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The physiological mechanisms of calcium chloride application on broad bean plants grown under salinity stress

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Salinity stress decreases the dry mass of shoot and root, the percentage of this decrease varied between the two plant organs. Roots seemed to be more salt sensitive than shoots. This was accompanied with a lower Na^+ content in the shoots than roots. The organic cytosolutes (soluble carbohydrates, soluble proteins and proline) were much higher in shoots than roots. Treatment plants with CaCl_2 retarded the Na^+ and proline contents considerably in both plant organs. The amount of inorganic cytosolutes (K^+ and Ca^{++}) in general increased markedly. The amount of organic cytosolutes (soluble carbohydrates, soluble proteins and proline) is also enhanced markedly which in turn could increase the water status and consequently the dry matter yield when compared with the only salinized plants. Cell wall degrading enzymes cellulase, polygalacturonase (PG) and polymethylglacturonase (PMG) is significantly increased as salinity increases in tested plants. CaCl_2 treatments induced a significant decrease in the activity of cellulase, PG, and PMG.

Key words: Broad bean, salinity, CaCl_2 , cell wall degrading enzymes.

INTRODUCTION

Calcium is a divalent cation that is extremely important in maintaining the strength of stems and stalks of plants. It also regulates the absorption of nutrients across plasma cell membranes. Calcium functions in plant cell elongation and division, structure and permeability of cell membranes, nitrogen metabolism and carbohydrate translocation (Bor et al., 2003). Generally, Ca^{++} had an ameliorative effect on the growth of NaCl stressed plants, by modulating overall metabolism (Jaleel et al., 2008a). Line et al. (2008) reported that calcium is known to protect the integrity of cell membranes, reduce membrane permeability and prevent ion leakage caused by environmental stress. Specialized transport proteins, namely cation channels, are in charge of Ca^{++} influxes, which not only supply Ca^{++} for metabolic and structural needs but also function in the signaling mechanisms for many decades (Kitagawa and Yoshizaki, 1998). Na^+ in the soil plays a

major role in growth inhibition. High sodium levels disturb Ca^{++} nutrition and when accumulated in cytoplasm inhibits many enzymes (Hamdia et al., 2004). These effects are also due to a combination of adverse osmotic gradients, and the inhibitory effects of salts and ions on cell metabolism, as well as nutrient imbalance and such secondary stresses as oxidative stress linked to the production of toxic reactive oxygen intermediates (Iqbal et al., 2006). A variety of mechanisms contribute to salt tolerance, the common mechanism include ion compartmentation in vacuoles (Yeo, 1998), accumulation of compatible solutes in the cytoplasm (Hare et al., 1998). Compatible osmotica such as proline and glycine betaine are thought to function as osmoprotectants for proteins. One possible approach to reducing the effect of NaCl stress on plant productivity is through the addition of calcium supplements to irrigation in the case of salt

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stress (Nemoto and Sasakuma, 2002). Supplementing the medium with Ca^{++} alleviates growth inhibition by salt in glycophytic plants (Demiral and Turkan, 2005). Calcium sustains K^+ transport and K^+/Na^+ selectivity in Na^+ challenge plants (Lauchli, 1990). Studies on the combined effects of NaCl and CaCl_2 accumulation of proline and glycine betaine are scarce.

Thus, the present work was made to study further the interactive effects of salinity and CaCl_2 treatments on growth parameters, metabolic sequence and cell wall degrading, ethylene evolution and cell wall degrading enzymes of broad bean plants.

MATERIALS AND METHODS

Culture conditions, NaCl and CaCl_2 treatments of plants

Broad bean plants were grown in plastic pots containing air dried soil (sand:clay, w:w 1:2). NaCl salinization levels were corresponding to osmotic potentials (-0.23, -0.46, -0.69, -0.92 and -1.15 MPa), in addition to control level (0.0 NaCl) with three replicate each. Treatments of the plants with saline solutions began when seedlings were two weeks old. In order to adjust the osmotic potential of soil solutions as possible near the desired level, plants were irrigated with experimental solution every other day for three weeks and the soil moisture content was kept near the field capacity by addition of 100 ml. Thereafter, the salinized and non-salinized plants were irrigated every other day with Hoagland nutrient solution. The plants were allowed to be adjusted to the above treatments with 100 ppm of CaCl_2 . An ex-primary experiments result showed that CaCl_2 of 100 ppm would ameliorate the salinity stress. Each pot was irrigated with 100 ml of CaCl_2 solutions over one week for four intervals. A week later after the last CaCl_2 application plants were harvested for the following analysis after 45-days.

Measured growth parameters

Leaf area was determined as described by Watson and Watson (1953). Dry matter was determined after drying plants in an aerated oven at 70°C to constant mass.

Biochemical analyses

Dosage of pigments, saccharides, soluble amino acids, soluble protein and proline

The chlorophyll a, b and carotenoids content in leaves of broad bean were determined using a spectrophotometer following the procedure described by Metzner et al. (1965). For the dosage of saccharides, soluble amino acids, soluble protein and proline, 0.1 mg of plant dry matter was crushed to a fine powder in water. Saccharides were determined by the anthrone - sulphuric acid method (Fales, 1951). Total soluble amino acids were determined according to method of Moore and Stein (1948). Soluble protein was determined according to the method of Lowry et al. (1951). Proline was determined according to the method of Bates et al. (1951).

Ionic status

Sodium and potassium were determined according to method of

Williams and Twin (1960). Calcium and magnesium were determined by Flame-photometer according to method of Schwarzenbeck and Biederman (1948).

Enzyme extraction and activity assays

One gram of plant tissues was cut into small pieces and homogenized for 10 min in 0.1 M phosphate buffer (pH 6.6 - 7.0). The extract portion was filtered through 3 layer of cheese cloth and centrifuged for 20 min at 2000 rpm at 4°C . The clear supernatant was assayed for enzyme activity or stored at -20°C until use.

Polygalacturonase activity

Polygalacturonase (PGase) activity was determined according to the method of Nelson's method (1944) as modified by Somogi (1952). The reaction mixture contained 0.4 ml of 1% of substrate (sodium polypectate), 0.2 ml of 0.1 M acetate buffer (pH 4.5) and 0.4 ml of enzyme extract. The reaction mixture was incubated at 30°C for 1 h in a water bath. The reaction was stopped by adding Nelson's reagent, and the absorbance was measured at 520 nm. The enzyme activity was expressed as the amount of enzyme extract which releases 1 μmole of reducing groups in 1 h at 30°C and pH 4.5.

Cellulase activity

Cellulase (CMCase) activity was determined according to the method of Nelson (1944) as modified by Somogi (1952). The reaction mixture contained, 0.4 ml of 1% ml of enzyme preparation, and incubated at 30°C for 1 h in water bath. The enzyme activity was expressed as the amount of enzyme extract which releases 1 mg/ml of reducing groups in 1 h at 30°C and pH 4.5.

Polymethylgalacturonase activity

Polymethylgalacturonase (PMGase) activity was determined according to the method of Nelson method (1944) as modified by Somogi (1952). The reaction mixture contained 0.4 ml of 1% of substrate (pectin solution), 0.2 ml of 0.1 M acetate buffer (PH 4.5), and 0.4 ml of enzyme preparation. The reaction mixture was then incubated at 30°C for 1 h in water bath. The reaction was stopped by adding the Nelson's reagent and 1 ml of mixture of reagent A and B by ratio of (25:1 v/v). After 20 min boiling at (temperature $^\circ\text{C}$) the mixture was cooled under running tap water, and 1 ml of reagent C was added. The enzyme activity was determined using a spectrophotometer at 520 nm. The enzyme activity was expressed as the amount of enzyme extract which releases 1 μmole of reducing groups in 1 h at 30°C .

Statistical analysis

The experiment was performed 45-days. The experimental data were subjected to the one way analysis of variances (ANOVA test) using the SPSS version 11.0 to quantify and evaluate the source of variation and the means were separated by the least significant differences, L.S.D. at P level of 0.05% and 0.01% (Steel and Torrie, 1960).

The percentage presented in the following tables was calculated by the data of survival of plants and by the production of dry matter of shoot and root, leaf area, soluble sugar, protein amino acids, proline and minerals at control plants and at different salinization levels alone and with treatment with 100ppm CaCl_2 . The data was compared by plants grow at water content of F.C. as control and the other different salinization levels.

Table 1. Some growth parameters (fresh and dry matter mg g⁻¹ in shoot and root, leaf area Cm² and chlorophyll content (mg g⁻¹ d. m.) of broad bean under different salinization treatments and treatment with CaCl₂.

Parameter	Treatment	Shoot f. m.	%	Shoot d.m.	%	Root f.m.	%	Root % d.m.	Leaf area	%	Chl.a	%	
Salinity	0.00	18.10	100.0	4.40	100	2.40	100	0.38	100	100.6	100	18.10	100
	-0.23	17.30	95.6	3.60	81.8	2.30	95.8	0.32	84.2	81.90	81.90	17.30	95.5
	-0.46	12.30	68.0	2.90	65.9	1.70	70.8	0.21	55.3	87.6	87.6	12.30	67.9
	-0.69	11.10	61.3	2.40	54.5	1.30	54.2	0.17	44.7	69.2	69.2	11.10	61.3
	-0.92	8.10	44.8	0.96	21.8	0.91	37.9	0.11	28.9	70.9	70.9	8.10	44.8
	-1.15	6.20	34.3	0.78	17.7	0.62	25.8	0.08	21.1	40.1	40.1	6.20	34.2
Salinity + 100 ppm CaCl ₂	0.00	23.30	128.7	6.70	152.3	3.30	137.5	0.64	168.4	131.1	131.1	23.30	128.2
	-0.23	20.40	112.7	7.50	170.5	2.50	104.2	0.56	147.4	82.30	82.30	20.40	112.7
	-0.46	17.30	95.6	4.70	106.8	2.10	87.5	0.34	89.5	83.2	83.2	17.30	95.6
	-0.69	13.70	75.7	5.10	115.9	1.40	58.3	0.33	86.8	85.7	85.7	13.70	75.7
	-0.92	10.80	59.7	3.20	72.7	1.30	54.2	0.17	44.7	91.6	91.6	10.80	59.7
	-1.15	8.90	49.2	1.70	38.6	1.10	45.8	0.12	31.6	55.8	55.8	8.90	49.2
LSD	5%	1.52		1.22		0.53		0.08		2.8		1.52	
LSD	1%	2.06		1.67		0.71		0.11		3.9		2.06	

RESULTS

Morphological analysis

Data in Table 1 showed fresh, dry mass of shoots and roots of broad bean plants as well as leaf area which remained unchanged up to -0.23 MPa of NaCl, then they decline significantly with increasing salinity stress. The rate of decline in these parameters was greater at higher salinity levels. The percentage of reduction in fresh and dry mass of shoot at -1.15 MPa was about 66 and 75% respectively. For root organ the percentage of reduction of fresh and dry mass at that level was about 82% and 79% respectively. The percentage of reduction in leaf area at the same level was 60%. This means that salinity injury is more obvious in root organ than shoot of broad bean tested plant. For photosynthetic pigments, salinity induced a slight increase in the photosynthetic pigments especially at higher salinity levels (Table 1). Exogenous applications of CaCl₂ (100 ppm) alleviated the deleterious effect of salinity on fresh, dry mass, leaf area and photosynthetic pigments of the tested plants as compared with unstressed plants. Moreover, the values of fresh and dry mass in root organ were markedly higher than those of shoot organ in response to CaCl₂ treatments (Table 1).

Biochemical analysis

Soluble saccharides and proteins showed different responses to salinity stress in both shoot and root organs. In shoot, while soluble saccharides content decreased considerably, the soluble proteins significantly increased under salinity stress, the opposite trend held in roots. The magnitude of these contrasting effects was much higher

at the higher dose of the salt. The content of soluble saccharides increased while the soluble proteins decreased with increasing salinity levels. It is interesting to note that at -1.15 MPa NaCl levels, the root soluble saccharides increased by more than two folds, while soluble proteins decreased to about 40% in relation to the control plants.

CaCl₂ treatments induced a general accumulation of soluble saccharides and proteins in shoot and root as compared with control plants. The contents of amino acid reduced smoothly up to -0.92 MPa in shoot. This slight reduction seemed to be more or less constant (about 20%). However there is a sudden and sharp reduction at the highest salinity level used (about 46%). In roots, the contents of amino acids enhanced slightly up to -0.46 MPa, thereafter a highly significant reduction was recorded by the further increase in salinity stress. This reduction was about 43% at the level -1.15 MPa. It is interesting to note that CaCl₂ treatments induced a huge accumulation of amino acids contents of both shoots and roots. The percentage of this accumulation at -1.15 MPa in both shoot and root was 78% and 163% respectively. The data in Table 2 showed that there was a marked and progressive accumulation in proline content as salinity level increased. This accumulation was much more pronounced in root than in shoot. Proline content in roots reached more than seven fold at -1.15 MPa NaCl as compared with control plants.

Calcium chloride treatments retarded the accumulation of proline content of the two plant organs as compared with control and the corresponding salinized plants, but still higher than the control plants.

Ionic status analysis

Data in Table 3 showed the changes in mono and

Table 2. Accumulation of soluble sugar mg g^{-1} d. m. (S.S.), soluble protein g^{-1} d. m. (Prot.), amino acids g^{-1} d. m. (A.A.) and proline g^{-1} d. m. (Prol.) contents in both shoot and root of broad bean plant under different salinization treatments and treatment with CaCl_2 .

Parameter	Conc. MPa	Shoot S.S.	%	Root S.S.	%	Shoot Prot.	%	Root Prot.	%	Shoot A.A.	%	Root A.A.	%	Shoot Prol.	%	Root Prol.	%
Salinity	0.00	88.7	100.0	35.50	100.0	27.70	100.0	60.30	100.0	15.40	100.0	10.10	100.0	2.70	100.0	0.11	100.0
	-0.23	62.50	70.5	67.90	191.3	27.40	98.90	37.30	61.9	13.30	86.40	10.9	107.9	2.6	96.3	0.10	89.4
	-0.46	62.20	70.1	79.4	223.7	30.00	108.3	41.80	69.3	11.70	76.0	10.70	105.9	4.8	177.8	0.30	264.6
	-0.69	50.90	57.4	85.7	241.4	32.00	115.5	39.30	65.2	12.40	80.5	6.80	67.3	4.6	170.4	0.60	534.5
	-0.92	32.10	36.2	72.70	204.8	31.4	113.4	39.30	65.2	12.40	80.50	6.80	67.3	4.6	170.4	0.80	711.1
	-1.15	31.60	35.6	79.5	223.9	32.70	118.1	35.60	59.00	8.30	53.9	5.70	56.4	4.6	170.4	1.06	938.1
Salinity + 100 ppm CaCl_2	0.00	98.30	110.8	51.4	144.8	30.20	109.0	52.90	87.7	51.60	335.1	27.90	276.2	3.2	118.5	0.19	168.1
	-0.23	80.70	91.0	47.10	132.7	52.40	189.2	60.20	99.80	29.60	192.2	30.70	304.0	3.6	133.3	0.17	147.8
	-0.46	77.60	87.5	41.90	118.0	53.60	193.5	60.60	100.5	29.20	189.6	12.30	121.8	3.7	137.0	0.33	292.9
	-0.69	77.60	87.5	41.00	115.5	36.90	133.2	43.60	72.3	29.30	190.3	16.40	162.4	3.4	125.9	0.31	270.8
	-0.92	78.60	88.6	46.7	131.5	32.70	118.1	42.60	70.6	27.50	178.6	26.60	263.4	2.9	107.4	0.28	250.4
	-1.15	77.10	86.9	45.50	128.2	32.70	128.2	42.20	70.0	27.50	178.6	26.60	263.4	2.9	107.4	0.28	250.4
LSD	5%	1.25		0.979		0.726		0.611		1.41		1.75		1.24		0.211	
LSD	1%	1.69		1.33		0.976		0.822		1.29		2.37		1.68		0.285	

divalent cations (Na^+ , K^+ , Ca^{++} and Mg^{++}) as affected by salinity and CaCl_2 treatments. The data revealed that there is a marked and progressive increasing trend in shoots and roots of wheat plants, which was more pronounced at the higher dose of NaCl and in root than in shoots. At the level of -1.15 MPa, the percentage increase in Na^+ was about 68.2 and 183.3% in shoot and root respectively.

CaCl_2 treatment leads to a reduction in Na^+ concentration in shoots and roots as compared to the corresponding salinized plants. The data indicate that while, K^+ content increased markedly in shoots, it dropped severely in roots. Although Ca^{++} content decreased in shoots and roots by salinity stress, the percentage of this reduction varied among two plant organs. At the level of -1.15 MPa the percentage of reduction in CaCl_2 was 43% in roots and only about 20% in shoots.

While Mg^{++} increased considerably and unexpectedly in shoots, in roots it remain more or less unchanged up to -0.69 MPa, then a sudden and sharp reduction was obtained.

CaCl_2 treatments had no marked change in K^+ content of shoots and roots, while a marked and progressive increase in Ca^{++} content was detected in roots than shoots treated plants as compared with untreated plants. Mg^{++} showed a constant increase in shoot only compared with control plants.

Cell wall degrading enzymes analysis

Cell wall degrading enzymes (cellulase, PG and PMG) significantly increased as salinity increased of tested plants (Figure 1) as compared with control plant. The absolute increasing values were about 60, 40 and 13 for PG, PMG and cellulase.

This activity was in order of PG activity > activity of PMG > cellulase activity.

CaCl_2 treatments induced a significant decrease in the activity of cellulase, PG, and PMG. This reduction was more obvious in the activity of cellulase and PG especially at lower salinity levels.

DISCUSSION

Although salinity stress decreased the dry mass of shoot and root, the percentage of this decrease varied between the two plant organs. Roots seemed to be the more salt sensitive than shoots, whatever the salinity level used. This was accompanied with a lower Na^+ content in the shoots than roots which indicated that while Na^+ content increased smoothly up to -0.92 MPa in shoot (about only 13.6% increase), in root it increased

Table 3. Accumulation of some mineral mg g⁻¹ d.m. in both shoot and root of broad bean plant under different salinization treatments and treatment with CaCl₂.

Parameter	Conc. MPa	Shoot Na ⁺	%	Root Na ⁺	%	Shoot K ⁺	%	Root K ⁺	%	Shoot Ca ⁺⁺	%	Root Ca ⁺⁺	%	Shoot Mg ⁺⁺	%	Root Mg ⁺⁺	%
Salinity	0.00	8.80	100.0	6.0	100.0	4.30	100.0	6.3	100.0	16.5	100.0	10.0	100.0	1.1	100.0	7.5	100.0
	-0.23	9.5	108.0	14.0	233.3	6.5	151.2	1.9	30.2	11.3	68.5	10.0	100.0	3.0	272.7	7.5	100.0
	-0.46	9.8	111.4	20.5	341.7	5.3	123.3	2.5	39.7	9.4	57.0	7.5	75.0	4.5	409.1	6.4	85.3
	-0.69	10.0	113.6	21.8	363.3	9.3	216.3	2.5	34.9	12.5	75.8	6.1	61.0	3.0	272.7	7.1	94.7
	-0.92	10.0	168.2	16.0	266.7	5.0	116.3	1.5	23.8	12.5	75.8	5.0	50.0	3.0	272.7	2.1	28.0
	-1.15	14.8	170.5	17.0	283.3	5.0	116.3	1.0	15.9	13.1	79.4	5.6	56.0	1.9	172.7	2.6	34.7
Salinity + 100 ppm CaCl ₂	0.00	8.8	90.9	7.8	130.0	5.5	127.9	4.5	71.4	11.3	68.5	6.9	69.0	6.8	618.2	7.9	105.3
	-0.23	8.0	90.9	7.0	116.7	5.3	123.3	2.5	39.7	13.8	83.6	8.1	81.0	6.3	572.7	6.8	90.7
	-0.46	8.8	113.6	3.7	61.7	5.8	134.9	3.5	55.6	10.7	64.8	12.5	125.0	6.8	618.2	3.4	45.3
	-0.69	10.0	128.4	3.7	53.3	7.5	174.4	2.9	19.0	15.0	90.0	11.9	119.0	6.10	554.5	3.4	45.3
	-0.92	11.30	128.4	3.2	63.3	5.3	123.3	2.3	20.6	14.5	87.9	10.7	107.0	6.8	518.2	3.1	41.3
	-1.15	11.30		3.8		4.5	104.7	1.4	22.2	9.9	60.0	11.3	113.0	4.1	372.7	3.4	45.3
LSD	5%	0.28		2.8		1.5		1.4		0.58		0.61		1.4		1.1	
LSD	1%	0.29		3.8		2.2		1.4		0.78		0.83		1.7		1.5	

by 66.7% at the same salinization level which indicated the differences in salt tolerance among the two plant organs was closely associated with the differences in the Na⁺ content. In conformity K⁺ and Ca⁺⁺ contents were much higher in shoots than roots. Thus, this plant restricted the translocation of Na⁺ from root to shoot and on the other hand, it transported a lot amount of K⁺ and Ca⁺⁺ into the aerial parts of the plant. Hedge and Joshi (1975), Janardan et al., (1976) and Hamdia et al. (2004), working with rice, cotton and wheat cultivars, respectively found that K⁺/Na⁺ ratio was high in salt tolerant than sensitive cultivars and recommended it as a suitable selection criterion for salt tolerance. Al- Alfocea et al. (1993) and Hamdia et al. (2004) reported that K⁺ nutrition is not affected by excessive Na⁺ in salt tolerant tomato and wheat plants respectively. This situation was interpreted by Garacia et al. (1997) who reported that in rice there was no correlation between K⁺ and

Na⁺ transport and concluded that the genes affecting Na⁺ uptake had not apparently related with those involved in K⁺ uptake. However, this situation contrasts with that in triticeae (Gorham, 1990). Antagonistic relations between Na⁺ and K⁺ or negative effects of salinity on K⁺ uptake in different plants were recorded by other authors (Hamdia and Shaddad, 1996; Carjaval et al. 2000; Grieve and Poss, 2000; Hamdia et al. 2004). The mechanisms of ion distribution increased the osmotic pressure of the shoot which facilitates the steepness of osmoregulation towards the aerial parts which in turn increases the water flow from the root to the shoots which in turn maintained the water status (and the conservation and utilization). Along with this the organic cytosolutes (soluble carbohydrates, soluble proteins and proline) were much higher in shoots than roots, which again confirmed that broad bean plant considered its strategy of salt tolerance in shoot organ than in

root. In conformity to the above results and discussion, these plants were treated with CaCl₂ as:

1. Na⁺ and proline content retarded considerably in both plant organs.
2. The amount of inorganic cytosolutes (K⁺ and Ca⁺⁺) in general increased markedly.
3. The amount of organic cyto-sloutes (soluble carbohydrates, soluble proteins and proline) also enhanced markedly which in turn could increase the water status and consequently the dry matter yield when compared with the only salinized plants.
4. Consequently, there is a major difference in K⁺ / Na⁺ ratio and Ca⁺⁺ / Na⁺ ratio among the two plant organs. These differences were recorded to be important in the determination of the values of salt tolerance of glycophytes; in general Na⁺ ratio and Ca⁺⁺/Na⁺ ratio were used as a suitable selection criterion for the salt tolerance of glycophytes. There

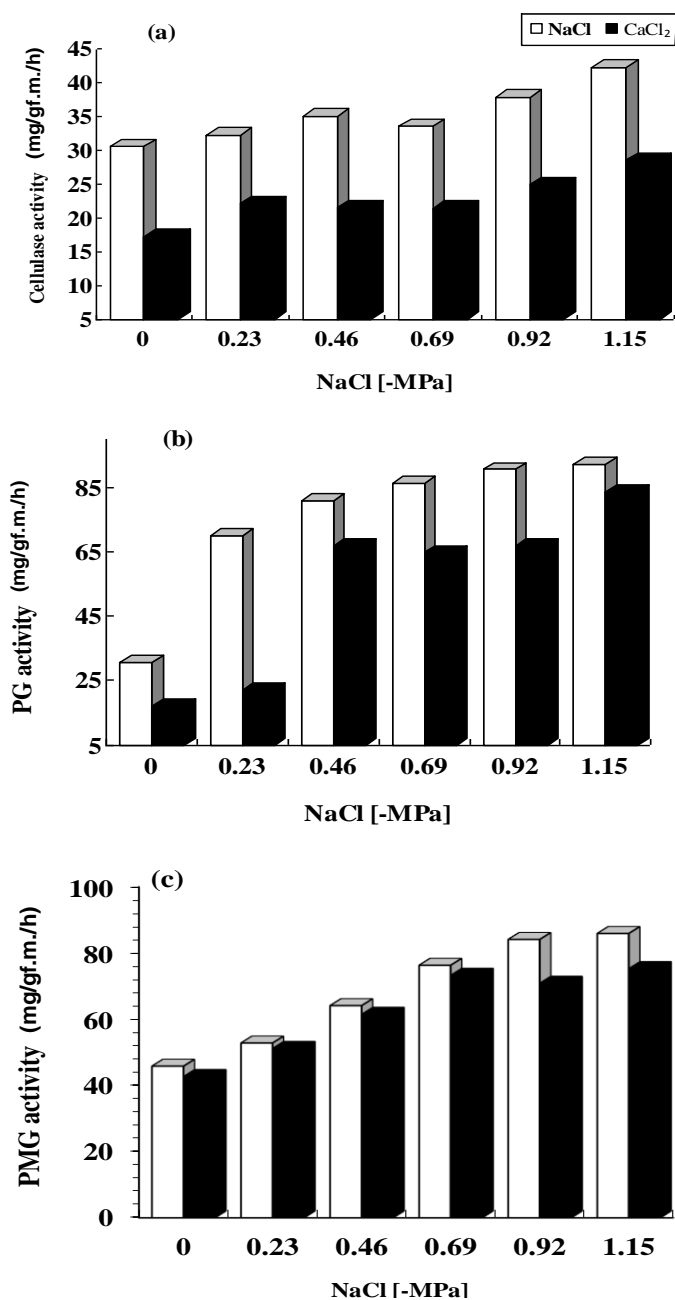


Figure 1. represented howed cell wall degrading, cellulase (a), PG (b) and PMG (c) enzymes activitL : (a) L.S.D. 5% 0.3, (b) L.S.D. 5% 0.5, (c) L.S.D. 5% 0.6.

There is no antagonistic effect between Na⁺ and K⁺ in shoots because both of them increased by salt stress. Interestingly, the opposite occurred in root where Na⁺ antagonized considerably in the absorption of K⁺.

These differences in salt tolerance were accompanied by a large variation in the organic solutes, which consequently promoted differences in the water status (conservation and utilization) of the two wheat cultivars. In cv. Giza 168, soluble saccharides and soluble protein in both roots and leaves were in most cases increased by salinity

stress. This suggests that the salt adapted characteristics of cv. Giza 168 included saccharides and protein involved in tissue growth and osmoregulation (Yordanov et al., 2003; Hamdia, et al., 2004; Jaleel et al. 2008b). The relative ability of a plant to increase the concentration of solutes in its tissues (osmotic adjustment) will partially determine its tolerance to stress condition (Richardson and McCree, 1985; Tester and Davenport, 2003; Arshi et al. 2005; Gobinathan et al., 2009).

Cell wall degrading enzymes (cellulase, PG and PMG) significantly increased as salinity increased. This in accordance with Singh and Prasad (2009) stated that reduction in cell size and thickening of cell wall resulted in stunted growth of *Arachis hypogaea* seedlings were due to overall extensibility of cell wall grown under the level 50 to 100 mM NaCl. Also, Keutgen and Pawelzik (2007) showed that strawberry cultivars differ in their sensitivity to NaCl; fruits of cv. Elsanta suffer from softening, whereas those of cv. Korona retain their firmness. Elsanta is in line with the general observation that severe osmotic stress results in slower cell expansion and weaker cell walls.

CaCl₂ treatments resulted in a significant decrease in the activity of cellulase, PG, and PMG. This effect was more obvious in the activity of cellulase and PG especially at lower salinity levels. This reduction was also concomitant with the increase in the growth parameters, metabolic components and minerals support as the significant role of CaCl₂ in enhancement broad bean plant under salinity stress.

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