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Effect of Salinity on Growth, Proline Accumulation and Chlorophyll Content during Vegetative Growth, Flowering and Seed Formation of *Brassica juncea* L.

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Abstract. *Brassica juncea* L (Kraniti) plants were grown in solution cultures having three levels of salinity concentrations (40, 70 and 150 mM w/w)). Higher levels of salinity were achieved by addition of the NaCl, $CaCl_2$ and $MgCl_2$ dissolved in one-fourth strength Hoagland solution. The effect of these treatments on plants growth, proline accumulation and chlorophyll content during vegetative growth, flowering and seed formation were studied. Increasing salinity affected fresh and dry weight of the leaves.These effects were accompanied by a marked increase in the free proline content of the leaves, inflorescences and siliquaes. The rate of accumulation was the greatest, in the leaf, during the period of seed formation. Chlorophyll concentrations in leaves was reduced as a result of increasing salinity, this decrease was greater during the period of seed formation than during vegetative growth and flowering.

Introduction

Proline accumulation in response to salinity has been extensively documented in higher plants [1-5]. Barnett and Naylor [6] and Stewart *et al.*[7] suggested that proline may act as storage compound for energy and reduced nitrogen and carbon to be used post-stress metabolism, whereas Stewart and Lee [3] suggested that proline might act as acyteoplasmic osmoticums counteracting the effect of salt accumulated in the vacuole.

As regards the chlorophyll content of the salinized plant, it is apparent that the chlorophyll content was reduced as a result of increasing salinity[8]. Strogonov *et al.*[9] suggested that the chlorophyll content of salt stressed plants depend on the biological processes and development stages of the plant and also on the type and concentration of the salts.The present work was carried out to elucidate the effect of salinity on growth, ,proline accumulation and chlorophyll concentration, in the critical periods of development of *Brassica Juncea* L.(cv. Kraniti).

Materials and Methods

Seeds of *Brassica Juncea* L.(cv. Kraniti) were soaked overnight in aerated water and then germinated on rockwool cubs. Approximately one week after germination plants, were transferred to pots (110 mm) standing in one-fourth strength Hoagland solution and grown in a glasshouse at 27°C day , 20°C night temperature. Control plants continued to grow in the original solution, while treated plants were grown on solution cultures having three levels of salinity concentrations (40, 70 and 150 mM (w/w)). Higher levels of salinity were achieved by addition of the NaCl,$CaCl_2$ and $MgCl_2$ (Table 1).Salts were added gradually at 40 mol m^{-3} d^{-1} to plants of the treated groups to final concentration of either 40 mM or 70 mM or 150 mM. The experiment was carried out in randomized block design. Each treatment was replicated four times. Each replicate consisted of four plants each. Thereafter material was taken for analysis at different stages of growth as follows (36 days from sowing - vegetative stage, 46 days old - flowering stage and 56 days old at seeding time). For analyses, leaves of the 4th pair; flowers from the central part of the inflorescence and siliquas, unripe seeds from the central part of infructescenes were taken.

Table 1. Weights(g) of salt to be dissolved in tank volume 110 L of one- fourth strength Hoagland solution to give the concentration (mM) of salt in nutrient solution for cropping

Weight of salt	Concentration (mM)		
(g)	40	70	150
NaCl	128.57	224.99	482.13
$CaCl_2.2H_2o$	64.69	113.21	242.58
$Mg\ Cl_2 6H_2o$	134.18	234.81	503.17

Proline content in leaves, inflorescence and siliquas was determined as described by Singh *et al.*[10]. The samples (about 200 mg fresh wt) were frozen in liquid nitrogen and homogenized in 2ml of methanol: chloroform: water (12:5:1, v/v/v) at room temperature. The homogenate was centrifuged for 10 min, and the supernatant collected. The pellet was dissolved in 2 ml of the same solution and centrifuged again. One ml chloroform and 1.5 ml water were added to the combined supernatants, and the mixture was centrifuged once more. This final extract was then subjected to spectrophotometric and measurement was carried out at 520 nm. Each extract was assayed in duplicate or triplicate, and in each batch processed triplicate standards of 0, 10, 50, and 100 ug / ml proline dissolved in extraction solvent were included at all stages, including initial partition against chloroform and water.

Three replicate samples were measured for every treatment analyzed, and proline concentration is expressed on a dry weight (dw) basis. This was obtained by multiplying fw concentration by the fw:dw ratio calculated after drying the remaining plants at 85°C for 24 h.

Chlorophyll of leaves and siliquas was extracted with 80% acetone and determined according to Arnon's method, thereby spectrum absorption was measured at 645 and 663 nm [11].

Data were subjected to statistical analysis of analysis variance and Duncan's multiple range tests using Minitab [12].

Results

The effect of salinity (40, 70, 150 mM) was tested over a period of 56 days. Prior to salinization, the fresh weight of the leaf of a sample of four plants was 0.596 ± 0.086 g per leaf (mean ± standard error of the mean) with an average of 7.5 ± 0.2 leaves per plant. Results presented in Table 2 show that it was significantly reduced by the presence of salinity. The leaves' fresh weight of the plants growing in 150 mM of salinity had a mean fresh weight 68% that of the control group. The dry weight of the leaves was more affected than the fresh weight (Table 2), although the differences were barely significant at the 95% probability level.At the highest level of salinity(150 mM), the decrease in growth of the plant was, in part,as a consequence of the reduced number of leaves per plant (Table 2).

Table 2. Effect of salinity on the leaf growth of the *Brassica juncea* L. (Kraniti.V) during vegetative stage

Treatments NaCl,$CaCl_2$ and $MgCl_2$ (mM)	Fresh weight per leaf (g)	Dry weight per leaf (g)	No. of leaves per plant	Water content in the leaf (gg^{-1} dry wt.)	Ratio fresh/dry wt.
Control	0.596 ± 0.086[aa]	0.077± 0.019 [a]	8.75 ± 0.352 [a]	6.740± 0.442 [b]	7.740 ± 0.525
40	0.502± 0.073 [a]	0.061±0.017 [a]	8.00 ± 0.241 [ab]	7.216± 0.491 [b]	8.216 ± 0.722
70	0.463±0.069 [ab]	0.049 ±0.013 [b]	7.50 ± 0.311 [b]	8.334 ±0.622 [a]	9.335 ± 0.797
150	0.405± 0.033 [b]	0.041 ±0.012 [b]	6.70 ± 0.280 [c]	8.854± 0.727 [a]	9.84 ±0.950

* Means, within a column followed by the same letter do not differ significantly at 5% level of probability according to Duncan's Range Test.

The relative insensitivity of the shoot fresh weight, as compared to the dry weight to salinity, was due to a pronounced rise in the amount of water per unit dry weight (water concentration) for plants grown in 150 mM (Table 2).

Table 3 shows that the control plants maintained on basal medium had a lower concentration of proline in the leaves during the different stage of growth. A much higher level of proline was noticed in inflorescnces and siliquas (2.821 and 2.220 mg/g dwt respectively). Under the lower salinity (40 mM), the level of proline in leaves was only just above that of the control and remained relatively unchanged during the

experiment (during flowering and seed formation).

Table 3. Effect of salinity on proline accumulation (mg/g dw) in the leaves and siliquae of *Brassica juncea* L (Kraniti.V) during vegetative, flowering and seed formation stages

Treatments NaCl, $CaCl_2$ and $MgCl_2$ (mM)	Vegetative	Flowering		Seed formation	
	Proline mg/g Dwt				
	Leaves	Leaves	Inflorescencs	Leaves	Siliquas
Control	1.352±0.035 [c] *	1.452 ±0.052 [d]	2.821±0.225 [c]	1.64±0.0281 [ab]	2.220 ±0.023 [c]
40	1.970±0.041 [b]	1.996 ±0.032 [c]	2.911±0.191 [c]	1.720 ±0.037 [a]	2.401 ±0.041 [c]
70	3.860 ±0.052 [a]	2.450 ±0.041 [b]	3.910±0.131 [b]	1.650 ±0.041 [b]	4.520 ±0.062 [b]
150	4.330 ±0.071 [a]	2.740 ±0.05 [a]	4.330±0.140 [a]	1.480 ±0.022 [c]	6.440 ±0.091 [a]

* Means, within a column followed by the same letter do not differ significantly at 5% level of probability according to Duncan's Range Test.

In the period of vegetative stage, proline accumulation in leaves increased gradually with the increase of salts level in the media. The highest proline concentration in leaves at the highest level of salinity was about 68% higher as compared to the control. While in period of flowering, the proline concentration in the leaves increased as external salts levels increased (Table3). But during seed formation, it decreased. On the other hand, proline content of inflorescences and siliquas increased gradually with the increase of salts levels. The range of increase in proline about the control values was 34% in inflorescences and 65% at the highest level of salinity treatment (150 mM).

Chlorophyll concentration in leaves was also affected by salinity, and this effect depends on the levels of salinity and the stage of the plants growth (Table 4). The chlorophyll level diminished significantly in the developmental stage of growth as salinity increased. This decrease in the period of seed formation was more pronounced than during vegetative and flowering. In leaves under 150 mM of salinity. The decrease of chlorophyll, in comparison to control treatment was 45% in the vegetative stage;51% flowering and 57% for seed formation. In the siliquas, the chlorophyll concentration was rather constant and was not affected by salinity treatments (Table 4).

Discussion

The growth variation obtained here for *Brassica juncea* may be attributed to the physiological scarcity of water due to increased osmotic pressure which is so common in saline soils[13-15]. In the present study, the growth of plants influenced by salinity levels, showed a general tendency towards a decrease fresh weight, dry weight and No.

of leaves per plants with an increase in water content (measured as gg^{-1} dw) in the leaf (Table 2). The yield and yield attributes of same varieties of crop plants decreased significantly with increase in the level of salinity irrigation water[16]. Malibari *et al.* [17] observed an increase in water content of alfalfa plants at all salinity levels. Polijakoff-Mayber and Gale [18] stated that salinity increased the concentration of abscissic acid, which in turn induces stomatal closure, and as a result the rate of transpiration decreases and consequently an increase in the water content of plants. Nieman [19] observed that NaCl increased the succulence of leaves (water content per unit area) in all species except onion, which suggests an increase in cell size. This difference in the response of fresh and dry weights to salt was to a change succulence[20].

Table 4. Effect of salinity on chlorophyll concentration in leaves and siliques of *Brassica juncea* L.(cv. Kraniti) during vegetative growth, flowering and seed formation

Treatments NaCl, $CaCl_2$ and $MgCl_2$ (mM)	Chlorophyll concentration mg/g dw			
	Vegetative leaves	Flowering leaves	Seed formation	
			Leaves	Siliquas
Control	15.65 ±0.521 [a]*	15.23 ±1.032 [a]	14.62 ±0.961 [a]	1.78 ±0.341 [a]
40	14.82 ±0.740 [a]	12.44 ±1.061 [b]	10.42 ±0.660 [b]	1.64 ±0.221 [a]
70	10.77 ±0.641 [b]	9.50 ±0.983 [c]	8.28 ±0.840 [c]	1.63 ±0.403 [a]
150	8.59 ±0.951 [c]	7.43 ±1.302 [d]	6.26 ±0.942 [d]	1.44 ±0.642 [a]

* Means, within a column followed by the same letter do not differ significantly at 5% level of probability according to Duncan's Range Test.

Salt-tolerance in plants is considered as complex polygenic trait and its expression may be dependent upon tissue and ion developmental stage of plant [21-23]. Plants are known to respond to salt stress by increasing the net synthesis and accumulation of proline [3,4]. From the results for proline content (Table3), it is clear that the process of proline accumulation in rape plants growing under condition of salinity occurred with intensity in the three tested development stages. Proline accumulation was noted in leaves, inflorescence and siliques. The present study showed that only in the inflorescences and siliques of rape, to which physiologically active substance and metabolites flow in the course of seed development from the mother plants, the proline content was higher than in the leaves. Similar observations in other species have led to the suggestion that the process of intensive proline accumulation is directly related to osmotic stress[24-26; 27, pp 609-635; 28, pp 243-259]. In the leaves of other crop plants, for instance Soya, three-fold lower proline level was found on the 10th day of stress than in rape leaves and it was indicated that these accumulations may be the consequence of protein hydrolysis [29].The results showed that the chlorophyll

concentration was reduced significantly in the three tested development stages as a result of increasing salinity (Table 4).

Analysis in detail of the influence of the salinity on proline accumulation and chlorophyll concentration in the leaves in the period of vegetative stage, indicates that proline accumulation is associated with a decrease of the chlorophyll concentration. During seed formation, however, proline accumulation was noted as salinity increased at the time the chlorophyll concentration decreased. It is possible that with the loss of the physiological function of the leaves, proline migrated from the dying leaves to other organs[10,20]. It may also be due to the suppression of the specific enzyme which is responsible for synthesis of green pigments [9]. Further research is necessary to investigate the relation between sensitivity to salinity and drought of various genotapes and proline accumulation.

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أثر الملوحة على تراكم البرولين و المحتوى اليخضوري خلال مراحل النمو الخضري و الإزهار وإثمار نباتات الخردل

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ملخص البحث. نميت نباتات الخردل في مزارع سائلة ذات ثلاث مستويات من الملوحة (٤٠،٧٠،١٥٠ مل مول وزن / وزن) ، ولقد جهزت المحاليل الملحية زات المستويات المختلفة من مخلوط كلوريد الصوديوم ،كلوريد والكالسيوم، كلوريد المغنسيوم المذابه فى محلول هوجلند.ولقد درس أثر هذه المعالجات على كل من النمو النباتي وتراكم الحمضى الأمينى برولين ، والمحتوى من اليخضور ، وذلك أثناء مراحل النمو الخضري وفترة التزهير و الإثمار للنباتات المعالجة . ولقد تبين من الدراسة أن زيادة ملوحة المزرعة يؤثر على نمو النبات لكل من الوزن الطازج ، والوزن الجاف للنباتات خلال مراحل النمو . هذا التأثير كان مصحوباً بزيادة ملحوظة في محتوى الحمضى الأمين برولين الطليق لكل من الأوراق و الإزهار و الإثمار. وكان معدل تراكمه في أوراق النباتات أعلى ما يمكن أثناء فترة تكوين البذور .ولقد نقص المحتوي اليخضور لأوراق النباتات وذلك بزيادة مستويات الملوحة،وكان نقص المحتوى اليخضوري في أوراق النباتات أعلى أثناء فترة تكوين البذور مقارنة بفترة النمو الخضرى والتزهير .