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Stress Evokes Changes in Response to Sakha-69 Wheat According to Sodium and Calcium Anions

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Abstract: Accumulation of Na^+ in the soil represents a problem for agricultural production. This may be alleviated by calcium. Our objective was to determine the relative effects of Cl^- and SO_4^{-2} on Ca^+ alleviation of Na^+ stress. We treated *Triticum aestivum* L Sakha-69 seedlings growing under natural conditions to NaCl or Na_2SO_4 at the level of 15,30 and 45 mM/L either alone or in combination with 15 and 30mM $CaCl_2$ or $CaSO_4$ in addition to a reference control. Nutrient solution with added treatments was applied once a week. Increasing NaCl concentration decreased Chl a concentration at the tillering stage, the reduction in case of Na_2SO_4 was more than the previous case, while carotenoids concentration was significantly increased. Activity of amylases and concentration of soluble protein were depressed, while concentration of reducing sugars was significantly increased with increasing NaCl concentration. The response of Sakha-69 wheat for such parameters, on applying Na_2SO_4, was completely inverted. Sucrose concentration was increased concomitant with a decrease in polysaccharide concentration at the heading stage for both treatments. Addition of $CaSO_4$ to NaCl alleviated the depressive effect of salinity on Chl a, carbohydrate fractions as well as soluble protein. On the other hand, addition of $CaCl_2$ to NaCl was more beneficial for alpha amylase activity. It is observed that alternative anions had a better effect on the concentration of soluble protein. It is also noticed that the depressive effect of Cl^- at early growth stage was diminished at the advanced stage. Application of $CaCl_2$ at both concentrations with 30mM Na_2SO_4 highly significantly increased polysaccharide concentration compared to both corresponding and reference control.

Key wards: Na^+, Ca^{2+}, Cl^-, SO_4^{2-}, wheat, Chl, carotenoids, soluble protein, sugars, amylases.

INTRODUCTION

Nearly 20% of the world's cultivated area and approximately half of the world's irrigated lands are affected by salinity (Zhu, 2001). It was estimated that salinization reduces the world's irrigated area for crop production by 1–2% every year (FAO, 2002).

Salinity is a major abiotic stress in plants worldwide. Salt stress causes an initial water-deficit, due to the relatively high solute concentrations in the soil, and also ion-specific stresses resulting from changes in K^+/Na^+ ratios. Thus, it leads to increased Na^+ and Cl^- concentrations that are detrimental to plants (Yamaguchi and Blumwald, 2005). Salt-stressed plants exhibit a decrease in their photosynthetic efficiency(Ma *et al*., 1997). Salt also affects photosynthetic components such as enzymes, chlorophylls, and carotenoids, cytosolic enzyme activities, and metabolism (Niu *et al*., 1995).

Soil salinity in nature is normally a mixture of different salt species, where sulphate and chloride salts often dominate in saline soils (Wang *et al*., 1991). Sulphate and chloride anions are very different in physiochemical behaviors. Sulphate salts were less toxic to nitrification than Cl^-(Bohn *et al*., 1985).

Salt stress tolerance in plants is a complex phenomenon that may involve developmental changes as well as physiological and biochemical processes (Hare and Cress, 1997). In halophytes, salt tolerance is a result of inorganic ion accumulation, mainly Na^+ and Cl^-, which are compartmentalized in the vacuole, while organic solutes accumulate in cytoplasm balancing water potential through several cellular compartments (Serraj and Sinclair, 2002). In addition to their role in cell water relations, organic solute accumulation may also contribute to the maintenance of ionic homeostasis and stabilization of some macromolecules and organelles such as proteins, protein complexes and membranes (Bray *et al*., 2000).

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Calcium plays an essential role in processes that preserve the structural and functional integrity of plant membranes, stabilizes cell wall structures, regulates ion transport and selectivity, and controls ion-exchange behaviour as well as cell wall enzyme activities. Because calcium appears to be readily displaced from its membrane binding sites by other cations, these functions may become seriously impaired. Maintaining an adequate supply of calcium in saline soil solutions is an important factor in controlling the severity of specific ion toxicities, particularly in crops which are susceptible to sodium and chloride injury (Grattan and Grieve, 1999). Although the solubility and mobility of the Ca-salt ion in the soil had been studied, little research has examined whether different forms of Ca vary in their beneficial effects on plant and soil.

The aim of this study was to investigate the role of the anions, SO_4^{2-} and Cl^- on the ability of different forms of Ca to ameliorate salinity . Effects were assessed by examining photosynthetic and some antioxidative pigments, some osmolytes, carbohydrate metabolism and activities of alpha & beta amylases.

MATERIALS AND METHODS

The Egyptian cultivar Sakha-69 of wheat (*Triticum aestivum* L) was obtained from Gemmiza Agricultural Research Station, Gharbia. Grains of wheat were germinated in acid-washed sand and allowed to grow in black plastic bags. The total number of bags used in the experiment was 155 (31 sets each set comprised 5 replicates) arranged in complete randomized block design. Grains were watered regularly with excess of tap water for 14 days till the grains were germinated. Treatments were then applied. The nutrient solution as described by Hogland and Arnon (1950).

The applied treatments were made of two series of three concentrations of Na salts (NaCl or Na_2SO_4, at the rate of 15, 30 and 45 mM/L) were applied alone or in combination with two concentrations for both Ca salts ($CaCl_2$ or $CaSO_4$, at the rate of 15 and 30mM/L). In addition to one common control for both series (neither Na nor excess Ca addition). All these treatments were added to the nutrient solutions and applied once a week. The experiment was carried out in an experimental garden at Botany Department, Faculty of Science, Tanta University, under the normal environmental conditions.

Some plants from each treatment were randomly selected and harvested at the tillering and heading stages. The harvested plants were washed thoroughly with tap water, then with distilled water. Fresh samples were used for the determination of photosynthetic pigments and enzyme activity. Estimation of photosynthetic pigments was carried out according to the method of Metzner *et al*.(1965). The procedure mentioned by Rick and Stegbauer (1974) was used to assay alpha & beta amylases. The plant samples were dried in an air-forced oven at 60^{o}c to a constant weight. The dried materials were ground using an electric mixer and the fine powders were kept in paper bags for further analyses .The soluble sugars and starch concentrations in the plant shoot were estimated quantitatively using the modified Nelson's method (1944) by Naguib (1963). Concentration of total soluble proteins was estimated quantitatively using the method described by Bradford (1976).

Statistical Analysis:

The obtained data were subjected to statistical analysis according to Gomez and Gomez (1984). Means of treatments were compared using new least significant differences (NLSD) as described by Waller and Duncan (1969).

RESULTS AND DISCUSSION

Concentration of Pigments:

The obtained results showed that there was a significant reduction in the concentration of Chl a in leaves of both NaCl (except 15mM treatment) and Na_2SO_4 treated plants at the tillering stage with increase in the concentration of Na salts in the nutrient solution, while it was significantly increased up to 30mM(NaCl treatment),the same concentration for Na_2SO_4 treatment showed non-significant effect at the heading stage (Fig.1 a&b).

The results showed that addition of 30mM $CaCl_2$ to 45mM NaCl increased Chl a concentration by 15.3 % at heading stage compared to its corresponding control. It's observed that using $CaSO_4$ was more effective in ameliorating the depressive effect of salinity on Chl a concentration than $CaCl_2$. At the tillering stage, Chl a concentration for the treatment of 30mM NaCl+30mM $CaSO_4$ was 2 times that of comparable $CaCl_2$. In this respect, Awada *et al*.(1995) stated that calcium sulfate treatments ameliorated Na-induced salinity in snap beans more than did comparable calcium chloride treatments.

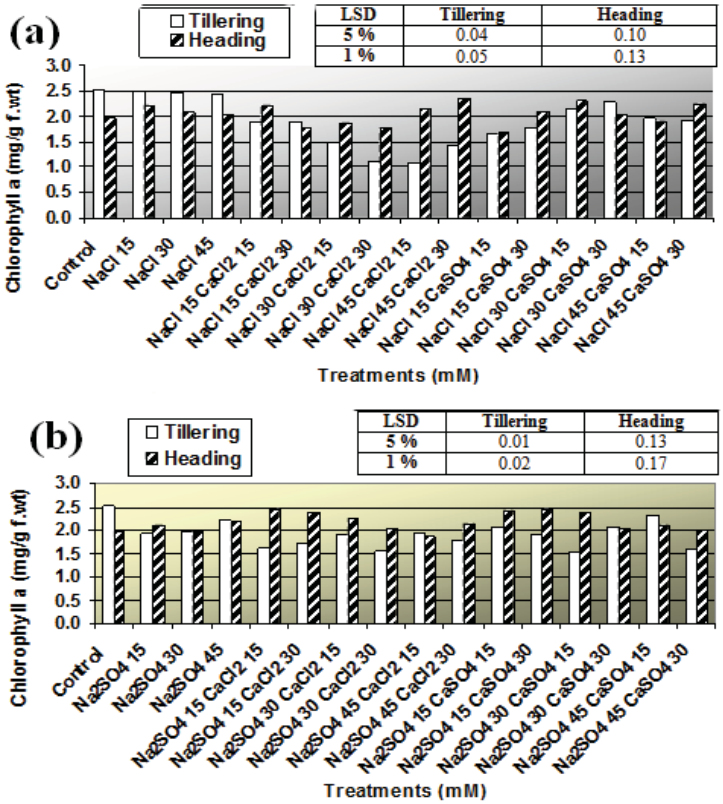

Fig. 1: Concentration of chlorophyll a (mg/g f.wt) in leaves of wheat plants affected by NaCl(a), Na_2SO_4 (b) and their combination with the two concentrations of $CaCl_2$ or $CaSO_4$ at tillering and heading stages of growth.

Concentration of chlorophyll b (Chl b) was non-significantly affected at the tillering stage due to NaCl treatments (Fig. 2). Application of 30mM NaCl significantly increased concentration of Chl b by 16.5% with regard to the control at the heading stage. The highest concentration of Na_2SO_4 recorded an increase by 6.3 % at the same stage.

Concerning the effect of $CaSO_4$, Chl b value for the treatment of 30mM NaCl +15Mm $CaSO_4$ exceeded the control value by 17.7%. Also, the increase by 16.9% was due to 15mM Na_2SO_4 + 30mM $CaSO_4$ treatment at heading stage compared to its corresponding control.

The results in Fig. 3 showed that there was a significant increase in the concentration of carotenoids in leaves of the treated plants at the tillering stage with increase in the concentration of NaCl. The concentrations of carotenoids in plants irrigated with 30mM NaCl were increased by 45.7&10.2 % in comparison with the control at tillering and heading stages, respectively.

The obtained results showed that at the heading stage, the addition of 30mM $CaCl_2$ to 15mM NaCl increased carotenoids concentration by 14.3%, while the addition of 30mM $CaSO_4$ to 15mM NaCl increased its concentration by 7.9 % with regard to their corresponding control. On the other hand, addition of 30mM $CaCl_2$ to 15mM Na_2SO_4 increased concentration of carotenoids by 4.8%, while 30mM $CaSO_4$ + 15mM Na_2SO_4 increased it by 11.3% with regard to their corresponding control.

The reduction in chlorophyll content observed in salt-stressed plants may be either due to salt induced retardation of synthesis of the pigments or/and acceleration of pigment degradation (Santos, 2004), in addition to the instability of the pigment protein complex (Jaleel *et al*., 2007). As salinity adversely influenced the photosynthetic process, photosynthetic production (e.g. sugar) was inhibited. The reduction in chlorophyll content concurrently with the increase in soluble protein contents led to the suggestion that nitrogen may be shifted to the synthesis of protein instead of chlorophyll. It is well established that carotenoids offer protection against photooxidation (Mittler, 2002).

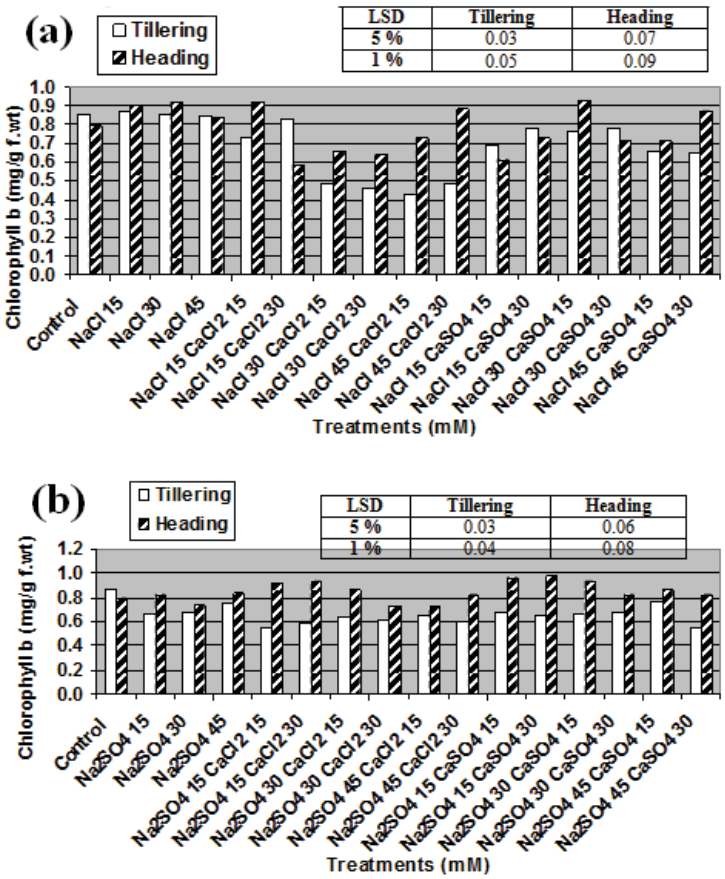

Fig. 2: Concentration of chlorophyll b (mg/g f.wt) in leaves of wheat plants affected by NaCl(a), Na_2SO_4(b) and their combination with the two concentrations of $CaCl_2$ or $CaSO_4$ at tillering and heading stages of growth.

Carbohydrate Fractions:

It is observed that all carbohydrate fractions were reduced with age. It is also noticed that there was a significant increase in the concentration of reducing sugars with increasing NaCl concentration from zero up to 45mM at both tillering and heading stages of growth(treatment of 15mM at heading stage was non-significantly affected). On the contrary, concentration of reducing sugars was significantly decreased with increasing Na_2SO_4 concentration in the nutrient solution during various growth stages compared to the control (Fig. 4).

Carbohydrates accumulate in various plants under salinity conditions. It is well known that carbohydrate metabolism in salt affected plants differs greatly than that of non-salinized ones. Moderate salt stress caused a sharp increase in invertase activity and an accumulation of reducing sugars (Youssef, 1994). Osmotic adaptation to salinity and drought via soluble sugar accumulation are well documented (Patakas *et al*., 2002). Accumulation of certain carbohydrate fractions or of the total carbohydrate content may be induced in the stressed plants (Parida *et al*., 2002 A. Parida, A.B. Das and P. Das, NaCl stress causes changes in photosynthetic pigments, proteins and other metabolic components in the leaves of a true mangrove, *Bruguiera parviflora*, in hydroponic cultures, *J. Plant Biol.* 45 (2002), pp. 28–36.Parida *et al*., 2002). On the other hand, other researchers reported a marked reduction in certain carbohydrate fractions or in the total carbohydrate content of the stressed plants (Perry *et al*., 1987; Gadallah, 1999).

Combination between either NaCl or Na_2SO_4 and $CaCl_2$ or $CaSO_4$ for both revealed high significant reduction for reducing sugars at the tillering stage compared to the control. Treatment of (30mM) for both NaCl and $CaCl_2$ showed a significant increase over the control. Concentration of reducing sugars for the

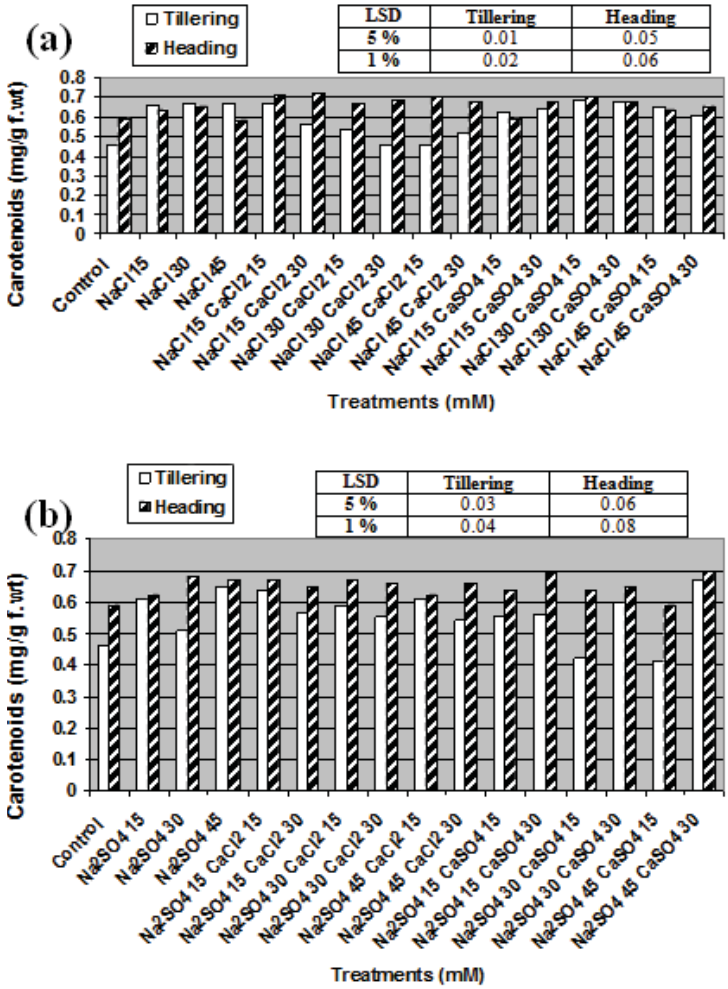

Fig. 3: Concentration of carotenoids (mg/g f.wt) in leaves of wheat plants affected by NaCl(a), Na_2SO_4 (b) and their combination with the two concentrations of $CaCl_2$ or $CaSO_4$ at tillering and heading stages of growth.

treatment of 30mM Na_2SO_4+15mM $CaSO_4$ was increased by 27.5% over its corresponding control. At heading stage, the concentration of reducing sugars was significantly increased under the different treatments of NaCl as well as Na_2SO_4 combined with $CaCl_2$ or $CaSO_4$ for both with some exceptions (Fig.4a&b).

At the tillering stage, concentration of sucrose was highly significantly decreased as the concentration of NaCl was increased from zero to 45mM (Fig.5). On the contrary, mean values of sucrose at heading stage were significantly increased, the increase due to15mM NaCl was non- significant. All Na_2SO_4 treatments highly significantly depressed sucrose concentration. At heading stage, 15mM Na_2SO_4 significantly decreased sucrose concentration, while it was highly significantly increased due to 30mM Na_2SO_4 treatment.

Plastid starch represents a reserve of sugars and is rapidly converted to sucrose under stress conditions. On the other hand, Al-Hakimi and Hamada (2001) found that, in wheat (*Triticum aestivum* L.), soluble sugars of shoots were lowered with the increase of NaCl concentration. However, the increase in soluble sugars was considered as a protective mechanism against protein denaturation (Todd, 1972). It is known that drought tolerance can be partly attributed to the accumulation of soluble sugars (Pelah *et al*., 1997) as they are able to protect the structural integrity of membranes during dehydration by preventing membrane fusion, phase transition and phase separation (Crowe and Crowe, 1992). Addition of $CaCl_2$ to NaCl resulted in a significant reduction in sucrose concentration except treatments of 15Mm NaCl with either 15or30mM $CaCl_2$ which

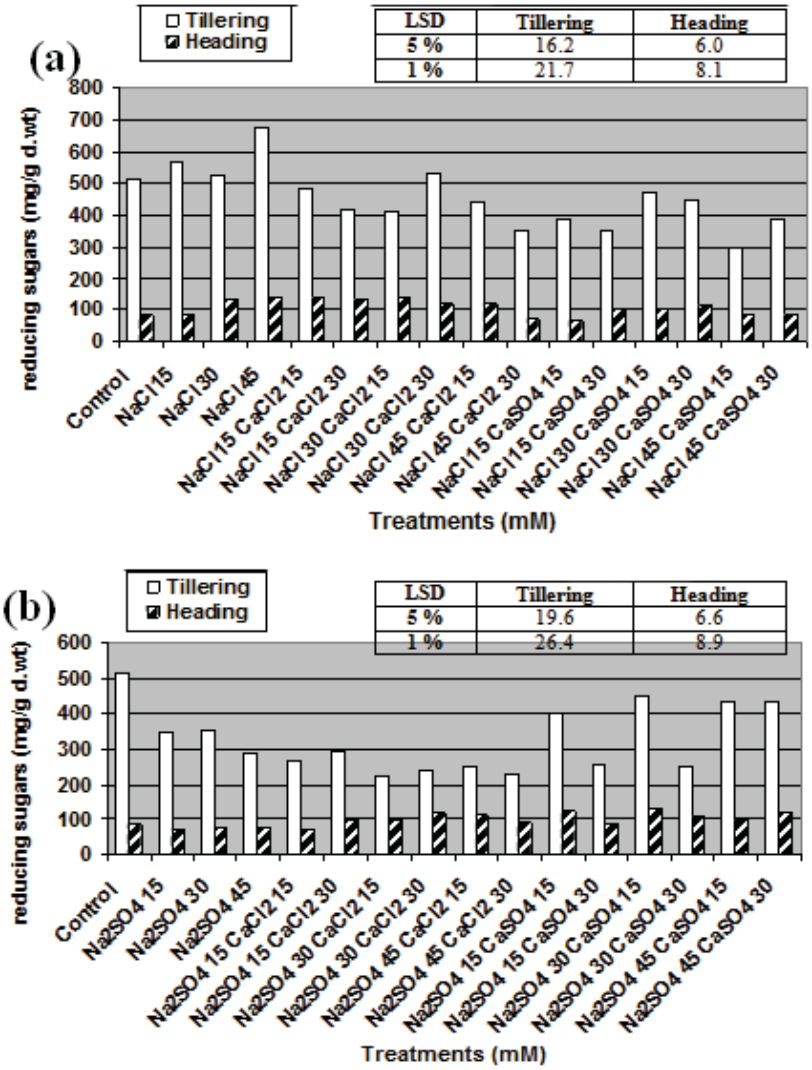

Fig. 4: Concentration of reducing sugars (mg/g d.wt) in shoot of wheat plants affected by NaCl(a), Na_2SO_4 (b) and their combination with the two concentrations of $CaCl_2$ or $CaSO_4$ at tillering and heading stages of growth.

revealed high significant increase at tillering stage. On the contrary, all $CaSO_4$ addition to NaCl resulted in high significant increase in sucrose concentration, except 30mM $CaSO_4$ treatments.

Treatment of 30mM Na_2SO_4+15mM $CaCl_2$ (at tillering stage) recorded high significant increase compared to the control. It boosted its corresponding control by 32.0 %. However, it recorded high significant reduction at heading stage (accumulated the highest significant value of starch at that stage).

The data showed that there were non-significant and high significant reductions in the concentration of polysaccharide at both tillering and heading stages of growth, respectively with increase of NaCl concentration (Fig. 6). It is also observed that Na_2SO_4 had a non-significant effect on polysaccharide concentration at the tillering stage, while such response was highly significantly depressed during heading stage (15mM Na_2SO_4 had a high significant increasing effect).

A decrease in starch content and an increase in both reducing and non-reducing sugars have been reported in leaves of *Bruguiera parviflora* (Parida *et al*., 2002). The contents of reducing and non-reducing sugars and the activity of sucrose phosphate synthase were increased under salt stress, whereas starch phosphorylase activity was decreased (Dubey and Singh, 1999).

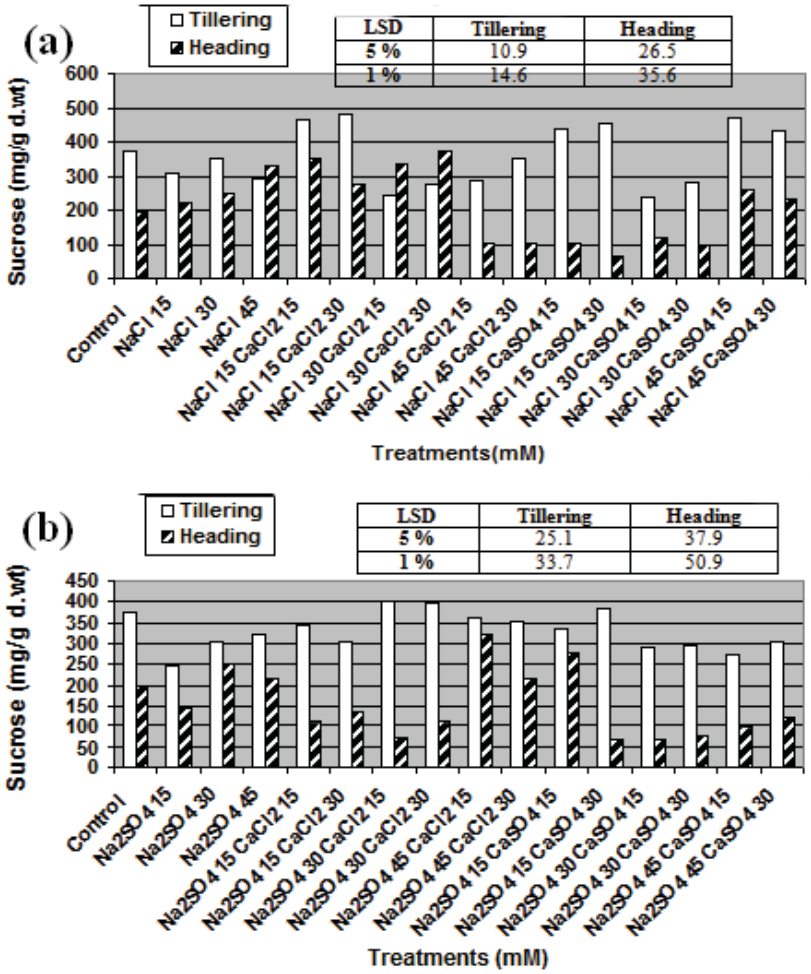

Fig. 5: Concentration of sucrose (mg/g d.wt) in shoot of wheat plants affected by NaCl(a) ,Na_2SO_4 (b) and their combination with the two concentrations of $CaCl_2$ or $CaSO_4$ at tillering and heading stages of growth.

At the tillering stage, combination of 15 or 30mM $CaSO_4$ with different concentrations of NaCl had stimulatory effect on polysaccharide concentration .They were non-significantly differed compared to the experimental control. Application of $CaCl_2$ at both concentrations with 30mM Na_2SO_4 highly significantly increased polysaccharide concentration compared to both corresponding and reference control. Addition of $CaSO_4$ to Na_2SO_4 at the highest level stimulates polysaccharide concentration by 1.6 fold compared to its corresponding control.

The aforementioned results revealed that the concentration of reducing sugars was increased with increasing NaCl concentration (at tillering and heading stages), while it was decreased as the concentration of Na_2SO_4 was increased. It is also observed that at the tillering stage concentration of sucrose was decreased for both NaCl and Na_2SO_4 treatments, meanwhile polysaccharide concentration was increased for the latter treatment. On the contrary, sucrose concentration was increased concomitant with a decrease in polysaccharide concentration at the heading stage for both treatments.

Restricted utilization of carbohydrates in salinized plants seems likely to be linked with sucrose metabolism .Water stress is known to alter carbon assimilate partitioning between sucrose and starch, resulting in an increase in sucrose concentration (Chaves, 1991). In this connection, Patakas *et al*. (2002) reported an increase in sucrose concentration in stressed leaves as a consequence of greater sucrose synthesis plus starch degradation.

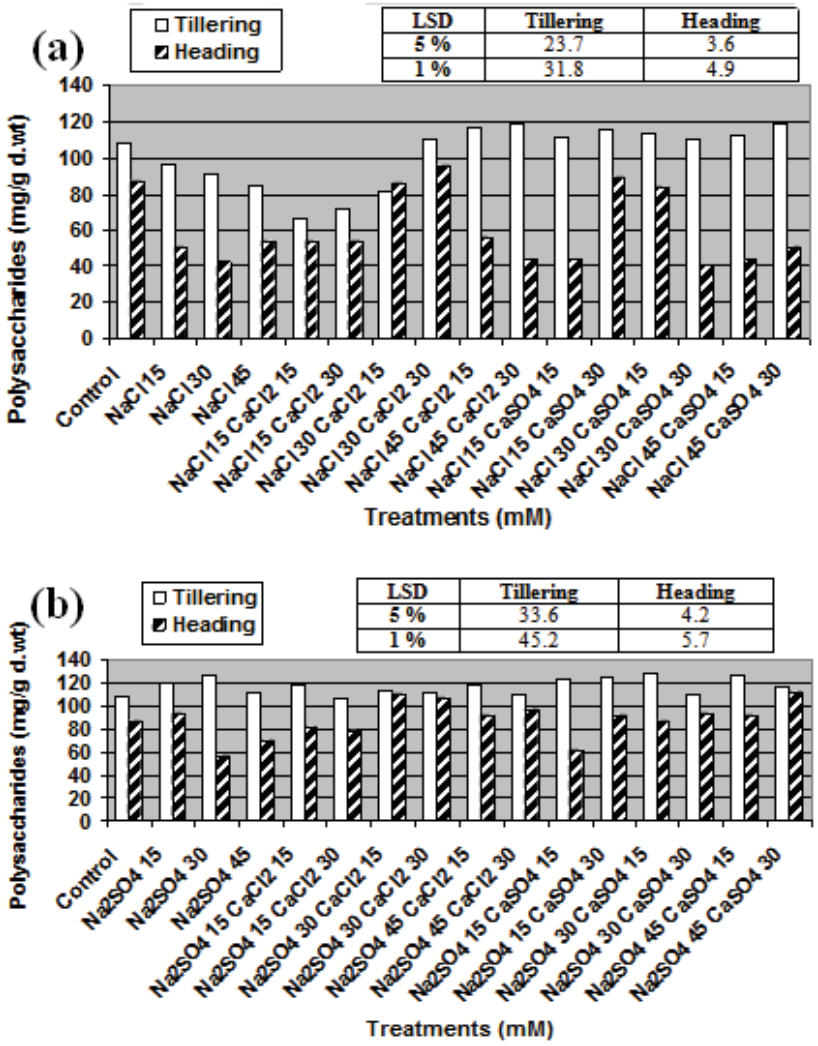

Fig. 6: Concentration of polysaccharides (mg/g d.wt) in shoot of wheat plants affected by NaCl(a) ,Na_2SO_4 (b) and their combination with the two concentrations of $CaCl_2$ or $CaSO_4$ at tillering and heading stages of growth

Amylase Activity:

Alpha amylase activity exceeds that of beta amylase especially at tillering stage (Figures 7& 8) . The average mean value of alpha amylase for NaCl treatments represents 1.6 fold that of beta amylase at the tillering stage.

By acting at random locations along the starch chain, α-amylase breaks down long-chain carbohydrates, ultimately yielding maltotriose and maltose from amylose, or maltose, glucose and "limit dextrin" from amylopectin. Because it can act anywhere on the substrate, α-amylase tends to be faster-acting than β-amylase. β-amylase working from the non-reducing end, it catalyzes the hydrolysis of the second α-1,4 glycosidic bond, cleaving off two glucose units (maltose).

α-Amylase (1,4-α-D-glucan glucanohydrolase):

Alpha amylase activity was decreased from the tillering to the heading stage. This is because of active vigorous growth at early growth stages to cope with sink demand (carbon skeleton and energy release).

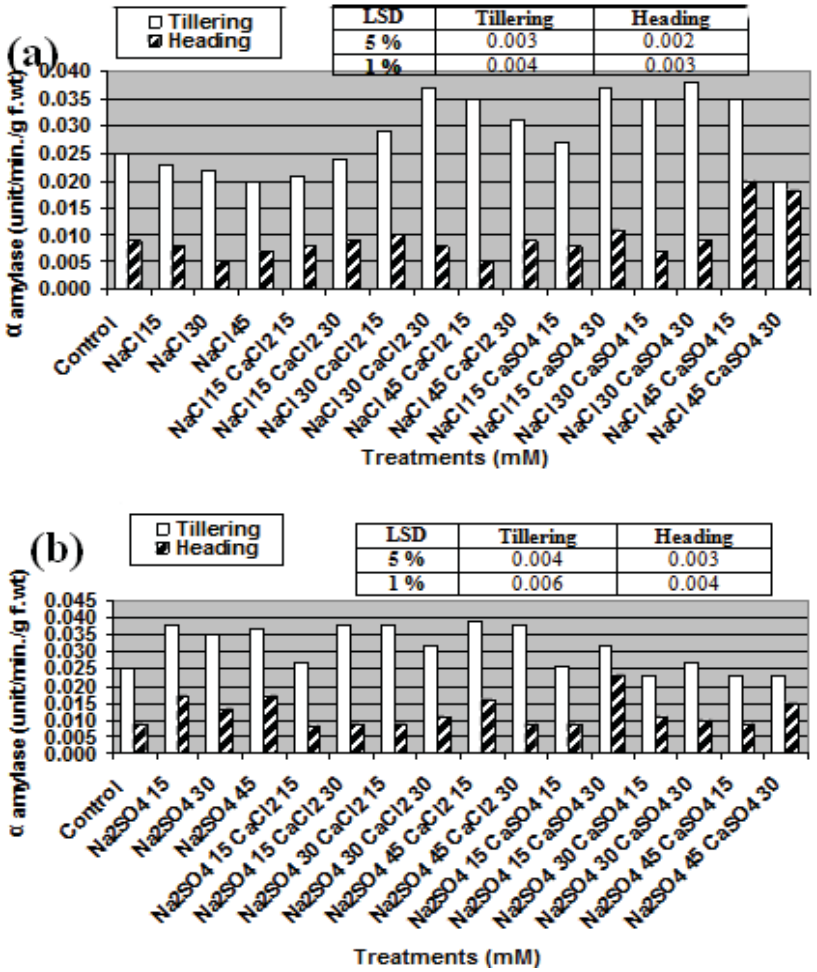

Fig. 7: Alpha (α) amylase activity (unit/min./g f.wt) in shoot of wheat plants affected by NaCl(a), Na_2SO_4 (b) and their combination with the two concentrations of $CaCl_2$ or $CaSO_4$ at tillering and heading stages of growth.

In case of NaCl treatments, the enzyme activity was significantly decreased with increase in NaCl concentration, the reduction was non-significant for 15mM NaCl treatment at both tillering and heading stages. On the contrary, increase of Na_2SO_4 concentration highly significantly increased α-amylase activity during tillering and heading stages of growth.

It is reported that salinity reduces protein hydration (Kramer, 1983) and induces changes in the activities of many enzymes in germinating seeds (Garg *et al*., 1993). Sodium salts suppress or stimulate the enzyme activity in plants. The effect of salinity varies with the stage of plant growth, plant organ, type of salinity and the studied enzyme (Sheoran and Garg, 1978).

Sodium is the primary cause of ion specific damage, resulting in a range of disorders in enzyme activation and protein synthesis [5] M. Tester and R. Davenport, Na^+ resistance and Na^+ transport in higher plants, *Ann. Bot.* 91 (2003), pp. 1–25.. High salt concentrations inhibit enzymes by impeding the balance of forces controlling the protein structure (Serrano *et al*., 1999). Several investigations showed that NaCl lowered the activity of different enzymes by decreasing or increasing the rate of transcription or translation (Ostrem *et al*., 1987) as well as the turn over rate of enzymes (Karlekar *et al*., 1985).

It is observed from Fig. 7 that α-amylase activity, for the treatments of 30mM NaCl + 30mM either $CaCl_2$ or $CaSO_4$, was 1.7 times that of corresponding control. The increase may be due to the antagonistic effect of Ca against deleterious effects of Na. Also, α-amylases are calcium metallo-enzymes, completely unable to function in the absence of calcium.

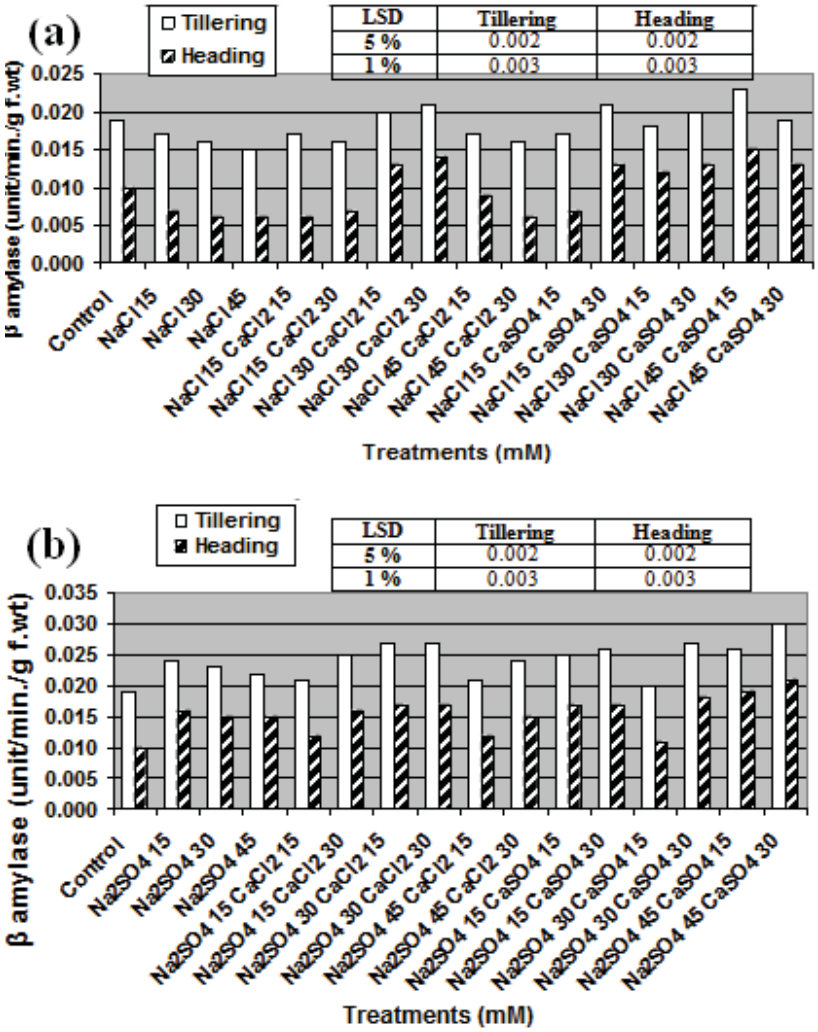

Fig. 8: Beta (β) amylase activity (unit/min./g f.wt*)* in shoot of wheat plants affected by NaCl(a) ,Na_2SO_4 (b) and their combination with the two concentrations of $CaCl_2$ or $CaSO_4$ at tillering and heading stages of growth

Calcium chloride was more better for α-amylase activity than calcium sulphate with the same molarity because α-amylase possesses chloride ion. For instance, treatment of 45mM NaCl +30mM $CaCl_2$ increased α-amylase activity by 63.2%, while that of 45mM NaCl +30mM $CaSO_4$ treatment was 5.3% only compared to their control (45mM NaCl).

β-Amylase (1,4-α-D-glucan Maltohydrolase) Another Form of Amylase:

It is observed that activity of β-amylase was significantly decreased as the concentration of NaCl was increased from zero to 45mM at the tillering and heading stages, while it was significantly increased due to Na_2SO_4 application.

Treatments of 30 mM NaCl+30 mM $CaCl_2$ and 45 mM NaCl + 15mM $CaSO_4$ recorded the highest values for b amylase activity at the tillering stage. They had increasing significant effects over their corresponding controls. Application of Na_2SO_4 + $CaSO_4$ at the highest level for both scored the highest value for β-amylase activity at the tillering and heading stages of growth.

Soluble Protein:

The results show that there was a significant reduction in the concentration of soluble protein in shoots of wheat plants at the tillering stage with increase in the concentration of NaCl in the nutrient solution. The concentrations of protein in plants irrigated with 45mM NaCl were decreased by 44.8 &10.0 % compared to the control at the tillering and heading stages of growth, respectively. This indicates that the depression in protein concentration was more marked during early growth stage. On the contrary, the increase in Na_2SO_4 concentrations had a significant increasing effect on protein concentration of plants at the tillering stage. It is also observed that at the same molarity, soluble protein concentration of plants treated with Na_2SO_4 was greater than that of plants subjected to NaCl. For instance, treatment of 45mM Na_2SO_4, its protein concentration was 2.6 fold that of the same NaCl concentration (Fig. 9 a &b).

Soluble protein contents of leaves were decreased in response to salinity. The protein reduction by NaCl could be through inhibition of its synthesis and increase in its hydrolysis in many crop plants (Debouba *et al*., 2006). The protease activity in salt stressed plants appears to be of adaptive significance because it leads to the accumulation of free amino acids (Ramanjulu *et al*., 1994). Further, denaturation of enzymes involved in protein synthesis (Jaleel *et al*., 2007). Shortage of nitrogen supply due to the exposure to NaCl in which Cl^- inhibits NO_3 uptake (Deane-Drummond and Glauss, 1982). Reduced RNA content which is needed for protein synthesis. On the contrary, Agastian *et al*., 2000 P. Agastian, S.J. Kingsley and M. Vivekanandan, Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes, *Photosynthetica* 38 (2000), pp. 287–290. Full Text via CrossRef | View Record in Scopus | Cited By in Scopus (14)Agastian *et al*. (2000) have reported that soluble protein was increased at low salinity and decreased at high salinity in mulberry. Similar results were observed by Keutgen and Pawelzik (2008) on strawberry .

Sulphate and chloride anions are very different in physiochemical behaviors. Sulphate has larger hydration diameter than Cl^- and is more prone to forming ion pairs than Cl^- in soil solution (Bohn *et al*., 1985). As a result, SO_4^{2-} and Cl^- have different activities in soil solution. For example, SO_4^{2-} salts were less toxic to nitrification than Cl^- salts (Heilman, 1975).

A sever inhibition of protein synthesis was observed by Langdale *et al* .(1973)in spite of adequate NO_3 absorption .This means that nitrate reduction was restricted either due to reduced concentration or inhibition of nitrate reductase (NR) activity. It is known that all eukaryotic molybdenum enzymes share a common Mo atom that is bound to two S atoms of a pyranopterin derivative called molybdopterin. Recently, Fischer *et al*. (2005) found in the high resolution structure of NR-Mo2, one out of two sulfate molecules is well defined in close proximity to the active site. This anion (Mo-S) binding is accompanied by several conformational changes relative to NR-Mo1 with no sulfate bound.

The data showed that addition of 30mM $CaCl_2$ to 45mM NaCl increased concentration of soluble protein by 14.1% compared to its corresponding control. It is also observed that application of $CaCl_2$ at the lowest rate was more effective in ameliorating the depressive effect of 30mM NaCl on soluble protein than the highest rate, it was 2.2 times. The opposite was observed for $CaSO_4$ (Fig.9).

As regards the effect of anions of Ca salts (Cl^- & SO_4^{--}), it is observed that using $CaSO_4$ was more effective in ameliorating the depressive effect of salinity on soluble protein than $CaCl_2$. At the tillering stage, protein concentration for the treatment of 30mM NaCl + 30mM $CaSO_4$ was 3.2 times that of the same $CaCl_2$ molarity. At concentration of 30mM for both Na salts and also for both Ca salts, protein concentration was arranged in descending order as follows:

$NaCl+CaSO_4 > Na_2SO_4+CaCl_2 > Na_2SO_4+CaSO_4 > NaCl+CaCl_2$. So, it is observed that alternative anions had a better effect on the concentration of soluble protein. It is also noticed that SO_4^{--} anion either for Na or Ca is better than Cl^- anion . The role of Ca^{2+} as a second messenger in many biological systems, indicates that plants are able to adjust to high salt environments by activating a signal transduction system involving Ca^{2+} (Hasegawa *et al*., 2000). Stress-induced proteins to play a role in stress tolerance.

Salts can also affect the electrostatic interactions among the macromolecules, contributing through the ionic force (Fennema, 1993). The effect of salts on protein solubility in aqueous solutions is a function of the ionic species present . In this connection, Kinsella (1982) showed that a salt concentration of 0.15 mol/l is sufficient to change the structure of the water and conformation of the proteins. However, this will depend on the content and type of salt present in the medium. Different salts and in particular SO_4^{2-} has been shown to promote amyloid formation for a number of proteins (Klement *et al*., 2007).

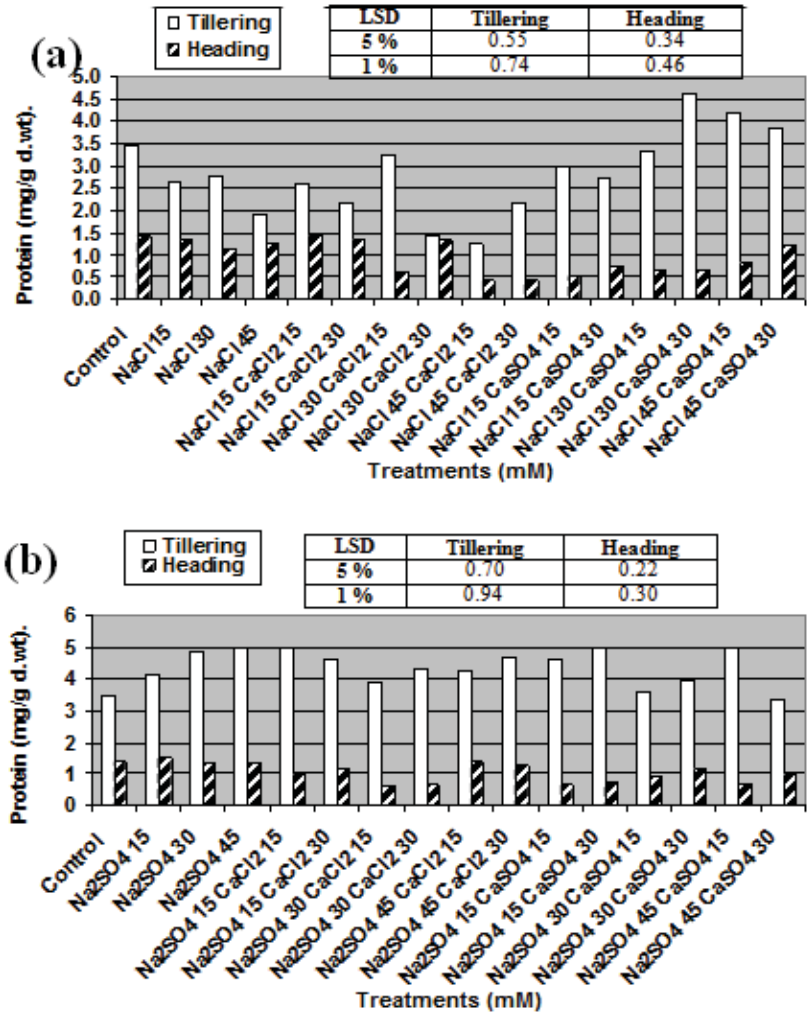

Fig. 9: Concentration of soluble protein (mg/g d.wt) in shoot of wheat plants affected by NaCl(a),Na_2SO_4 (b) and their combination with the two concentrations of $CaCl_2$ or $CaSO_4$ at tillering and heading stages of growth.

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