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ORIGINAL ARTICLE

Impact of Calcium Supplementation on Photosynthetic Pigments, Compatible Osmolytes Contents and Membrane Stability Index in Triticale (x *Triticosecale* Wittmack) Exposed to Salinity Stress

Malihe Jahani[*1,2], Mohammad Reza Hadi[2], Mojtaba Jafarinia[2], Sedighe Jahani[1]

[1]*Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*

[2]*Department of Biology, Marvdasht Branch, Islamic Azad University, Marvdasht, Iran*

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KEYWORDS

Ameliorative impact; Carbohydrate; Sodium-calcium interactions; Triticale

ABSTRACT: In many areas, salinization is considered as one of the most serious dangers to environmental resources and human health. Calcium has a crucial role in plant resistance to salinity stress. In order to investigate the impact of calcium supplementation on photosynthetic pigments, compatible osmolytes contents and membrane stability index (MSI) in triticale (x *Triticosecale* Wittmack) exposed to salinity stress, an experiment as a completely randomized design with 3 replications in greenhouse condition (25 ± 2˚C, 35% relative humidity, 16-hour photoperiod) was conducted. The seeds were germinated in soil. One week old triticale seedlings (with two leaves) were imposed by 0, 50, 100 and 150 mmol L^{-1} NaCl and 0, 6 and 10 mmol L^{-1} $CaCl_2$ for 5 weeks and assayed for some morpho-physiological parameters including fresh weight (FW) and dry weight (DW) of shoot, photosynthetic pigments (chlorophyll (Chl) a and Chl b, total Chl and carotenoids (Car)) contents, proline and glycine betaine (GB) contents, soluble sugars and starch contents and MSI in leaves. Results showed that with incrementing salinity meaningfully decremented FW and DW of shoot, photosynthetic pigments, starch content and MSI while proline, GB and soluble sugars contents incremented in leaves. Calcium treatment meaningfully incremented FW and DW of shoot, photosynthetic pigments, starch content and MSI but caused a meaningful decline in proline, GB and soluble sugars contents in leaves. It can be concluded that calcium had exerted an ameliorative impact on triticale under salinity stress. Maximum ameliorative impact of calcium was observed in plants exposed to 6 mmol L^{-1} $CaCl_2$.

INTRODUCTION

At the current, most agricultural areas are seriously affected by salinity, which has emerged as a serious global issue owing to the potential detrimental impacts on human and animal health [1]. Calcium is considered as an essential element in most plants processes [2]. Moreover, calcium is as a crucial mineral for human health and it aids in the formation of teeth and bones. Also, calcium assists muscle movement and nerve communication [3]. Calcium (Ca^{2+}) has been demonstrated to be a major indicator of plant salt resistance, conferring preserving benefits on plants growing in sodic soils [4]. Ca^{2+} is required for the functional and structural integrity of cell membranes of plant, as well as the stabilization of cell wall structures, the regulation of ion transport and selectivity, and the control of ion-exchange behavior and cell wall enzyme activities [5]. Depending on the genotype of the plant, the nature of these reactions will

*Corresponding author: malihe.jahani2009@gmail.com (M. Jahani)
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differ [4, 5]. Ca^{2+} regulates the function, structure, and signaling of membranes by forming bonds with the phospholipid bilayer, therefore maintaining and enhancing the structural integrity of membrane organelles in plants under stress [6]. Ca^{2+} serves as a second messenger in signaling of stress, which is one of its most important roles [4,7].

Plant growth is slowed by salinity stress, which is one of the most major osmotic stresses [8]. The adverse impact of salinity stress in plants is its osmotic impacts, ion toxicity impact and interference in nutrient absorption [9, 1]. In a study on rice, it was reported that salt stress diminished the fresh weight (FW) and dry weight (DW) of shoot, while the addition of calcium treatment diminished the adverse impacts of salinity stress and incremented FW and DW of shoot [10].

Salinity stress promotes the buildup of reactive oxygen species (ROS), which is a critical factor in plant growth retarding [8, 11]. Plants create a multitude of compatible solutes and antioxidant components that are triggered to give secondary protection against oxidative stress in order to minimize cellular damage caused by ROS buildup [12].

The buildup of adaptive solutions is one of the strategies against salinity stress in plants [11]. These solutions include essential metal ions such as K^+ and they are basically organic solutions. The most major organic osmotic solutions which accumulate in plants due to salinity include soluble sugars, proline, glycine betaine (GB), and etc. [13, 14].

Salinity stress raises proline synthesis in the plant [15]. Proline is an essential amino acid and important osmolyte that is soluble in water and it is a response to the initial defense to balance osmotic pressure in cells under salinity stress [9]. It was reported that salinity stress raised proline levels in cotton, while addition of calcium treatment to the saline environment diminished proline levels [16].

Photosynthesis is one of the important physiological processes in plants, which is affected by genetic and environmental factors [11]. It was reported that the addition of calcium treatment to sweet sorghum exposed to salt stress diminished the adverse impacts of stress and raised the chlorophyll (Chl) level [17].

Triticale (×*Triticosecale* Wittmack) is a human-made cereal that combines the functionality and high production of wheat (*Triticum* spp.) with the durability of rye (*Secale cereale* L.) [18]. Triticale has been planted all over the world, mostly for grain and fodder production, but also for bioenergy generation in recent years [19]. Furthermore, in the human food market, it likewise has a developing potential [20]. Triticale contains a lot of non-starch polysaccharides (such as arabinoxylans), phenolic acid, and anthocyanin, which can cause to prevent heart disease, cancer, diabetes, and neurodegenerative disorders [21]. Also, triticale has antioxidant and antihypertensive properties [22]. It will become a key cereal crop for world populations in the near future [19]. Due to the value of triticale as a cereal crop plant with great developmental potential in the food market and the expansion of saline land areas, identifying a component for salinity stress tolerance in triticale is critical.

Since salinity is one of the most major problems in different regions of the world and calcium plays a crucial role in resistance of plants to salinity, it is necessary to study the sodium-calcium interaction in plants. To the best of the authors' knowledge, there is no study on the interaction of sodium-calcium with triticale. This study provides baseline information on the influence of calcium chloride on triticale exposed to salinity stress. The present research was carried out to investigate the impact of calcium supplementation on photosynthetic pigments, compatible osmolytes contents and membrane stability index (MSI) in triticale (x *Triticosecale* Wittmack) exposed to salinity stress. This will provide a simple, cheap and economic solution to deal with salinity and further enhance sustainable agriculture.

MATERIALS AND METHODS

Growth condition and treatments

To investigate the interactive impact of sodium chloride and calcium chloride on physio-biochemical parameters in triticale leaves, a research as a completely randomized design with three replications was conducted in greenhouse condition (temperature 25±2°C, relative humidity 35%, with a 16-hour photoperiod). Seeds of triticale cultivar Moreno

were planted in soil. Physico-chemical properties of soil are shown in Table 1. One week after seed cultivation in the soil, seedlings were subjected to various doses of sodium chloride and calcium chloride. Treatments included 0, 50, 100 and 150 mmol L^{-1} NaCl levels and 0, 6 and 10mmol L^{-1} $CaCl_2$ levels. Applying the treatments (as addition to the soil) was done for 5 weeks. After the treatment period, 42-day-old plants (Figure 1-4) harvested and then evaluated some morpho-physiological parameters including FW and DW of shoot, photosynthetic pigments, proline and GB, soluble sugars and starch contents and MSI in the leaves.

Table 1. Physico-chemical properties of soil.

EC (dS m^{-1})	pH	Organic matter (%)	Saturation percentage (%)	Sand (%)	Silt (%)	Clay (%)	Texture
0.838	7.31	1.231	54.713	63.5	26.5	10	Sandy Loam

Soluble Cations (mEq 100 g^{-1} soil)				Soluble Anions (mEq 100 g^{-1} soil)		
Na^+	K^+	Ca^{+2}	Mg^{+2}	SO_4^{-2}	Cl^-	HCO_3^-
0.059	0.037	0.353	0.1105	0.074	0.0191	0.402

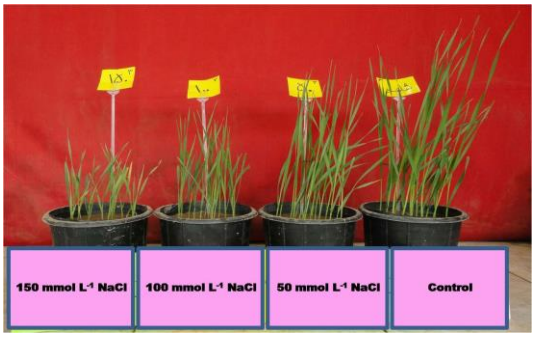

Figure 1. The impact of various doses of sodium chloride on vegetative growth of triticale plants (photograph of 42-day-old plants).

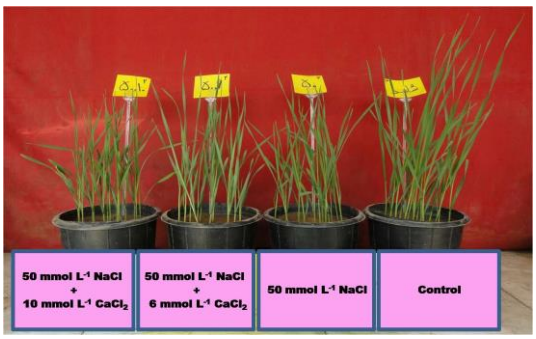

Figure 2. The impact of various doses of calcium chloride on vegetative growth of triticale plants exposed 50 mmol L^{-1} sodium chloride treatment (photograph of 42-day-old plants).

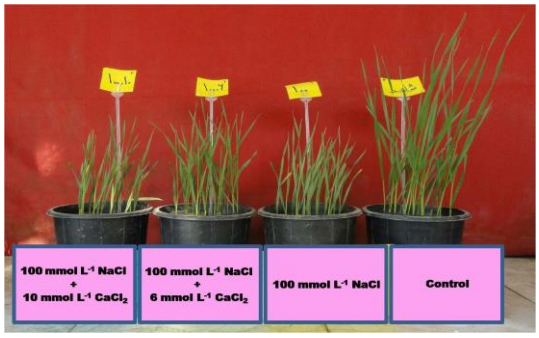

Figure 3. The impact of various doses of calcium chloride on vegetative growth of triticale plants exposed 100 mmol L^{-1} sodium chloride treatment (photograph of 42-day-old plants).

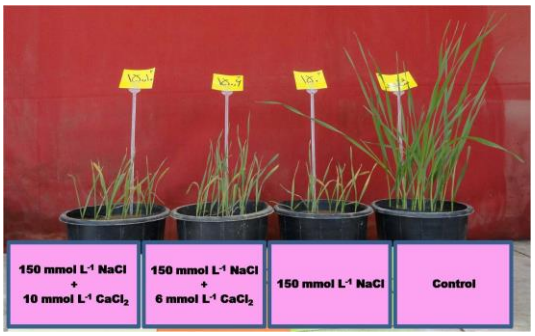

Figure 4. The impact of various doses of calcium chloride on vegetative growth of triticale plants exposed 150 mmol L^{-1} sodium chloride treatment (photograph of 42-day-old plants).

Growth analysis

To determine shoot FW, FW of each sample was measured with a Sartorius digital scale model TE214S with an accuracy level of 0.0001 g. Then for measuring shoot DW, each sample was separately inserted into an aluminum foil and dried in an oven at 70°C for 48 h and then weighed with the same digital scale.

Physio-biochemical analysis

The contents of Chls (Chl-a, Chl-b, and total Chl) and Car were assessed by a spectrophotometric method at wavelengths of 646.8, 663.2, and 470 nm [23].

The amount of proline was assayed by a spectrophotometric method [24]. Utilized technique focuses on the creation of a color combination under acidic circumstances at 100°C via a reaction between the proline and ninhydrin reagent. At 520 nm, the proline content was measured and finally computed utilizing a standard curve. Moreover, amount of GB was analyzed at 365 nm utilizing a spectrophotometer [25].

The phenol sulfuric acid technique [26] was utilized to assess the amount of soluble sugars and insoluble sugars (starch). A spectrophotometer was utilized to detect the absorbance at 485 nm, and the soluble sugars and starch content were computed utilizing a standard glucose curve.

For MSI estimation, leaf discs were washed in diH_2O and put in two groups of vials containing 15 mL of ddH_2O. At 25°C, one group was incubated for 2 hours. After that, the solution's electrical conductivity (EC_1) was estimated. The conductivity (EC_2) of the second group was estimated after it was heated in a water bath for 20 minutes at 95°C. Afterwards, MSI was defined utilizing the following formula [27]:

$$MSI = [1 - (EC_1 / EC_2)] \times 100$$

Statistical analysis

The experiment was conducted in a completely randomised design with three independent repetitions. Statistical analyses were performed utilizing analysis of variance (ANOVA) by SPSS v.22 software and were expressed as the mean values ± SD. The significance of differences between treatments was evaluated utilizing Tukey's test at 5% probability level.

RESULTS

The results showed that, as salinity incremented, shoot FW and DW diminished meaningfully, but addition of calcium treatment to the salinity environment incremented FW and DW of shoot meaningfully. The highest FW and DW was related to the control plant, and the lowest FW and DW was related to the plant imposed by 150 mmol L^{-1} sodium chloride, which meaningfully diminished 67% and 68.67% respectively compared to the control plant (Figure 5).

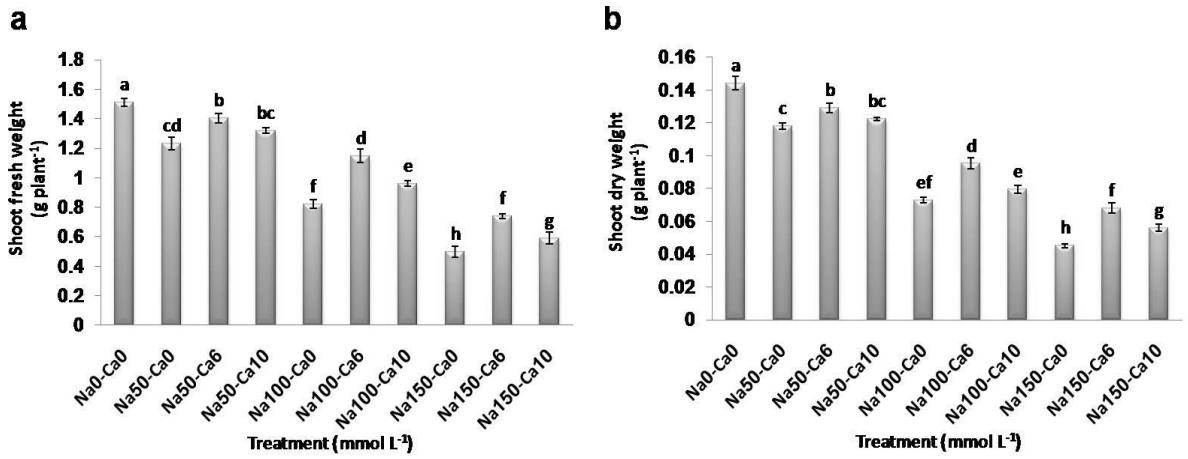

Figure 5. The interaction impacts of NaCl and $CaCl_2$ on (A) fresh weight and (B) dry weight of triticale shoot. Values are mean of three replicates ± SD. Different letters represent a meaningful difference between treatments ($P \leq 0.05$).

The results showed that, as salinity incremented, Chl-a, Chl-b, total Chl and Car content in leaf diminished meaningfully, while addition of calcium treatment to the saline environment incremented Chl-a, Chl-b and total Chl content in leaf meaningfully (Figure 6).

The highest Chl-a, Chl-b and total Chl content was observed in the control and the lowest Chl-a content was observed in the plant imposed by 150 mmol L^{-1} sodium chloride, had decremented 53.33% respectively compared with the control plant (Figure 6). The lowest Chl-b content was observed in the plant imposed by 150 mmol L^{-1} sodium chloride which decremented 79.22% compared to the control plant (Figure 6).

The lowest total Chl content was observed in the plant imposed by 150 mmol L^{-1} sodium chloride which decremented 59.60% compared to the control plant (Figure 6). The highest carotenoid content was observed in the control and the plant imposed by 50 mmol L^{-1} sodium chloride and 6 mmol L^{-1} calcium chloride. The lowest carotenoid content was observed in the plant imposed by 150 mmol L^{-1} sodium chloride and the plant imposed by 150 mmol L^{-1} sodium chloride and 10 mmol L^{-1} calcium chloride, had decremented 68.44% and 63.53% respectively compared with the control plant (Figure 6).

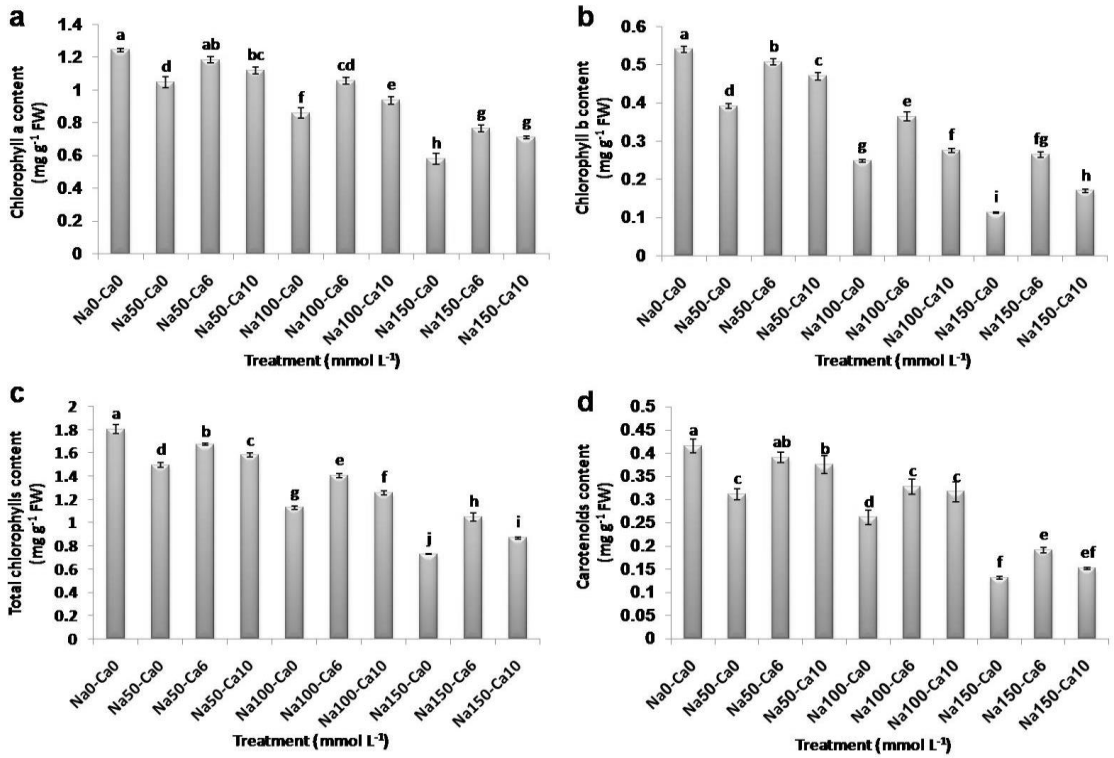

Figure 6. Interaction impacts of NaCl and $CaCl_2$ on (A) chlorophyll (Chl) a, (B) Chl-b, (C) total Chl and (D) carotenoids (Car) contents in triticale leaf. Values are mean of three replicates ± SD. Different letters represent a meaningful difference between treatments (P≤0.05).

Results showed that the highest proline and GB contents were observed in the plant exposed to 150 mmol L^{-1} sodium chloride with an increment of 2.28 and 2.08 times respectively compared with the control plant and the lowest proline content was related to the control plant (Figure 7).

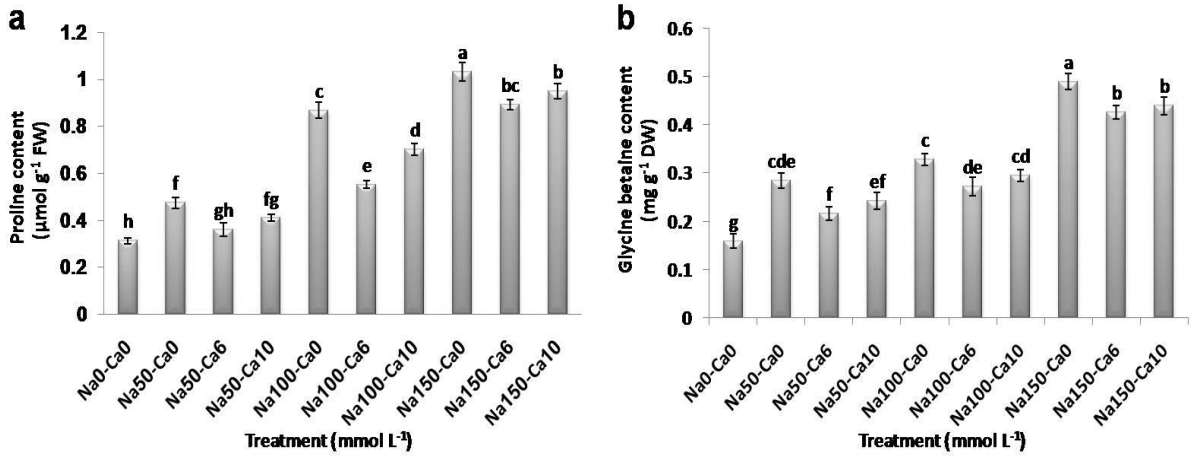

Figure 7. Interaction impacts of NaCl and $CaCl_2$ on (A) the proline and (B) glycine betaine contents in triticale leaf. Values are mean of three replicates ± SD. Different letters represent a meaningful difference between treatments (P≤0.05).

The results showed that, as salinity incremented, soluble sugars contents incremented meaningfully, but addition of calcium treatment to the salinity environment decremented soluble sugars content meaningfully. The highest soluble sugars content was related to the plant exposed to 150 mmol L^{-1} sodium chloride, which meaningfully incremented 1.38 times compared to the control plant, and the lowest

soluble sugars content was related to the control plant (Figure 8).

Also, the results showed that starch content decremented under salinity stress but incremented slightly with calcium supplementation. The highest strach content was observed in the control and the lowest starch content was observed in the plant imposed by 150 mmol L^{-1} sodium chloride with a diminish of 39.26% compared with the control plant (Figure 8).

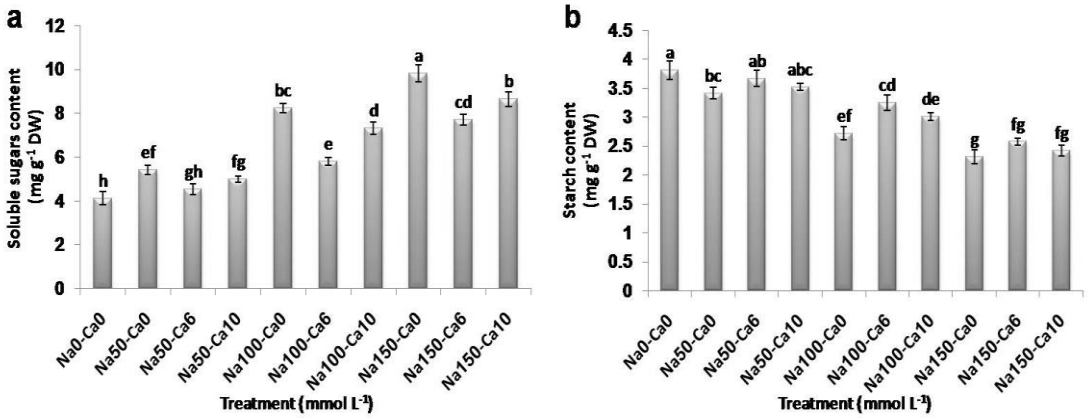

Figure 8. Interaction impacts of NaCl and $CaCl_2$ on (A) the soluble sugars and (B) starch contents in triticale leaf. Values are mean of three replicates ± SD. Different letters represent a meaningful difference between treatments (P≤0.05).

The results showed that leaf MSI decremented under salinity stress but raised with calcium addition. The highest MSI was related to the control plant and the lowest MSI was related to the plant exposed to 150 mmol L^{-1} sodium chloride, which meaningfully decremented 32.24% compared to the control plant (Figure 9).

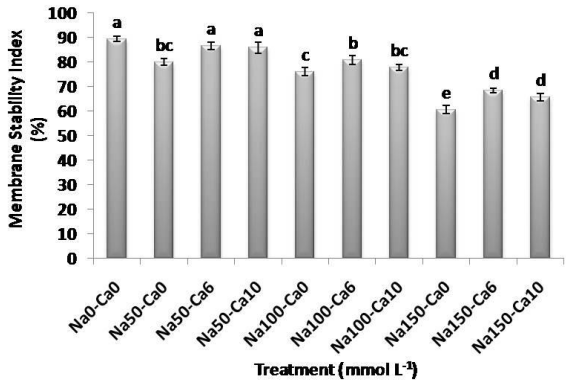

Figure 9. Interaction impacts of NaCl and $CaCl_2$ on the membrane stability index (MSI) in triticale leaf. Values are mean of three replicates ± SD. Different letters represent a meaningful difference between treatments (P≤0.05).

DISCUSSION

In the present study, salinity resulted in a meaningful decline in the shoot biomass while the addition of calcium incremented the shoot biomass meaningfully (Figure 5). The reason for the weight loss caused by salinity could probably be due to the presence of many harmful ions such as Na^+ and Cl^- in the saline environment where these ions are harmful themselves or cause interference in other nutrients absorption, for example the competition of K^+ and Na^+ or Cl^- and NO^{-3} causes interference in nutrients absorption in the plant [28].

In studies conducted on tomato [29, 30, 31], barley [32], sorghum [33], rice [34], cotton [16] and soybeen [35], it was reported that salinity stress decremented shoot FW and DW, while calcium addition to the saline environment incremented shoot FW and DW, which is in agreement with the results of the present study. Also, salinity stress may

cause a secondary osmotic stress or a physiological drought stress in the plant which prevents water lost by the closure of stomata in the leaves. Stress also diminishes leaf area and ultimately diminishes photosynthesis and dry matter [28].

It has been reported that the addition of calcium to saline environments decrements the adverse impacts of salinity on plant growth, by maintaining the structure and integrity of cell membrane and incrementing cell division, diminishing sodium absorption and transport to the upper organs, incrementing potassium absorption and therefore incrementing the potassium to sodium ratio in the plant, enhancing nitrogen metabolism and photosynthetic activity [36, 5].

In the current study, parallel to the inhibitory impacts of salinity on growth, there was a meaningful decrement in the photosynthetic pigments content under salt stress but addition of calcium treatment incremented meaningfully photosynthetic pigments content in treated seedlings (Figure 6). In studies conducted on soybean [35], wheat [37] indian mustard [38], chili pepper [39], tomato [40] and rice [34, 41], it was reported that salinity stress diminished the Chl content while calcium addition to the saline environments incremented the Chl content, which is consistent with the results of the current research.

In salinity condition, the decline in photosynthesis and biomass has been related to stomatal closure [42], and the incremented generation of ROS in chloroplasts [43]. Calcium application under salt stress could cause to simultaneous increment in stomatal conductance and intercellular CO_2 dose and finally cause to increment of photosynthesis and plant biomass [44].

Data presented here showed that salinity treatments led to rising proline content in the treated triticale seedlings while addition of calcium treatment meaningfully diminished the proline content in seedlings (Figure 7a). Proline is an osmotic regulator that responds to salinity stress by incrementing [1, 15]. The incremented proline during stress is probably due to the increment of the proline biosynthesis enzymes activities such as P5CS (proline-5-carboxylate synthase) and P5CR (proline-5-carboxylate reductase) and decrement of the proline degradation enzymes activities such as PROX (proline oxidase) and PDH (proline dehydrogenase) [45, 46].

In study on *Catharanthus roseus* seedlings, salt stress caused to increment in P5CS activity and proline content and decrement in PROX activity while addition of calcium to salinity-exposed plants caused to diminish in proline content by incrementing in PROX activity and decrement in P5CS activity [47]. Calcium application under salt stress could cause to increment in PROX activity and decrement in P5CS activity and finally cause to diminish in proline content [47]. In studies carried out on rice [41, 48], mung bean [49], peanut [50] and broad bean [51], it was reported that salinity stress incremented proline content, while addition of calcium to saline environments diminished the proline content, which is consistent with the results of the current research.

Furthermore, in the present study, salinity resulted in a meaningful increment in GB while the addition of calcium decremented GB meaningfully (Figure 7b). As compatible osmolytes, GB and proline preserve plants against environmental stressors [52, 53]. According to this study, the incremented amounts of proline and GB in response to salt stress might represent a metabolic adaptation to scavenge ROS. Similarly, a rise in GB levels owing to salt stress has been documented in tomato [54].

In the present study, salinity resulted in a meaningful increment in soluble sugars and a meaningful decrement in starch whiles the addition of calcium supplementation meaningfully decremented soluble sugars and slightly incremented starch (Figure 8). Probably the reason for the accumulation of soluble sugars during salinity stress is the insoluble sugars (starch) decompose and form soluble sugars to maintain osmotic potential and reduce the risk of dehydration [55]. In addition, stopping the growth and synthesis of sugars by non-photosynthetic pathway can be another factor in incrementing the concentration of soluble sugars in during of stress [55]. Calcium may decrement salinity stress in the plant due to reduced sodium accumulation, thereby reducing the need for the plant to accumulate soluble sugars as osmotic protectors [36]. Similarly, it was reported that salinity stress raised the amount of soluble sugars in rice [56]. Also, in cowpea, it

was reported that salinity stress incremented the soluble sugars content while adding calcium supplementation diminished the soluble sugars content [57].

Data presented here showed that MSI decremented under salinity stress but incremented with calcium addition (Figure 9). Here, the heightened Na^+ levels owing to salinity stress may compromise cell membrane integrity, which results in ion leakage and disruption of cellular metabolism. Incremented permeability for ions, which may be easily detected by electrolyte leakage, is a great sign of cellular membrane disorder caused by salt stress [50]. Calcium ions may compete for membrane-binding sites with sodium ions. As a result, it's been proposed that high calcium levels can preserve cell membranes against the negative impacts of salt [5, 58]. The plasma membrane permeability to Na^+ can be diminished by high Ca^{2+} levels. The passive influx of Na^+ is decreased when membrane permeability to Na^+ is decreased by Ca^{2+} [58]. Similarly, it was reported a diminish in MSI in tomato subjected to salinity stress and an improvement in MSI by calcium supplementation [40]. Also, in olive plant subjected to salinity stress was documented a decrement in electrolyte leakage by supplementary calcium [59].

CONCLUSIONS

Calcium is a crucial mineral nutrient, and it is effective in diminishing the adverse impacts of salinity. In addition, because of the importance of triticale as a cultivating plant and the increment of extents of salty lands, determination of the optimal concentration of calcium is important for plants resistance in stress conditions. Results of this research revealed that the addition of calcium to saline environments decrements the adverse impacts of salinity stress and the maximum improving impacts of calcium was observed at a concentration of 6 mmol L^{-1} calcium chloride which can be a simple, cheap and economical strategy to deal with salinity stress and incrementing soil productivity and it may be recommended for utilize by farmers as a suitable option against salinity stress.

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Conflict of interests

The authors declare that there are no conflicts of interest related to this article.

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