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RESEARCH ARTICLE

Amelioration of the Growth, Yield and Chemical Constituents of Canola Plants Grown under Salinity Stress Condition by Exogenous Application of Proline

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Abstract

Two field experiments were carried out in the Research Farm of Faculty of Agriculture, Cairo University, Wady El-Notron Province, El Behaira Governorate, Egypt, during two successive winter seasons of 2012-2013 and 2013-2014 to study the effect of different concentrations of proline on growth parameters, yield and yield attributes and some chemical constituents of canola under saline conditions. The results showed that plant height, number of leaves.plant⁻¹, number of branches.plant⁻¹ and dry weight.plant⁻¹ were significantly affected by increasing the proline concentration from 0 to 100 µM. Increasing proline concentrations from 0 to 100 µM with both varieties Serw 4 and Serw 6 significantly increased all yield attributes as compared with control plant (0 proline) except number of seed.siliqua⁻¹, 1000-seed weight and seed yield.plant⁻¹. However, Serw 6 with 75 µM proline concentration records the highest values of number of siliqua.plant⁻¹, 1000-seed weight, seed yield.plant⁻¹, seed and biological yield.ha⁻¹, while the highest recorded values of plant height and number of seed.siliqua⁻¹ were recorded at 100 µM proline with Serw 6. Serw 4 + 50 µM of proline records the highest values of Ca, Mg, K, Na and Fe. Also, proline application at 50 µM with the same variety records the highest value of protein per cent, while the highest seed oil content recorded by Serw 4 + proline concentration at 75 µM.

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INTRODUCTION

Salinity is an ever increasing environmental problem and is a substantial restraint to agriculture. Agricultural productivity in arid and semiarid regions of the world is very low due to accumulation of salts in soils (Ashraf and Sarwar, 2002; Munns, 2002). Saline causes many adverse effects on plant growth, which is due to low osmotic potential of soil solution (osmotic stress) specific ion effects (ionic stress), nutritional imbalance or a combination of these factors (Marschner, 1995; Ashraf, 2004). Although salt stress affects all growth stages of a plant, seed germination and seedling growth stages are known to be more sensitive in most plant species. Furthermore, germination and seedling stage is predictive of plant growth responses to salinity (Cuartero et al., 2006). Several authors, who have studied the effects of salinity on canola report reductions in plant height, shoot and root dry weight, leaf number, leaf area, pod number.plant⁻¹, seed number.pod⁻¹, 100 seed weight, seed yield.plant⁻¹, oil and protein content in the seeds (Redmann et al., 1994; Hashem et al., 1998).

Exogenous application of plant growth regulating compounds is an efficient and technically simpler approach to cope with the deleterious effects of salinity on plants (Khan et al., 2006; Ashraf et al., 2010) and these exogenous application of plant growth regulators, fertilizers, and nonenzymatic antioxidants has been successfully used to minimize the adverse effects of salinity on plant growth and yield (Tuna et al., 2008; Kaya et al., 2010). In salt stressed plants osmotic potential of vacuole decreased by proline accumulation. It was thought that accumulated proline under environmental stress do not inhibit biochemical reactions and plays a role as an osmoprotectant during

osmotic stress (Beltagi, 2008). Proline is the major amino acid associated with environmental stresses (salinity, extreme temperatures, UV radiation and heavy metals). When exposed to a high salt stress, many plants accumulate high amounts of proline, in some cases several times the sum of all the other amino acids (Ashraf and Foolad, 2007). The proline functions as an osmolyte, radical scavenger, electron sink, stabilizer of macromolecules and a cell wall component (Matysik et al., 2002). Increased accumulation of proline leads to the increase of enzyme activity of glutamate kinase and therefore increases proline biosynthesis (Vašáková and Štefl, 1982). Proline and hydroxyproline are found in specific compounds, many of these compounds have specific characteristics and these proteins help to overcome plant stress. This is consistent with the general argument that proline is one of the major organic osmolytes. Therefore, rapid accumulation of free proline in plants is a typical response to a wide range of environmental stresses (Pavlíková et al., 2008). Exogenous application of proline counteracted the adverse effects of salt stress by stimulating growth of cells and plants (Ali et al., 2008) improving metabolism (Rana and Rana, 1996) and reducing oxidation of membrane lipids (Okuma et al., 2004; Yazici et al., 2007) under stress conditions.

Canola (*Brassica napus* L.) grown mainly for edible oil purpose is a moderately salt tolerant crop (Francois, 1994). In view of increasing awareness of the health advantages of canola oil and existing salt tolerance potential, its demand has undoubtedly increased during the last two decades. This has resulted into increased cultivation of canola on soils where salinity problems already exist. Thus, there is a need for further improvement in the salt tolerance of canola (Steppuhn et al., 2001; Ashraf and McNeilly, 2004) in order to improve crop salt tolerance that will result into enhanced productivity on salt affected soils, different perspective strategies have been proposed by various plant scientists (Ashraf et al., 2008). Of them, exogenous use of compatible organic solutes has gained a considerable ground as a shotgun approach to ameliorate the adverse effects of salt stress on plants (Ashraf and Foolad, 2007). For instance, foliar spray of proline counteracted the growth inhibition induced by NaCl in rape (Makela et al., 1999). Thus, exogenous application of proline improves the growth, survival, and tolerance of a wide variety of plants under various stress conditions (Ashraf and Foolad, 2007). The aim of this work is to study the effect of different concentrations of proline on growth parameters, yield and yield attributes and some chemical analysis of canola varieties under saline conditions.

MATERIALS AND METHODS

Two field experiments were carried out in the Research Farm of Faculty of Agriculture, Cairo University, Wady El-Notron Province, El Behaira Governorate, Egypt, during two successive winter seasons of 2012-2013 and 2013-2014 to study the effect of different concentrations of proline on growth parameters, yield and yield attributes and some chemical constituents of canola varieties under saline conditions. The soil texture of the experimental site was sand and some physical and chemical properties according to Chapman and Pratt (1978) are representative in Table (1). Analysis of irrigation water is presented in Table (2).

The soil was ploughed twice, ridged and divided into plots. During seed preparation, 200 kg.ha⁻¹ calcium superphosphate (15.5% P₂O₅) and 100 kg.ha⁻¹ potassium sulphate (48 % K₂O) were applied, while, 160 N kg.ha⁻¹ as ammonium sulfate (20.6% N) was added in four equal doses weekly. Canola varieties (Serw 4 and Serw 6) are a moderate salt tolerance and induced by Oilseed Crops Institute, Agriculture Research Center, Egypt. The proline treatments (0, 50, 75 and 100 µM) were distributed as spraying in a randomized complete block design (RCBD) with three replications. Each plot consisted of 15 rows (20 cm spacing) of 3.5 meter length, i.e. 10.5 m², with seed rate of 8 kg.ha⁻¹. Planting date was 20th and 25th November in first and second season, respectively. Sprinkler irrigation took place immediately after sowing, then every one week intervals according to agronomic practices in the district.

Data recorded

At 90 days after sowing, ten plants were randomly taken from each plot to determine plant height (cm), number of leaves.plant⁻¹, number of branches.plant⁻¹ and dry weight.plant⁻¹ (g). At harvest, ten plants were sampled randomly to estimate, plant height, number of siliqua.plant⁻¹, number of seeds.siliqua⁻¹, 1000-seed weight (g), and seed yield.plant⁻¹ (g), seed, straw and biological yield.ha⁻¹ (kg/ha). The content of sodium and potassium were determined in the digested material using Jenway flame photometer as described by Eppendorf and Hing (1970). Calcium and magnesium were determined by Versinte method according to Jackson (1967). The dried plants were then thoroughly ground to fine powder and total nitrogen percentage was determined according to the method described by A.O.A.C. (1975). Seed protein content calculated by multiplying N (%) by 5.75. Seed oil content was estimated by using Soxhelt apparatus and petroleum ether 60-80°C as a solvent. Combined analysis of the two growing seasons was carried out according to procedure outlined by Snedecor and Cochran (1990). Mean values of the recorded data were compared by using the least significant differences (L.S.D. 5%).

Table 1. Mechanical and chemical analysis of the experimental soil site (average of both seasons).

Depth (cm)	Mechanical analysis				Chemical analysis											
	Sand %	Silt %	Clay %	Soil texture	pH	EC dS/m	CaCO ₃	Organic Matter %	Soluble cations meq/l				Soluble anions meq/l			
									Na ⁺	K ⁺	Mg ⁺⁺	Ca ⁺⁺	CO ₃ ⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻
0-30	90.97	7.30	1.73	Sandy	8.00	0.50	12.25	0.15	0.83	0.28	3.62	2.61	--	1.36	3.52	2.46
30-60	89.51	8.61	1.88	Sandy	8.20	0.70	10.52	0.23	0.86	0.35	2.95	2.77	--	1.50	3.39	2.04

Table 2. Chemical analysis of irrigation water (average of both seasons).

Characters	pH	EC dS.m ⁻¹	Soluble cations meq.L ⁻¹				Soluble anions meq.L ⁻¹			
			Na ⁺	K ⁺	Mg ⁺⁺	Ca ⁺⁺	CO ₃ ⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻
	7.8	5.00	26.30	0.71	4.45	13.20	0.02	4.52	35.10	5.02

RESULTS AND DISCUSSION

Growth parameters

Data presented in Table (3) show that plant height, number of leaves.plant⁻¹, number of branches.plant⁻¹ and dry weight.plant⁻¹ at 90 days after sowing were affected by increasing the proline concentration from 0 to 100 µM. However, plant height and dry weight.plant⁻¹ significantly affected by increasing the proline concentration from 0 to 100 µM. However, 100 µM proline concentration recorded the highest values in most studied characters with significant differences in plant height for both varieties, with no significant differences between 100 and 75 µM in number of leaves.plant⁻¹, number of branches.plant⁻¹ and dry weight.plant⁻¹ for both varieties. Among the most importance factors responsible of retarded growth, under the influence of salinity, is the decline in photosynthetic performance (Saffan, 2008). Thus, salinity usually causes a reduction in the leaf area which generally leads to a drastic reduction in net CO₂ assimilation (Franco et al., 1991; Benzioni et al., 1992).

Table 3. Effect of increasing proline concentration on some growth characters of canola plants grown under saline condition at 90 days after sowing (combined analysis of both seasons).

Varieties	Proline concentration µM	Plant height (cm)	Number of leaves plant ⁻¹	Number of branches plant ⁻¹	Dry weight. plant ⁻¹
Serw 4	0.0	101.15	8.25	4.24	24.25
	50	98.25	8.39	4.54	25.12
	75	104.23	9.38	5.21	25.99
	100	109.23	9.94	5.39	26.39
Serw 6	0.0	99.59	7.98	4.58	25.11
	50	103.5	8.38	5.11	25.23
	75	107.23	9.25	5.21	26.98
	100	110.29	9.57	5.39	26.35
LSD 5%		1.74	NS	NS	1.12

The increased thickness of the leaf under salinity stress may also reduce the photosynthetic performance via decrease of CO₂ diffusion in the mesophyll cells (Laisk and Loreto, 1996; Lauteri et al., 1997). The osmotic effect resulting from soil salinity may cause disturbances in the water balance of the plant (Saffan, 2008), including a reduction of turgor and on inhibition of growth, as well as stomatal closure and reduction of photosynthesis (Poljakoff Mayber, 1982). At the whole plant level the effect of stress is usually perceived as a decrease in photosynthesis and growth (Rahdari and Hoseini, 2012) and is associated with alteration in carbon and nitrogen metabolism (Yordanov et al., 2003). Application of proline increased growth parameters under saline conditions as compared with the control plant (0 proline) with both varieties. The proteinogenic amino acid-proline functions as an osmolyte, radical scavenger, electron sink, stabilizer of macromolecules and a cell wall component (Matysik et

al., 2002). Increased accumulation of proline leads to the increase of enzyme activity of glutamate kinase and therefore increases proline biosynthesis (Vašáková and Štefl, 1982). Plants utilize increased content of proline to protein biosyntheses that have specific properties. Proline and hydroxyproline are found in specific compounds. Many of these compounds have specific characteristics and these proteins help to overcome plant stress. This reason for such stress may be soil salinity. For some stress proteins rich proline content is typical (Jofre and Becker, 2009; Roshandel and Flowers, 2009). This is consistent with the general argument that proline is one of the major organic osmolytes. Therefore, rapid accumulation of free proline in plants is a typical response to a wide range of environmental stresses (Pavlíková et al., 2008).

Chemical constituents of shoot

Data presented in Table (4) show some macro (Ca, Mg, K and Na) and micro (Fe, Mn and Zn) nutrients of canola shoot at 90 days after sowing. Significant differences were observed among different proline concentrations with both canola varieties (Serw 4 and Serw 6), except Ca, Fe, Mn and Zn where, Serw 4 plus the zero proline concentration surpassed in Ca, Mn and Zn elements while, the same variety with 50 μM proline concentration surpassed in K and Fe elements. Serw 6 plus zero proline concentration surpassed in Na concentration. Exogenous application of proline counteracted the adverse effects of salt stress by stimulating growth of cells and plants (Ali et al., 2008) improving metabolism (Alia et al., 1991; Rana and Rana, 1996) and reducing oxidation of membrane lipids (Okuma et al., 2004; Yazici et al., 2007) under stress conditions.

Table 4. Effect of increasing proline concentration on some chemical constituents of canola shoot grown under saline condition at 90 days after sowing (combined analysis of both seasons).

Varieties	Proline concentration μM	Macronutrients ($\text{mg}\cdot 100\text{g}^{-1}$)				Micronutrients (ppm)		
		Ca	Mg	K	Na	Fe	Mn	Zn
Serw 4	0.0	1.02	4.63	1.53	2.37	0.75	0.83	0.05
	50	1.02	4.53	1.80	2.25	0.84	0.82	0.03
	75	0.50	3.74	1.35	1.66	0.45	0.80	0.03
	100	0.67	4.80	1.36	1.92	0.57	0.80	0.03
Serw 6	0.0	0.99	4.45	1.49	2.38	0.71	0.82	0.04
	50	1.01	4.62	1.33	2.34	0.59	0.83	0.04
	75	0.59	4.71	1.32	2.13	0.55	0.78	0.03
	100	0.78	4.84	1.29	1.98	0.61	0.81	0.04
LSD 5%		NS	0.4	0.22	0.29	NS	NS	NS

Yield and yield attributes

Data presented in Table (5) show the effect of proline concentrations on some yield attributed characters (plant height, number of siliqua.plant⁻¹, number of seed.siliqua⁻¹, 1000-seed weight, seed yield.plant⁻¹, seed, straw and biological yield.ha⁻¹ of canola varieties (Serw 4 and Serw 6) grown under saline condition. Increasing proline concentrations from 0 to 100 μM with both varieties Serw 4 and Serw 6 significantly increased most of studied characters as compared with control plant (0 proline), except number of seed.siliqua⁻¹, 1000-seed weight and seed yield.plant⁻¹.

Table 5. Effect of increasing proline concentration on yield and yield attributes of canola varieties grown under saline condition (combined analysis of both seasons).

Varieties	Proline concentration μM	Plant height (cm)	No. of siliqua .plant ⁻¹	No. of seed.siliqua ⁻¹	1000- seed weight(g)	Seed yield.plant ⁻¹ (g)	Seed yield (kg.ha ⁻¹)	Straw yield (kg.ha ⁻¹)	Biological yield (kg.ha ⁻¹)
Serw 4	0.0	123.14	136.05	12.45	3.53	5.22	1714.65	3073.50	4792.65
	50	127.20	138.49	12.37	3.57	5.35	1743.34	3123.00	4861.84
	75	130.41	155.87	13.07	3.58	5.93	1772.39	3240.00	5012.39
	100	132.22	156.21	12.89	3.52	5.87	1798.31	3271.50	5069.81
Serw 6	0.0	121.11	140.34	11.99	3.67	5.10	1731.57	2981.25	4712.82
	50	126.70	153.35	12.28	3.67	6.46	1765.80	3222.00	4987.80
	75	131.19	157.45	12.57	3.86	6.52	1812.24	3334.50	5146.74
	100	133.45	155.25	13.12	3.84	5.98	1765.32	3368.25	5133.57
LSD 5%		1.74	2.92	NS	NS	NS	10.11	9.32	15.45

However, Serw 6 in addition to 75 μM proline concentration records the highest values of number of siliqua.plant⁻¹ (157.45), 1000- seed weight (3.86 g), seed yield.plant⁻¹ (6.52 g.plant⁻¹), seed yield.ha⁻¹ (1812.24 kg.ha⁻¹) and biological yield.ha⁻¹ (5146.74 kg.ha⁻¹), while the highest recorded values of plant height (133.45 cm), number of seed.siliqua⁻¹ (13.12) and straw yield.ha⁻¹ (3368.25 kg.ha⁻¹) were recorded by 100 μM proline with Serw 6. The lowest yield attributes of canola plants in control plants (0 proline) reflected the detrimental effect of salinity on soil and irrigation water (Tables 1 and 2), where, the increment of some elements (i.e. Na, Ca and Cl) and the electrical conductivity (EC) of soil and irrigation water tended to negative effects on yield and yield attributes in control treatment (0 proline). This also closely related to the decrease in growth parameters showed in (Table 3). Moreover, salinity is one of the most serious stress factors that limit crop production. It can disrupt the plants' metabolic functions and can be easily noticed on the entire plant subsequently leading to decrease in productivity or even plant death. Proline is an amino acid and is one of the most commonly occurring compatible solutes and plays a crucial major role in osmoregulation and osmotolerance (Rhodes and Hanson, 1993; Hasegawa et al., 2000). It protects membranes and proteins against the destabilizing effects of dehydration during abiotic stress. In addition, it has some ability to scavenge free radicals generated under stress conditions (Ashraf and Foolad, 2007). Increasing yield attributes and economic yields as a result of proline application may be attributed to the increase in plant growth (Table 3) and decreasing uptake on mineral ions specially Na. It also may be due to increasing Mg concentration which accompanied with increasing photosynthetic pigment.

Chemical constituents of canola seed

Data presented in Table (6) illustrate that the effect of increasing proline concentration from 0 to 100 μM on some macro (Ca, Mg, K and Na), micro (Fe, Mn and Zn) nutrients, seed oil content and protein content of some canola varieties (Serw 4 and Serw 6), the data illustrated no significance effect on most of the studied characters by increasing proline concentration except Mg, Na and Fe. Treatment Serw 4 + 50 μM of proline records the highest values of Ca, Mg, K, Na and Fe. Also, proline application at 50 μM with the same variety records the highest value (with no significant difference) of protein per cent as compared with other treatments, while the highest seed oil content recorded by Serw 4 and proline application at 75 μM .

Table 6. Effect of increasing proline concentration on chemical constituents of yielded canola varieties seed grown under saline condition (combined analysis of both seasons).

Varieties	Proline concentration μM	Macronutrients ($\text{mg}\cdot 100\text{g}^{-1}$)				Micronutrients (ppm)			Seed protein (%)	Seed oil %
		Ca	Mg	K	Na	Fe	Mn	Zn		
Serw 4	0.0	0.16	2.80	0.38	0.84	0.35	0.83	0.06	21.05	48.55
	50	0.15	2.67	0.35	0.80	0.22	0.81	0.05	22.00	48.71
	75	0.19	2.84	0.43	0.99	0.49	0.81	0.05	21.40	49.51
	100	0.17	2.82	0.43	0.90	0.44	0.81	0.05	21.70	49.78
Serw 6	0.0	0.14	2.63	0.39	0.84	0.34	0.80	0.05	22.01	48.35
	50	0.15	2.66	0.41	0.88	0.35	0.81	0.06	21.89	48.53
	75	0.16	2.75	0.41	0.89	0.45	0.81	0.05	22.40	48.95
	100	0.16	2.81	0.42	0.88	0.49	0.80	0.06	22.35	48.97
LSD 5%		NS	0.12	NS	0.10	0.11	NS	NS	NS	NS

The low protein values (non significant) of yielded canola seeds grown under saline conditions reflect the inhibitory effect of salinity stress on plant growth, yield and its attributes (Tables 3, 4 and 5). The growth regulators effect of proline in alleviating the harmful effect of salinity stress may be reflected in the mineral contents of yielded seeds and the seed protein per cent which appeared slight increase as compared with control plant (0 proline). Proline act as osmoregulator, maintain membrane integrity and affect the solubility of various proteins due to its interaction with hydrophobic residues on the protein surface under the conditions of reduced water availability. The increase in the total hydrophilic area of the protein stabilizes it by increasing its solubility in an environment of low water availability. As the overall protein synthesis declines during drought stress, proline biosynthesis may substitute for protein synthesis (Hare, 1995).

CONCLUSION

Through the previous results could be concluded that use of exogenous application of plant growth regulating compounds like proline alleviation the adverse effects of salt stress on canola varieties by improving metabolism and stimulating growth of plants this tended to significantly increase in the most of growth, yield and yield attributes as well as some chemical constituents.

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