, 0.1, 1.5mM) of PAs (Put, Spd, Spm) and treated with NaCl had significantly increased soluble carbohydrates when compared with low and moderate concentrations of NaCl. Seeds of *O. majorana* presoaked in different concentrations of PAs (Put, Spd, Spm) and treated with NaCl had significantly decreased soluble carbohydrates when compared with respective NaCl treatments.

At all NaCl levels, *Ch. recutita* and *O. majorana* seedlings showed a decrease in soluble protein. In case of *Ch. recutita* seeds presoaked in different concentrations of Put and treated with NaCl the soluble protein at low and moderate levels of NaCl increased significantly when compared with respective NaCl treatments; however, in case of *O. majorana* seeds the soluble protein decreased significantly when compared with respective NaCl treatments. Moreover, presoaking both plants seeds in Spd had a favorable effect on the increase of soluble protein up to 100mM NaCl, whereas at high NaCl levels, soluble protein showed little response to the spermidine treatments which mostly induced in significant changes. *Ch. recutita* seeds presoaked in Spm (0.01mM) treated with NaCl had significantly increased soluble protein when compared with respective NaCl treatments; at the levels of 0.1, 1, 5mM (Spm) the soluble protein decreased when compared with the corresponding treatments with NaCl. In case of *O. majorana* seeds presoaked in 0.01, 0.1mM Spm and treated with NaCl had significantly decreased soluble protein, while at the levels of 1 and 5mM Spm soluble protein increased compared to the corresponding treatments with NaCl.

NaCl-treated *Ch. recutita* and *O. majorana* seedlings showed the production of proline increased up to 50mM NaCl and above that it decreased. Also, presoaking of seeds in any of the applied PAs was generally associated with a marked decrease in production of proline. Presoaking the seeds in different concentrations of PAs (Put, Spd, Spm) and treated with NaCl significantly decreased the proline when compared with control or the corresponding NaCl treatments. In case of salinized *O. majorana* seedlings the production of total FAA other than proline was retarded more prominently at high levels of salinity, while in case of *Ch. recutita* seedlings the production of total FAA increased up to 75mM NaCl and above that it decreased. Generally, the total FAA contents in both plants exhibited a highly significant increase with the rise of Spd levels, but in case of *O. majorana* the total amino acids only decreased at the level of 5mM Spd; in case of *Ch. recutita*, Spd treatments completely alleviated the inhibitory effect of NaCl up to 50mM and above these contents they tended to decrease with increasing NaCl. Spm treatments completely alleviated the inhibitory effect of NaCl at low levels (25 and 50mM), above these they significantly decreased with increasing salinity. In case of *O. majorana*, seeds presoaked in different concentrations of Spd or Spm and treated with NaCl increased the total FAA compared with the corresponding NaCl treatments.

Salinity interaction with complex plant physiological processes depends on salt type and concentration, plant genotype, growth stage and environmental conditions (Ghoulam and Fares 2001). To ameliorate the harmful effects of salinity on plant growth and the relevant metabolic activities, several seed pretreatments were used. In this respect, presoaking seeds in certain PAs as antioxidants was found to be beneficial to seed germination and growth of seedlings under saline conditions (Ali 2000, Ali et al. 2007, Dasgan et al. 2009, Kovacik et al. 2009). It is well known that PAs metabolism is altered in response to diverse kinds of environmental stresses (Nayyar et al. 2005, Jimenez-Bremont et al. 2007). The present investigation was conducted to study the response of *Ch. recutita* and *O. majorana* seeds treated with various levels of PAs to various levels of NaCl. The inhibitory effects of NaCl on the growth of salt-stressed seedlings support the results obtained by other investigators using various plant species (Ali 2000, Ali and Abbas 2003, Parida and Das 2005, Ali et al. 2007). PAs pretreatment led to a marked increase in the seedling growth (Ali 2000, Ali et al. 2007). Moreover, compared with corresponding treatments with NaCl, PAs (Put, Spd, Spm) when added together with NaCl effectively elevated the seedling growth in both plants (Ali 2000, Ali et al. 2007).

The effect of seed presoaking in polyamines as antioxidants on the biosynthesis of some cellular components in the salinized seedlings was also followed in the current studies. In this respect, it could be noticed that although *Ch. recutita* and *O. majorana* seedlings were treated with the same salinity levels, the trend of osmotic adjustment varied in the two experimental plants. It can be suggested that the osmotic adjustment in *Ch. recutita* seedlings was mediated by the accumulation of total FAA and proline at low and moderate salinity levels, while in *O. majorana* seedlings it was manifested by the accumulation of soluble carbohydrates and proline. Allosteric regulation of glutamate kinase activity

by free proline enables to increase the content of glutamate that is used in synthesis of glutathione and phytochelatins in plant cells. Similar results were obtained by Pavlíková et al. (2007, 2008) under the effect of heavy metals. Elimination of allosteric regulation of glutamate kinase and icreased proline biosynthesis is important for stress metabolism induced by cold (Štefl and Vašáková 1982), and/or by soil salinity (Štefl and Vašáková 1984). Expression of the genes encoding cell wall proteins (proline rich protein and extensin) and cellulose synthase was induced in barley roots by salt stress (Ueda et al. 2007). A novel OSPGYRP gene encoding a rice proline-, glycine- and tyrosine-rich protein was isolated from cold-stress treated rice seedlings using suppression subtractive hybridization. The expression of the OSPGYRP gene was induced by cold, salt, and osmotic stress (Li et al. 2009). Overexpression of MtZpt2-1 in roots conferred salt tolerance and affected the expression of three putative targets in the predicted manner: a cold-regulated A (CORA) homolog, a flower-promoting factor (FPF1) homolog and an auxin-induced proline-rich protein (PRP) gene (Merchan et al. 2007). High contents of hydroxyproline-rich glycoproteins as well as proline-rich proteins are associated with pollen fertility (Rubinstein et al. 1995, Showalter 2001, Bosch et al. 2003).

In conclusion, the present results clearly demonstrate that seed presoaking in PAs as antioxidant compounds partially or completely counteracted the suppressive effects of moderate or high salinity levels on seedling growth. These stimulatory effects were generally accompanied by the accumulation of some soluble cellular constituents in salt-stressed tissues, a response of significance in connection with the osmoregulatory role of these constituents in increasing the salt tolerance of the test seedlings. Thus, the harmful effects of salt stress on seedling growth and the relevant physiological activities could be alleviated or modified to some extent by presoaking the seeds in the proper concentrations of the applied antioxidants.

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