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ORIGINAL ARTICLE

Ameliorative effects of Ca^{2+} on the growth, metabolism, cationic status and cell wall degrading enzymes of induced salinity stress *Vicia faba* L.

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Vicia faba L. Plant was grown in a pot experiment to study the positive role of $CaCl_2$ on NaCl induced stress in terms of growth parameters, metabolic, cation contents and cell wall degrading enzymes in different plant organs. The salinity treatments were having an osmotic potential of (0.0; -0.23;-0.46; -0.92 and -1.15 MPa), respectively. A hundred mL of 10 mM $CaCl_2$ were added to the previous concentrations and harvested after 11 weeks old. The data revealed that, NaCl treatments reduced the growth parameters; which most sensitive in root than shoot. Organic cytosolutes were much higher in root than shoot organ except for protein accumulation. The amount of inorganic cytosolutes (Na^+ and Ca^{2+}) in general increased markedly in shoot than root and vice versa for K^+ and Mg^{2+}. $CaCl_2$ treatment alone induces these parameters than control one. Mixed salts of NaCL and $CaCl_2$ positively improve the aforementioned parameters with varying degrees depending on the organs. While root seems to be the more sensitive organ for growth parameters measured, it also seems most accumulator organ than shoot for many metabolites. For the ionic contents, shoot and root varies between the mono and divalent cations. Cell wall degrading enzymes significantly and progressively increased as salinity level of treated plants increased. However, $CaCl_2$ treatments induced a significant reduction in the activity of these enzymes when compared with their respective NaCL treatments. The ameliorative percentage due to calcium application of stressed faba bean on growth parameters ranges from 17.53 to 79.55 %; for metabolites from 8.69 to 194.91; for ionic status from 9.94 to 56.67 %, and for cell wall degrading enzymes from 16.76 to 39.15 %. These data leads to strongly recommend adding $CaCl_2$ to saline environment to decrease the deleterious effects of salinity.

Key words: *Vicia faba, growth performance, organic cyto-sloutes, cations, cell wall degrading enzymes, cellulase, polygalacturnase, polymethylegalacturnase*

Abbreviations Amino acid: A.A.; Cell wall degrading enzyme: CWDE; cellulase: CMCase; dry mass: dr. m. ; fresh mass: fr.m.; pectinmethylegalacturonase: PMGase.; polygalacturonase: PGase; Proline : Prol.; Protein: prot.; Root : Rt.; Shoot: Sh.;soluble sugar : S.S.

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Salinity stress is a major environmental constraint to most of the cash crop productivity in the arid and semiarid regions of the World (López et al. 2011). It has affected about 20% of the agriculture sector on globe as reported by (FAO 2000; Demiral and Türkan 2006; Tavili and Biniaz

2009; Tavarini et al. 2008 and Nemati et al. 2011). Due to this, large areas of arable lands are substantially or partially unproductive (Tunçtürk et al. 2011).

Salinity-induced inhibition of plant growth results from osmotic and ionic effects (Sumithra et al. 2006 and Bhattacharjee 2008). Many plant species have developed different mechanisms to cope with these effects. A variety of mechanisms contribute to salt tolerance which most of them not yet clear, the common mechanisms include ion compartmentation in vacuoles (Yeo 1998) and accumulation of compatible solutes in the cytoplasm (Hare et al. 1998). The osmotic adjustment is an important mechanism of salt tolerance in plants, which is the typical first response of all plants to salt stress (Tunçtürk et al. 2011). The reduction in osmotic potential in salt-stressed plants may stem from accumulation of inorganic ions (Na^+, Cl^-, K^+ and Ca^{2+}) and compatible solutes (soluble carbohydrates, amino acids and proline) (Hasegawa et al. 2000; Sulochana et al. 2002 and Kavi et al. 2005).

Chemical treatment and agronomical crop management practices have been tried to alleviate the salt stress. 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 stress (Girija et al. 2002;Nemoto and Sasakuma 2002 and Abdel Latef 2011).

Calcium is a divalent cation regulating the absorption of nutrients across plasma cell membranes. Its functions in plant cell elongation and division, structure and permeability of cell membranes, nitrogen metabolism and carbohydrate translocation (Bor et al. 2003). Thus, supplementing the medium with Ca^{2+} alleviates growth inhibition by salt in glycophyte plants (Demiral and Türkan 2006 and Abdel Latef 2011). Calcium has a role in building salt tolerance in plants. Adequate levels of calcium are necessary for the membrane to function normally (Jaleel et al. 2007). Most of the interest in calcium in plants has centered on its role in the cytoplasm in controlling the developmental processes (Arshi et al. 2010). Addition of calcium salt might restore adequate levels of potassium and calcium (Kent and Läuchli 1985). It also controls guard-cell turgor and stomatal aperture and helps in turgor maintenance (Bhattacharjee 2009).

Externally supplied Ca^{2+} reduces the toxic effects of NaCl, presumably by facilitating a high K^+/Na^+ selectivity (Liu and Zhu 1998 and Abdel Latef, 2011). High salinity results in increased cytosolic Ca^{2+}, which is transport from the apoplast and the intracellular compartments (Knight et al. 1997). This transient increase in the cytosolic Ca^{2+} initiates stress-signal transduction leading to salt adaptation. The accumulation of osmoprotectant as proline thought to function as osmoprotectant for proteins (Prochazkova et al. 2001;Misra and Gupta 2005), and provides an ecological compatible with the macromolecular structure, function and helps to adapt the salinity injury (Charest and Phan 1990). Generally, Ca^{2+} had an ameliorative effect on the growth of NaCl stressed plants, by modulating overall metabolism (Jaleel et al. 2008). Calcium is well known to protect the integrity of cell membranes, reduce membrane permeability and prevent ion leakage caused by environmental stress Line et al. (2008). The major cell wall degrading enzymes, are a group of enzymes such as polygalacturanase (PG), β-galactosidase, pectin methylesterase (PME) and cellulase (Tavarini et al. 2008) leading to solubilization of cell wall pectin. These enzymes may probably be significant to cell wall modifications at any stage of ripening (Almeida and Huber, 2007). However, several factors or

treatments can affect these enzymes activities. Ca^{2+} perhaps is one of the most effective treatments to reduce cell wall degrading enzymes activities (Wehr et al. 2004). Calcium is known to increase salinity tolerance and mitigate the adverse effects of saline conditions on plant growth (Jaleel et al. 2007 and Abdel Latef 2011). However, there is a little information on calcium chloride interaction with salinity stress and the resultant effects on growth and metabolism especially on faba bean. Therefore, the present work made to study further the ameliorative interactive effects of salinity and $CaCl_2$ treatments on growth performance, metabolic sequence, cationic status and the activity of cell wall degrading enzymes of salinity stressed faba bean plants.

MATERIALS AND METHODS

Broad bean (*Vicia faba* L.) was obtained from breeding program of seeds center, Beni Suef, Egypt. Faba bean seeds were surface sterilized by immersion in a mixture of ethanol 96% and H_2O_2 (1:1) for 3 times, followed by several washings with sterile distilled water. Ten seeds were sown per pots. Each pot containing two Kg of air-dried soil (sandy/clay, w/w 1:2). A total of 36 pots (6 treatment levels X 2 experimental sets X 3 replicates) were irrigated with normal tap water weekly basis to achieve soil water field capacity for three weeks. Treatments of the plants with corresponding saline solutions began gradually when seedlings were three weeks old and allowed to adjust to the above treatments for one week in the two sets.

Set I: only NaCl salinity levels corresponding to the following osmotic potentials:

(a) 0.0 MPa NaCl (control or reference group).

(b) -0.23 MPa NaCl.

(c) -0.46 MPa NaCl.

(d) -0.69 MPa NaCl.

(e) -0.92 MPa NaCl.

(f) -1.15 MPa NaCl.

Set II : (Combined salts or mixed salts) includes the following concentrations:

(a) 10 mM $CaCl_2$ + 0.0 MPa NaCl (Only $CaCl_2$ treatment group).

(b) 10 mM $CaCl_2$ + 0.23 MPa NaCl.

(c) 10 mM $CaCl_2$ + 0.46 MPa NaCl.

(d) 10 mM $CaCl_2$ + 0.69 MPa NaCl.

(e) 10 mM $CaCl_2$ + 0.92 MPa NaCl.

(f) 10 mM $CaCl_2$ + 1.15 MPa NaCl.

Plants were harvested after 7 weeks of treatments and dried in an oven at 70 ^{0}C to constant mass to make plant extract for the following analysis.

(a) Growth Performance

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

(b) Biochemical analysis

Saccharides were determined by the anthrone – sulphuric acid method as described by (Fales 1951). Soluble protein was determined according to the method of (Lowry et al. 1951). Whereas, proline was determined according to the method of (Bates et al. 1973)

(c) Cationic analysis

Sodium and potassium were determined by Flame-photometer according to the method of (Williams and Twine 1960). Calcium and magnesium were determined according to the method of (Schwarzenbach and Biedermann 1948) .

(d) Cell wall degrading enzymes analysis

Plant extract:

One gram of plant tissue was cut into small pieces and homogenized for 10 minutes in 0.1M phosphate buffer (pH 6.6 -7.0). The extract portion was filtered through 3 layer of cheesecloth 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.

1-Polygalacturonase activity:

Polygalacturonase(PGase) activity was assayed by measuring the increase in liberated reducing groups according to the method of (Nelson 1944) and modified by (Somogyi 1952). The reaction mixture contained 0.4 ml of 1% of substrate (sodium polypectate), 0.2 ml of 0.1 ml acetate buffer (pH 4.5) and 0.4 ml of enzyme extract. The reaction mixture incubated at 30 °C for 1 h in a water bath. After incubation, the reaction stopped by adding Nelson's reagent, and the absorbance was estimated at 520 nm. Enzyme activity was expressed as the amount of enzyme extract which releases 1 micro-mole of reducing groups in 1 h at 30 °C and pH (4.5).

2-Cellulase activity (CMCase):

Cellulase (CMCase) activity was assayed by measuring the increase in reducing groups according to the method of (Nelson 1944) and modified by (Somogyi 1952). The reaction mixture contained 0.4 mL of 1 % of enzyme extract and incubated at 30°C for 1 h in water bath. The enzyme activity was expressed as the amount of enzyme extract which releases 1mg mL^{-1} of reducing groups in 1 h at 30°C and pH 4.5

3-Polymethylgalacturonase activity:

Polymethylgalacturonase (PMGase) activity was assayed by measured the increase in reducing groups according to the method of (Nelson 1944) and modified by (Somogyi 1952). The reaction mixture contained 0.4 ml of 1% of substrate (pectin solution), 0.2 ml of 0.1ml of acetate buffer (pH 4.5), and 0.4 ml of enzyme extract.

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 1ml of mixture of reagent A and B by ratio of (25:1 v/v), then boiling for 20 min and cool under running tap water and 1ml of reagent C was added. Read the absorbance at 520 nm on spectrophotometer. Enzyme activity expressed as the amount of enzyme extract which releases 1 micro- mole of reducing groups in 1h at 30 °C.

Statistical analysis:

The results were statistically analyzed using SPSS statistical package software version 15.0 (Chicago, USA). Differences between treatments were determined by the application of analysis of variance (*ANOVA*) at $p \leq 0.05$ level and n=3. The mean comparisons between the two sets of treatments were determined by t- test.

RESULTS

Growth parameters

Analysis of variance (Table 1) for fresh, dry mass of shoot, root and leaf area were highly significant affected by $CaCl_2$ treatments ($P \leq 0.000$) with F-values 48.0, 41.3, 12.8, 23.5& 77.8 respectively. Fresh, dry mass of shoots and roots of broad bean plants as well as leaf area decreased with increasing NaCl salinity stress as compared with control plants. The rate of decline in these parameters was greater at highest salinity levels (-1.15 MPa).

Table 1. Analysis of variance for the effect of treatments on different parameters.

Source	F-value	Sign.	Source	F-value	Sign.
Sh_fr_m.	48.00***	.000	Rt_Prol.	21.38***	.000
Sh_dr_m.	41.28***	.000	Sh_ Na^+	11.24***	.000
Rt_ fr_m.	12.81***	.000	Rt_ Na^+	71.47***	.000
Rt_ dr_m.	23.49***	.000	Sh_ K^+	10.33***	.000
Leaf area	77.8***	.000	Rt_ K^+	19.17***	.000
Sh_S.S.	22.79***	.000	Sh_ Ca^{++}	6.06***	.000
Rt_S.S.	38.63***	.000	Rt_ Ca^{++}	22.93***	.000
Sh_S.P.	8.55***	.000	Sh_ Mg^{++}	7.66***	.000
Rt_S.P.	18.84***	.000	Rt_ Mg^{++}	46.63***	.000
Sh_A.A.	21.41***	.000	Cellulase	24.53***	.000
Rt_A.A.	51.32***	.000	PG.	108.08***	.000
Sh_Prol.	10.03***	.000	PMG.	38.86***	.000

***P<0.001.

Table 2. The ameliorative percentage of the measured parameters after $CaCl_2$ treatment from T test.

	Parameter of NaCl& $CaCl_2$ treatment.	Mean of NaCl treat.	Difference (NaCl-$CaCl_2$) treat	Amelioration %
Growth parameters	Sh_fr_m.	67.33	19.6	29.11
	Sh_dr_m.	60.97	48.50	79.55
	Rt_ fr_m.	63.88	17.37	27.19
	Rt_ dr_m.	55.70	39.03	70.08
	Leaf area	74.62	13.08	17.53
Metabolites	Sh_S.S.	62.16	22.75	36.61
	Rt_S.S.	187.23	364.93	194.91
	Sh_S.P.	115.04	25.45	22.12
	Rt_S.P.	75.46	6.55	8.69
	Sh_A.A.	77.16	63.10	81.77
	Rt_A.A.	74.16	92.05	124.13
	Sh_Prol.	133.98	7.69	5.74
	Rt_Prol.	188.33	48.33	25.66
Cationic status	Sh_ Na^+	117.21	11.65	9.94
	Rt_ Na^+	216.67	122.78	56.67
	Sh_ K^+	133.34	17.05	12.79
	Rt_ K^+	54.23	12.43	22.93
	Sh_ Ca^{++}	72.39	8.18	11.30
	Rt_ Ca^{++}	72.0	30.33	42.13
	Sh_ Mg^{++}	186.36	15.15	8.13
	Rt_ Mg^{++}	66.67	7.33	11.00
C.w. D.enz	Cellulase	39.17	15.33	39.15
	PG.	73.07	17.07	23.36
	PMG.	60.67	10.17	16.76

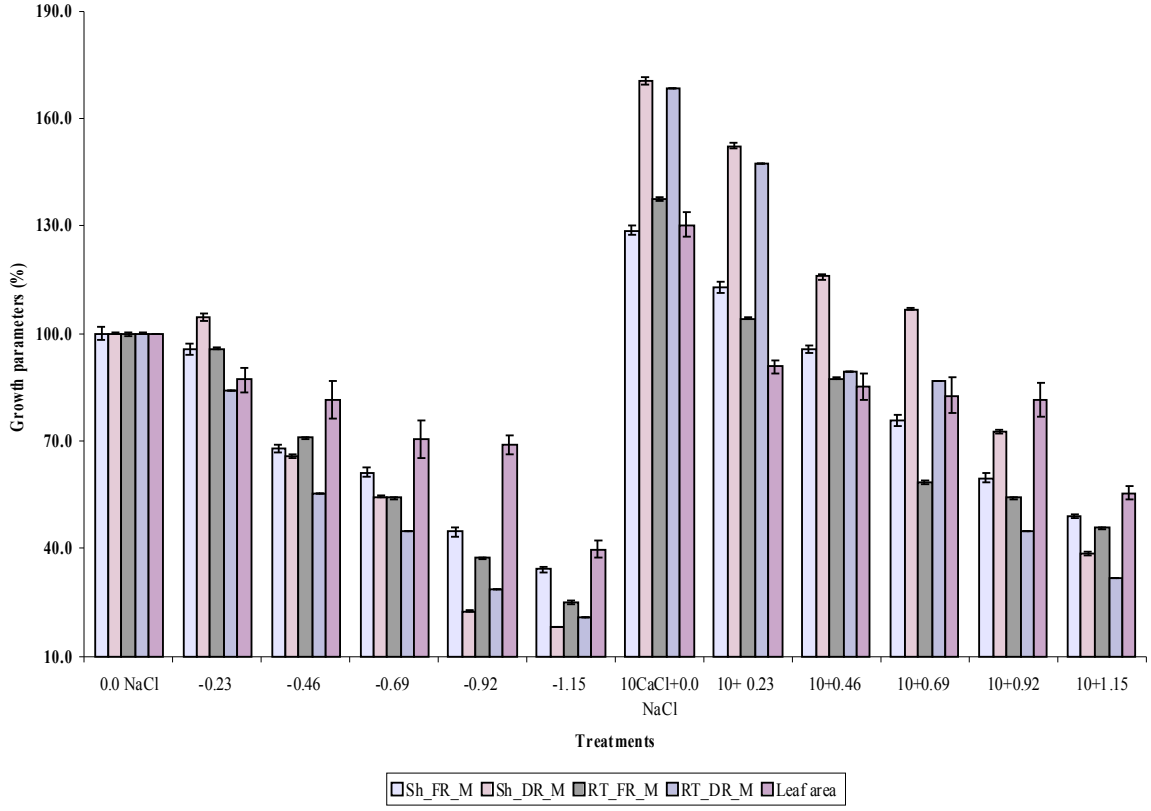

Figure 1. The effects of different levels of NaCl and mixed salts of NaCl+10 mM $CaCl_2$ on shoot, root fresh &dry mass and leaf area (%) of stressed faba bean.

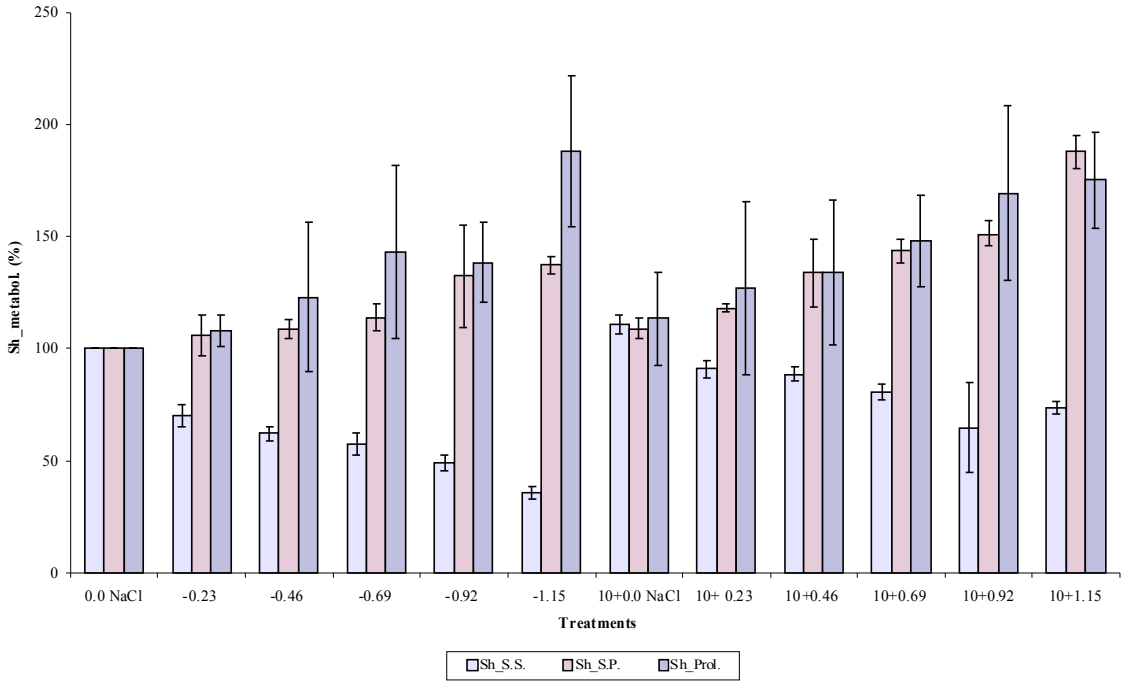

Figure 2. The effects of different levels of NaCL and mixed salts of NaCl+10 mM $CaCl_2$ on shoot S.S., S.P. and Proline accumulation (%) of stressed faba bean.

The percentage of reduction in fresh and dry mass of shoot at 1.15 MPa went to about 66 & 82% respectively. For root organ, the percentage of reduction of fresh and dry mass at that level was about 75& 79% respectively. The percentage of reduction in leaf area at the same level (-1.15 MPa) was 60%. However exogenous applications of $CaCl_2$ alone (10 mM + 0.0 NaCl) enhanced shoot fresh and dry mass by about 29.0, 70.5%, 37.5, 68.0 and 30% for root fresh and dry mass and leaf area respectively compared to controlled plant. The combination of salts (10 mM $CaCl_2$ + different concentration of NaCl) enhanced both shoot and root fresh and dry mass as well as leaf area, with 29.1,79.6, 27.2, 70.1 &17.5% respectively, but this reduction was less than those in NaCl; treatment alone (Table 2).

Biochemical analysis

ANOVA table (1) showed that $CaCl_2$ significantly affected (p-value≤ 0.000) soluble sugar accumulation of both shoot and root organs with F-value 22.8 &38.6 for shoot and root respectively. Soluble saccharides and proteins showed different responses toward salinity stress in both shoot and root organs. In shoot tissue, soluble saccharides content decreased considerably with maximum reduction about 64% at -1.15 MPa, the soluble proteins increased under salinity stress up to 37% compared with control (Fig. 2), while reverse was true for roots. The magnitude of these contrasting effects was increased at the higher dose of the salt treatments. At -1.15 MPa NaCl levels, the root soluble saccharides were increased by more than two folds, while soluble proteins decreased to about 40% in relation to the control plants (Fig. 3).On contrary, soluble saccharide in shoot treatments with $CaCl_2$ only enhanced soluble saccharide accumulation by 11% in shoot and more than that in root for three folds. Protein contents were ranged from 9 to 6% for protein accumulation in shoot and root respectively. The combined salts alleviate the deleterious effect of high salinity dose in shoot from 64% to 27% with enhancement percentage reaches about 36.6 % .While in root this alleviation was maximum at high salinity (from seven folds- to more than three) over control with enhanced about two times at that level of NaCl treatments (194.9%), (Table 2.)

For protein, analysis of variance showed the mixed salts of NaCl and $CaCl_2$ significantly affecting (P≤0.000) accumulation of protein in both tested organs of faba bean plant with F-value 8.55&18.84 for shoot and root respectively. The mixed salts improved shoot protein accumulation at high salinity level (-1.15 MPa) from 187.7 against 137.2% at NaCl only with amelioration percentage reached about 22.1 % in shoot tissue (Fig. 2). On the other hand, for mixed salts enhanced root protein accumulation from 66.7% compared to 59.0% at high salinity level of NaCl treatment, with enhancement ratio about 8.7 %, which less than that of shoot at control treatment (Table 2).

Proline accumulation was highly significant (P≤0.000) as affecting by the salt treatments, with F-values (10.0 &21.4) for shoot and root respectively. Proline content of shoot tissue was enhanced with only NaCl, ,$CaCl_2$ and their interactions but the maximum (88.1%) was recorded at (-1.15MPa NaCl) over the control under NaCl only, (13.6%) under only $CaCl_2$ treatment and 75.6% at high combined salinity (10 mM $CaCl_2$ +-1.15 MPa NaCl). In contrary to shoot, the enhancement in roots was maximum 333.6% (more than three folds) was observed with high combined salts treatments(10 mM $CaCl_2$ +1.15 MPa NaCl) over the

control one, more than two and half fold (263.9%) at high dose of only NaCl treatment (-1.15 MPa), and 163.9% at $CaCl_2$ alone (10 mM $CaCl_2$ + 0.0 NaCl) respectively. The accumulation of proline in root organ was higher than that in shoot. The proline in salt stressed root was accumulated in order of combined salts > only NaCl stress >Only $CaCl_2$ (Fig .3).

Cationic analysis

In our study, Na^+ accumulation was significantly effected by $CaCl_2$ treatments ($P \leq 0.000$) with F-value 11.2 &71.5 for shoot and root respectively (Table 1). In shoot, Na^+ was increased as salinity increased up to 59% at high salinity dose (-1.15 MPa) over the controlled plant. $CaCl_2$ alone (10 mM $CaCl_2$+0.0 NaCl) reduced the accumulation of Na^+ ion by about 12.5%. The combined salts alleviate the Na^+ injury in shoot by about 31% (Fig. 4). While root organ accumulate more Na^+ with increasing salinity reaching up to about 351% (three and half times) than unstressed plants The application of $CaCl_2$ only has no effect on Na^+ accumulation in root compared to control one. The combined salts application reduced Na^+ ion accumulation in root organs. The maximum accumulation of Na^+ takes place in the roots than shoots (Fig. 5). K^+ ions were highly significant ($P \leq 0.000$) with F- value 10.3&19.2 for both shoot and root respectively (Table 1). K^+ was much accumulator in shoot than root. It was increased with increasing salinity up to 72% at highest salinity (-1.15 MPa) against control. $CaCl_2$ alone increased K^+ content by about 7%, the combination of salts accumulate huge amount of K^+ reaching more than two folds at high salinity level compared to control, whereas it was decreased in root under all treatments (Fig. 5).Calcium (Ca^{2+}) contents also decreased under the influence of NaCl in both shoot and root organs. In the only $CaCl_2$ treatments the increased in Ca^{2+} accumulation ranged from 3.7 in shoot to 25.7% in root. In the combined treatments of $CaCl_2$ and NaCl, the ca accumulation was greater in root than shoot. Shoots were more severely affected than roots (Table, 2). In contrary to Ca^{2+}, in shoot Mg^{2+} was increased under all treatments and vise versa for root organs (Fig.4 & 5).

Cell wall degrading enzymes analysis:

Analysis of variance showed that all treatments significantly ($P \leq 0.000$) affecting cellulase, PG and PMG enzymes with F – value 24.5, 108.1 &38.9 respectively. Cell wall degrading enzymes (cellulase, PG and PMG) significantly increased as salinity of tested plants increased (Fig. 6) as compared with control plants. 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 (39.17%), PG, (17.07%) and PMG (16.76%). This reduction was more obvious in the activity of cellulase and PG especially at lower salinity levels (Table 2). Our findings highlight the positive role of $CaCl_2$ in salt stressed faba bean plant

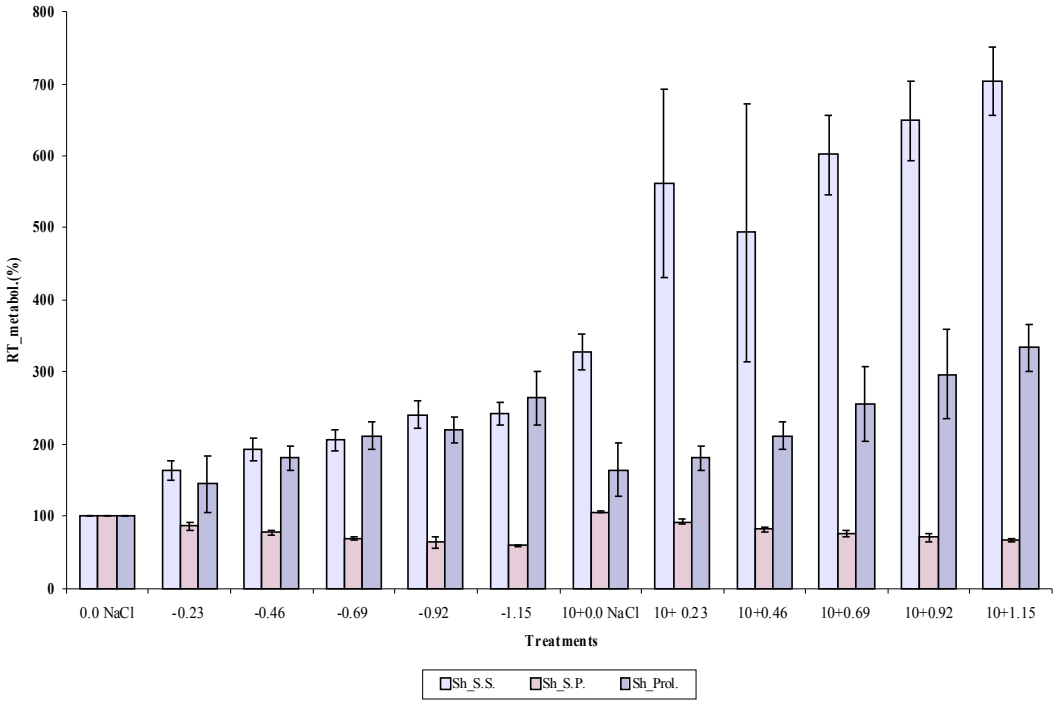

Figure 3 The effects of different levels of NaCL and mixed salts of NaCl+10 mM $CaCl_2$ on root S.S., S.P. and Proline accumulation (%) of stressed faba bean.

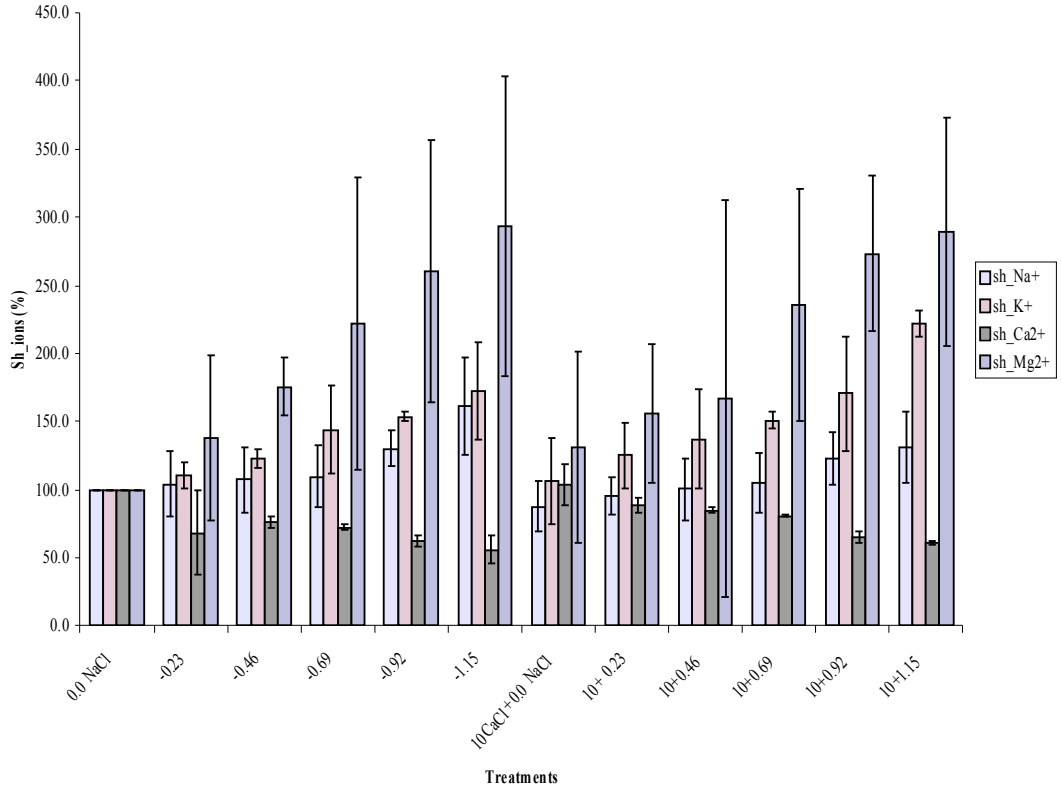

Figure 4. The effects of different levels of NaCL and mixed salts of NaCl+10 mM $CaCl_2$ on shoot Na^+, K^+ ,Ca^{2+} and Mg^{2+} concentration (%) of stressed faba bean.

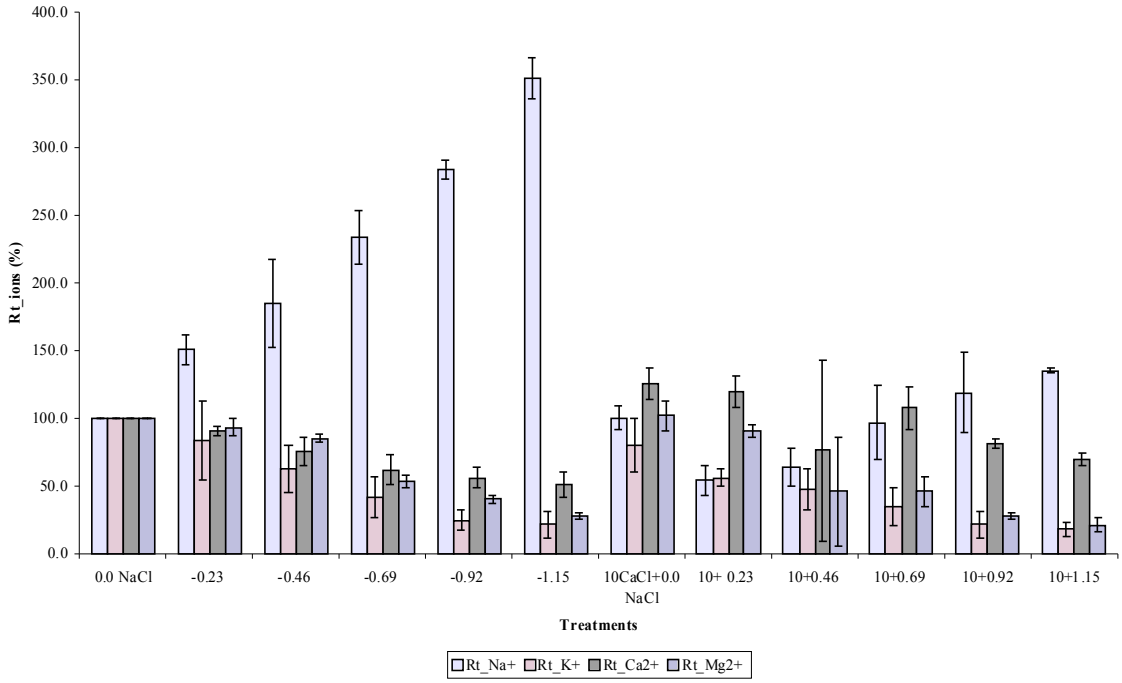

Figure 5. The effects of different levels of NaCL and mixed salts of NaCl+10 mM $CaCl_2$ on shoot Na^+, K^+ ,Ca^{2+} and Mg^{2+} concentration (%) of stressed faba bean.

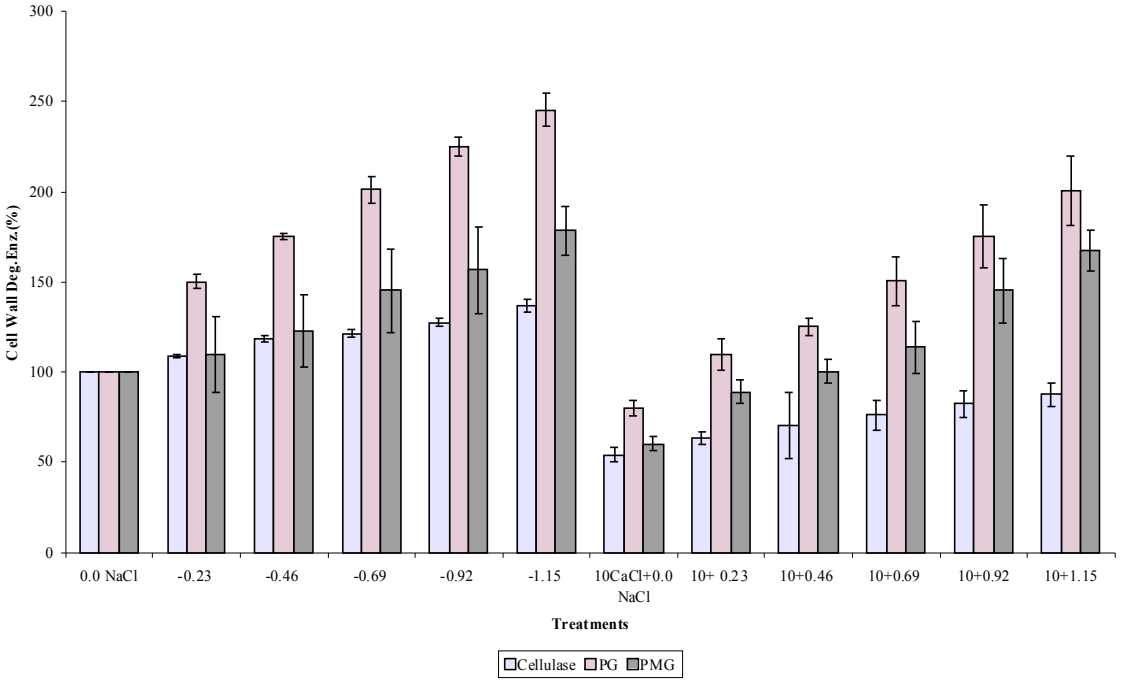

Figure 6. The effects of different levels of NaCL and mixed salts of NaCl+10 mM CaCl2 on cellulase, PG and PMG concentration (%) of stressed faba bean.

DISCUSSION

Regarding to the growth performance of faba bean in the present study, although salinity stress decreased the dry mass of shoot and root, the percentage of this reduction varied between the two plant organs. Root seemed to be the salt accumulator organ than shoot, whatever the salinity level was.

With the increasing concentration of NaCl, both of shoot dry weight as well as leaf area were markedly inhibited as compared with control. Similar reductions in leaf area were caused by salinity.

NaCl exhibited a dose-dependent inhibitory effect on leaf area might be due to inhibition of cell division and cell expansion under salt stress (Serraj and Sinclair 2002 and Abdel Latef 2011). This reduction in most crops of salt stress has also been reported (Li et al. 2006; Kusvuran et al. 2007 and Tunçtürk et al. 2008). Their results confirmed these earlier observations of growth reduction due to NaCl treatment. Probably the negative effect of salinity on plants provoked osmotic potential by salt in the culture medium, such that the root cells did not obtain the required water from the medium (Mer et al. 2000). Therefore, in plants, the uptakes of some mineral nutrients dissolved in water are also restricted (Tunçtürk et al. 2011). Thus, growth and development of plants were inhibited due to the occurring defect in metabolism. Other researchers thought that the growth reduction is the consequences of ion accumulation through the changing of membrane permeability (Grieve and Fujiyama 1987) .

As stated by (Munns 2002), suppression of plant growth under saline conditions may either be due to the decreasing availability of water or to the increasing toxicity of sodium and chloride associated with increasing salinity. The decreased root and shoot dry weights was apparently due to the accumulation of salts in the external medium, which increase the osmotic pressure of the soil solution and greatly decreases the availability of water to the roots. Calcium chloride application thus alleviated the NaCl toxicity and minimized the reduction in biomass caused by NaCl. Ca^{2+} acts as a secondary messenger for many biochemical processes within the cytosol (Mahajan and Tuteja 2005).

Addition of Ca^{2+} might be promoted plant growth and dry weight and minimized the adverse effect of NaCl in the present study, as it did also in the case of *Cassia angustifiolia* (Arshi et al. 2005).

$CaCl_2$ treatment improved both dry matter and leaf area with reference to the control. Thus, calcium appeared to mitigate the adverse effect of NaCl. While, salt stress inhibited cell division, cell enlargement and consequently leaf expansion. Calcium may favor cell elongation and cell expansion, ultimately increasing the leaf area (Hernandez and Almansa 2002). NaCl alone and in combination of $CaCl_2$ inhibited the root and shoot dry weights in comparison with the control. However, the combined salt treatment was less effective than NaCl treatment alone. The maximum reduction was 70% in the root dry weight and 79.55% in the shoot dry weight against all NaCl concentrations. Concerning the biochemical constituents, under salinity stress plants try to reduce their osmotic potential via increasing compatible solutes synthesis and mineral ions content to better water uptake. In the present study, the organic cyto-solutes (soluble carbohydrates, soluble proteins and proline) were much higher in roots than shoots, which confirmed that broad bean plant considered its strategy in root. Soluble sugar decreased with increased salinity in shoot and vice versa in root. Soluble carbohydrates are important solutes that are synthesized and accumulated in cytosol to maintained turgor for better growth under salt stress. Addition of calcium may increase α-amylase activity, which leads to the degradation of starch into sugars; α--amylase requires Ca^{2+} for its activity (Jaleel et al.2007). Regarding to protein degradation of root in a saline environment has been attribute to decreased protein synthesis, accelerated proteolysis, and denaturation of enzymes involved in protein synthesis (Levitt 1980). In this work, addition of

calcium increased the protein content of salt-stressed plants. Calcium treatment apparently may increases protein synthesis in salt-stressed plants. Combining $CaCl_2$ with NaCl treatment increased protein content versus NaCl stressed plants (Table. 2). Similar results have been reported in sorghum ((Jaleel et al.2007).In most plants, there is an increased in accumulation of amino acids and amines (e.g., proline, B-alanine, glycinebetaine) in their tissues in response to salt stress. The way these compounds are accumulating differs between species and ranges from only one to several different compounds is being accumulated. Generally, plant species that accumulate proline usually have low amounts of this amino acid when grown in well-watered and non-saline soils, increasing its contents upon imposition of salt stresses (Sakamoto and Murata 1998). The induction of proline accumulation may be due to an activation of proline synthesis through glutamate pathway as a tolerance mechanism to high salinity stress (Azooz et al. 2004). Although the precise role of proline accumulation is still debate, proline is often consider as a compatible solute involved in osmotic adjustment (Wang and Liang 1995). Accumulation of proline may occur through an increase in its synthesis constantly with inhibition of its catabolism (Jaleel et al. 2007), and may be a mechanism for stress tolerance. However, its role in imparting stress resistance under saline conditions is controversial. In the present work, proline content was enhanced with the NaCl alone, $CaCl_2$ alone and combined salts but the maximum (three-fold) enhancement over the control was observed with combined salts (NaCl + $CaCl_2$) treatments against each of the individual treatments of NaCl and $CaCl_2$. NaCl treatment alone caused two and half -times higher and $CaCl_2$ treatment alone caused one and half-times higher proline accumulation than reference plant respectively (Table 2).

Addition of $CaCl_2$ + NaCl increased the proline content mainly due to breakdown of proline-rich protein and fresh synthesis of proline and amino acids (Jun et al. 2000) . Increased proline in the stressed plants may be an adaptation mechanism to compensate the energy for growth and survival and thereby helps the plant to tolerate stress, as observed in spinach leaves (Ozturk and Demir 2003), *Cassia angustifolia* (Arshi et al. 2005) and *Catharanthus roseus* (Jaleel et al. 2007).

Regarding the cationic status, most of the aforementioned results of growth parameters and accumulation of inorganic cyto-solutes in the present work were accompanied with a lower Na^+ content in the shoots than roots. This may indicated that while Na^+ content increased smoothly up - 0.92 MPa in shoot (about only 13.6 % increase), the increased in root about 66.7 % at the same salinity level which means the differences in salt tolerance among the two plant organs was closely related to the differences in the Na^+ content. In conformity, K^+ and Ca^{2+} 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^{2+} into the aerial parts of the plant. Alfocea et al. (1993) and Hamdia et al. (2004) reported that excessive Na^+ in salt tolerant tomato and wheat plants respectively does not affect K^+ nutrition. This situation was interpreted by (Garacia et al. 1997) they 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. The K^+ content in plant tissues represents the main cation in plant cells, and it is an important component of the cell osmotic potential (Reggiani et al. 1995). One of the primary plant responses to salinity is the decrease in K^+ concentration in plant tissues (Elsamad and Shaddad

1997) and thus the substitution of K^+ by Na^+ may lead to nutritional imbalances. Both of these ions might compete for entry into plant root cells. This competition has significant negative effects on plant growth in saline soils, where concentrations of sodium often exceed those of potassium. Many authors (Carjaval et al.2000; Grieve and Poss 2000; Hamdia et al. 2004) recorded antagonistic relations between Na^+ and K^+ or negative effects of salinity on K^+ uptake in different plants. The mechanisms of ion distribution increased the osmotic pressure of the shoot, which facilitates the steepness of osmoregulation towards the aerial parts that in turn increases the water flow from the root to the shoots as a result of which water status maintained (the conservation and utilization). Calcium ions are known to have a regulatory role in the metabolism; Ca^{2+} ions may compete with Na^+ ions for membrane-binding sites and can protect the cell membrane from the adverse effect of salinity (Zidan et al. 1990 and Abdel Latef 2011). In the present study calcium (Ca^{2+}) contents also decreased under the influence of NaCl alone and combined treatments of NaCl + $CaCl_2$, but the reduction was less with combined treatments than with NaCl alone. Roots were more severely affected than shoot. On the contrary, $CaCl_2$ treatments increased the calcium content of these plant parts significantly

Along with these results, the organic cyto-solutes (soluble carbohydrates, soluble proteins and proline) was much higher in shoots than roots, which again confirmed that broad bean plant considered its strategy in shoot (Gobinathan et al. 2009). In conformity to the above results and discussion when these plants treated with $CaCl_2$ are:

1- Na^+ and proline content retarded considerably in both plant organs.

2- The amount of organic cyto-solutes (soluble carbohydrates, soluble proteins and proline) also improved markedly which in turn could increase the water status and consequently the dry matter yield when compared with the only salinized plants.

3- The amount of inorganic cyto-solutes (K^+ and $Ca2^+$) in general increased markedly.

The activity of cell wall degrading enzymes (cellulase, PG and PMG) significantly increased as salinity increased. This is also in accordance with (Singh and Prasad 2009). They 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. In addition, (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.

Results also showed that $CaCl_2$ treatments resulted in a significant decrease in the activity of cellulase, PG, and PMG as compared with the corresponding levels of salinity. 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 the significant role of $CaCl_2$ in enhancement broad bean plant under salinity stress.

Generally, supplementing the medium with Ca^{2+} alleviates growth inhibition by salt via increase halotolerance of some glycophytic plants and Ca^{2+} had an ameliorative effect on the growth of NaCl-stressed plants, by modulating overall metabolism.

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