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Physiological and biochemical evaluation of wheat genotypes (*Triticum aestivum*) under salinity stress

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Abstract

Cereals are the most important nutritional plants for populations worldwide. Wheat plants are essential in global food security in all countries. Herein, we investigate biochemical responses to salinity stress of selected wheat genotypes to identify the most contrasting salt-responsive genotypes and the mechanisms associated with their differential responses. Ten different Egyptian wheat genotypes were cultivated using soil system and treated for 70 days with diluted seawater (6000 ppm). Salt treatment induced a decrease in plant growth, this reduction showed variation, depending on response of genotypes and plant organs. Two genotypes (G8 and G10) exhibited low level of Na^+ accumulation, high K^+, Mg^{2+} and Ca^{2+}, and showed high chlorophyll concentration leading to high net photosynthesis, at salt stress as compared to the other genotypes. Also, they exhibited low level of lipid peroxidation, high total phenolics, total sugars and high proline concentration. These results enhanced reactive oxygen species (ROS) defense and osmotic adjustment. The obtained results confirmed variation in genotypes response to salinity stress, while both G8 and G10 response showed superiority when compared with the other studied genotypes and indicated that the physiological and biochemical traits could be used as screening criteria for selecting salt tolerant genotype.

Keywords: Wheat genotypes; *Triticum aestivum*; Salinity; Tolerance; Biochemical evaluation

1. Introduction

Wheat (*Triticum aestivum*) is the most important grain crop of all the cereals, ranking first among grain-producing crops worldwide, particularly for human consumption. Egypt, the Arab World's most populous country, is also the world's top importer of wheat [1]. Soil salinization is a worldwide problem that has harmed 833 million hectares (ha) of agricultural land in more than one hundred countries around the world. Globally, there are 833 million hectares of soils are salt-affected [2]. Around 20 % of the world's cultivated lands and 33% of irrigated lands affected by salt [3]. Soil salinization is spreading at a rate of 1–2 million ha/year worldwide, affecting a large amount of crop production and making land unsuitable for cultivation [4]. By 2050, salinization expected to affect more than 50% of all arable areas.

Furthermore, as the world's population continues to grow, global food security is under threat, since the world's food supply must be expanded by up to 70% [5]. Moreover, natural and artificial climate change factors have changed the pattern of precipitation and soil salinity resulting in sea levels elevation, occurrence of flood, increasing of evapotranspiration, increased resistance of pests and parasites, reducing productivity of plants [6, 7].

Among abiotic stresses, salinity stress especially in the arid and semi-arid regions of the world has emerged as one of the most important threats to the sustainability of wheat production [8]. It reduces germination, seedling growth as well as reproductive growth by disrupting numerous vital physiological and metabolic processes, which lead to sharp decline in yield and quality depending on frequency and extent of saline environment [9].

Salt tolerance of wheat known to change with growth stage. Identifying the multiple parameters associated with salt tolerance during different growth stages is important for evaluating wheat genotypes and improving their salt tolerance [10]. The response of plants to salinity can be described in two subsequent phases during the first phase, salinity causes osmotic

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stress because of a decrease in the soil water potential, which occurs within minutes to days, and is thought to be related to Na^+ sensing and signaling; [11].

The second phase develops within a few days or weeks depending on the severity of salinity and accumulation of Na^+ ions in different plant tissues, causing reduced yield and even plant death. Under salinity, Na^+ is the principle of toxic ion imposing both osmotic stress and ionic toxicity [12]. Salinity stress accelerates all phenological phases of wheat [13] and negatively affects wheat phenological developments such as reduces the number of fertile tillers, decreases the number of spikelets and spike number [14].

The saline environment disturbs plant water relations including relative water content, leaf water potential, water uptake, transpiration rate, water retention, and water use efficiency [15]. Salinity tolerant plants employ several physiological and biochemical mechanisms to adapt under salinity stress, there is a lack of robust salinity tolerant wheat cultivars globally. Plants develop various physiological and biochemical mechanisms to tolerate salinity stress.

Some of the most important mechanisms include, but are not limited to: (1) ion homeostasis and compartmentalization, (2) ions transport and uptake, (3) biosynthesis of osmoprotectants and compatible solutes, (4) activation of antioxidant enzyme and synthesis of antioxidant compounds, (5) synthesis of polyamines, (6) generation of nitric oxide (NO), and (7) hormone modulation [16]. All these parameters are used as screening criteria for salinity tolerance in a wide range of plant species. In fact, proline accumulation is used as one of the most important physiological indicators for salt tolerance [17, 18]. Such detrimental effects of salinity stress on the dry weight of shoot may be due to the direct impact on photosynthesis [19].

Salt stress influences cell ion homeostasis by altering ion balance, such as increased Na^+ and a simultaneous decreased Ca^{2+} and K^+ contents [20]. Indeed, previous studies revealed that malondialdehyde, MDA accumulation (a product of lipid peroxidation) should be considered as an indicator of oxidative stress and membrane integrity and could be used to discriminate between salt-tolerant and salt-sensitive genotypes [18, 21].

The present study was focused to understand the response generated at physiological and biochemical levels of ten wheat genotypes grown under the effects of salinity stress. This study will help in understanding the mechanisms involved in salt tolerance. The information generated from this study can be utilized for crop improvement in future.

2. Materials and methods

2.1. Plant material and growth conditions

Plant materials:

Ten genotypes of Egyptian spring wheat (*Triticum aestivum*), namely G1: Giza-G2: Giza - G3: Sohag - G4: Sharqia - G5: Giza - G6: Giza - G7: Monufia - G8: Giza - G9: Qena - G10: Sohag) were obtained from the National Gene Bank, Ministry of Agriculture, Giza, Egypt.

Growth conditions:

A pot experiment was conducted at the greenhouse of the National Gene Bank, Agriculture Research Center Egypt (N30, 06º. E31, 23º). The air temperature ranged from 20 to 25ºC during the day and 17 to 19ºC during the night, and the day light was adjusted for 16 hrs. Relative humidity fluctuated between 60 and 75 % day/night. Pots of 9 cm diameter were filled with 2 kg of peat moss/sand mixture, with an equal amount. Ten grains were sown at a depth of 2-3 cm in each pot, then after seven days, the seedlings were thinned to five seedlings per pot.

According to water holding capacity, each pot was irrigated using a non-saline Hoagland solution composed of 2.08 mM Ca $(NO_3)_2 \cdot 4H_2O$, 1.99 mM $MgSO_4 \cdot 7H_2O$, 2.00 mM $NH_4H_2PO_4$, 10.09 mM KNO_3, 46.26 µM H_3BO_3, 0.45 µM $Na_2MoO_4 \cdot 2H_2O$, 0.32 µM $CuSO_4 \cdot 5H_2O$, 9.19 µM $MnCl_2 \cdot 4H_2O$, 0.76 µM $ZnSO_4 \cdot 7H_2O$, 19.75 µM $FeSO_4 \cdot H_2O$ [22]. Per genotype, and 21 days after sowing, the experiment was divided into two treatments, each included five replicates. The control treatment was irrigated using tap water and the salt-stressed treatment was irrigated using diluted seawater (6000 ppm). Seawater was obtained from Ain Sokhna, red sea of Egypt and its electrical conductivity was 66.7 dsm-1.

Irrigation was performed as follows; two times per week with diluted seawater followed by one time using tap water until the end of experiment. Seventy days after sowing, five random plants from each group were collected to study whole plant dry weight, plant height, and flag leaf area. In addition, some biochemical parameters, proline concentration, lipid peroxidase, MDA and elements namely potassium, magnesium, calcium and sodium, were measured in shoots and roots. Whereas, chlorophyll (chl) concentration, total sugars and total phenolics were measured in shoot only.

2.2. Plant growth determination

After 70 days of salt treatment, plants were harvested, fresh weight (FW) and dry weight (DW) were determined after desiccation at 60°C for 72 h. Dried samples of shoots and roots were finely grounded and digested with sulphuric and nitric acid (1% (v/v) HNO3), according to the method of [23].

2.3. Minerals determination

Minerals determination was conducted on shoots and roots as reported by [24]. The cations (K^+, $Ca2^+$, and Na^+) were assayed by flame emission photometry, using an Eppendorf spectrophotometer (Eppendorf Geratebau Netherlerz). Magnesium ($Mg2^+$) was measured by atomic absorption spectrophotometer (Philips PU9100X).

2.4. Chlorophyll concentrations

Chlorophyll pigments were determined according to [25]. Briefly, 100 mg of fresh leaf discs (0.2 cm2) were immersed in 5 ml of 80% (v/v) acetone and kept in dark over night at 25°C, under room temperature. chl a, chl b and total chl concentrations were measured by spectrophotometry according to Lichtenthaler [25] equations:

$$Chl_a = 12.25A_{663} – 2.79A_{645}$$
$$Chl_b = 21.50A_{645} – 5.10A_{663}$$
$$Chl_{(a+b)} = 7.15A_{663} + 18.71A_{645}$$

2.5. Biochemical parameters

2.5.1. Total sugars

Total soluble sugars were determined using method given by [26]. Extraction of 100 mg plant material using 80% ethyl alcohol, then 1ml of extract was taken in test tube and added 1m of 5% phenol and immediately followed by the addition of 5 ml of concentrated sulfuric acid rapidly then the mixture was shaken gently and left to cool. OD of greenish brown color developed was taken at 490 nm in spectrophotometer. Distilled water was used instead of extract in blank. The quantity of sugars was calculated against the standard curve prepared by using pure glucose(10-100µg/ml) and expressed as mg g-1 dry weight.

2.5.2. Proline concentration

Proline concentration was determined using the method of [27]. Briefly, proline was extracted from dry shoots and roots (200 mg for each sample) with 10 ml of 3% Sulfosalicylic acid, at 70°C for 30 min. An aliquot of 1 ml of the extract was mixed with 1 ml of glacial acetic acid and 1 ml of acid ninhydrin. The mixture was heated at 90°C for 1 h in water bath and the reaction was stopped using ice. The mixture was extracted with toluene, and the absorbance of toluene fraction (aspired from the liquid phase) was measured at 520 nm using UV/VIS spectrophotometer (PerkinElmer, Norwalk, USA). Proline concentration was determined using calibration curve as µmol proline g−1 DW.

2.5.3. Lipid peroxidation

The level of lipid peroxidation was measured as 2-thiobarbituric acid-reactive substances (mainly malondialdehyde, MDA) according to [28]. Frozen samples (500 mg FW) were homogenized with a pre-chilled mortar and pestle with 2 ml of ice-cold trichloroacetic acid TCA (0.1%, w/v) and centrifuged at 15.000 × g for 15 min and at 4°C. Assay mixture containing 2 ml aliquot of supernatant and 2 ml of 0.67% (w/v) thiobarbituric acid (TBA), was heated at 95°C for 20 min and then rapidly cooled in ice bath. The samples were centrifuged (10.000 × g for 10 min at 4°C) and the supernatant absorbance was measured at 532 and 600 nm. The concentration of MDA was calculated from the difference between the two values using extinction coefficient (155 mM-1 cm-1).

2.5.4. Total phenolics

Total phenols were determined using the Folin-Ciocalteu method, modified as described by [29]. Amounts of 1.85 ml of distilled water, 0.125 ml of Folin-Ciocalteu reagent and 250 µl of a 14 % sodium carbonate solution were added to 250 µl of liquid extract sample in a test tube, making a final volume of 2.5 ml. The solution was homogenized and left to stand for 30 min, and the absorbance was determined at 750 nm. The total phenols were calculated as milligrams of gallic acid equivalents.

2.5. Statistical analysis

The statistical analysis was performed using 'Sigma Plot' software (version 12.0, www.systatsoftware.com). All means values and standard error (SE), of physiological and biochemical parameters were obtained from 5 replicates. Only differences with a P value < 0.05 were considered statistically significant using two-ways ANOVA with Duncan's multiple range tests at 95% confidence interval.

3. Results

3.1. Plant growth

Our results showed some differences between genotypes in biomass production under control conditions (Figure 1). Genotypes (G8, G9 and G10) showed better plant growth as revealed by the highest value of DW under control condition, as compared to the other Genotypes (Figure 1A). Under salt treatment conditions of diluted seawater (6000 ppm), plant decreased with salt treatment, depending on plant organs and genotypes. Whole plant DW, significantly decreased with increasing salt concentration (6000 ppm), as compared to control, in Genotypes G1, G2, G3, G4, and G9. At salt stress treatment, G5, G6, G7 and G9 showed the higher values of plant DW as compared with G1, G2, G3 and G4 but significantly lower than G8 and G10 (Figure 1A). Under salt treatment, G1, G2, G3, G4, and G9 exhibited the biggest loss in DW about 15, 16, 14 and 16%, as compared to control, while this decrease was not significant in G5, G6, G7, G8, and G10 as compared to control, same effect was observed in fresh weight (Figure 1D). The magnitude of salt effect was also linked to the plant height considered (Figure 1B). For example, G8 was the highest genotype increase of plant height under salt treatment as compared with the other genotypes, which was approximately 40 % as compared with control. Three more genotypes, G3, G4 and G5 showed increase with approximately 21, 28 and 23 %, respectively (Figure 1B). No significant variation was detected in plant height between control and salt treatment at all other genotypes. Salt treatment, induced a stimulation of flag leaf area: the

increase was about 38, 40 and 48%, respectively, for G5, G8 and G10 as compared to control. On the other hand, salinity caused a decrease of flag leaf area for G2, G3 and G9 which was approximately 40, 38 and 48% as compared to control (Figure 1C).

3.2. Mineral analysis

The addition of diluted seawater to the culture medium induced an accumulation of large amount of toxic ion Na^+ in all genotypes, salinity induced significant increases on shoot and root Na^+ concentrations. This increase was more pronounced in G1 in shoot and root Na^+ concentrations, no significant difference in shoot and root Na^+ concentration was detected between other genotypes, (Figure 2A; Figure 3A), with respect G8 and G10 that showed the lowest Na^+ concentration in shoot and root, suggesting that these two genotypes were able to control Na^+ accumulation in shoot and root.

In addition, this mechanism to control sodium toxicity was more obvious with genotypes G8 and 10 as compared with the other genotypes (Figure 2A; Figure 3A). Under salt treatment, G1, G2 and G9 genotypes showed a decrease in the shoot K^+ concentration, about 32, 18 and 28 %, respectively as compared with control. The root K^+ concentration decreased in G1, G2, G6, G7 and G9 about 49, 19,18, 19 and 20% respectively (Figure 2B; Figure 3B). On the other hand, salt stress had no additional decrease in shoot K^+ concentration in G8 and G10 genotypes which showed the highest value compared to other genotypes with an increase of K^+ concentration in shoot and root, about 34 and 23% in shoot and 27% in roots for both genotypes, (Figure 2B; Figure 3B).

In the same way of K^+ accumulation, G8 and G10 showed the highest value of shoot Ca^{2+} concentration under salt treatment (Figure 2C) as compared with the other genotypes. An increase of Ca^{2+} concentration was detected in both genotypes in shoot about 52 and 62% respectively. The same effect detected also in G5 that showed an increase in shoot Ca^{2+} concentration about 30% as compared with control (Figure 2C). It should be noted that salt treatment induced a significant decrease of Ca^{2+} concentration in shoot in G1 genotype that showed the lowest value as compared with the other genotypes with a decrease of 31% as compared with control (Figure 2C). Linked to the shoot the application of salinity affected negatively the root Ca^{2+} concentration with a decrease in all genotypes with respect G2, G8 and G10 that showed no significant difference as compared with control (Figure 3C).

Salt treatment gradually led to a significant decrease of shoot Mg^{2+} concentration in G1, G2, G3, G4 and G6 (Figure 2D). This decrease was about 43, 47, 69, 66, and 48%, respectively as compared with control. However, G5, G7, and G9 genotypes showed no significant change (Figure 2D). Nevertheless, there was a clear correlation between the previous salinity level and the increase of shoot Mg^{2+} concentration in G8 and G10 genotypes. A significant increase in shoot Mg^{2+} concentration was observed in both genotypes under salt treatment. This increase was about 56 and 61%, respectively (Figure 2D). G8 and G10 were qualified as the most tolerant genotypes showing the highest concentration of root Mg^{2+} concentration with an increase of about 73 and 56% as compared with other genotypes that showed a decrease of root Mg^{2+} about 40, 67, 54, 55% for respectively G1, G3, G5 and G6 (Figure 3D).

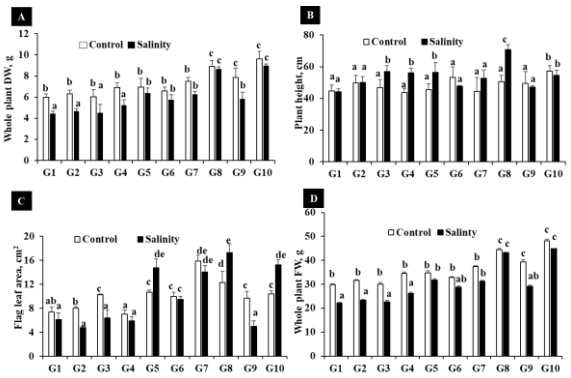

Figure 1. Varietal difference in plant growth parameters; Dry weight of whole plant (A), Plant height (B), Flag leaf area (C) and Fresh weight (D) of 10 wheat genotypes cultivated under control and 6000 ppm seawater concentration during 70 days of treatment. Data are means of 5 replicates ± SE. Means with similar letters are not different at $P < 0.05$ according to Duncan's multiple range test at 95% confidence interval.

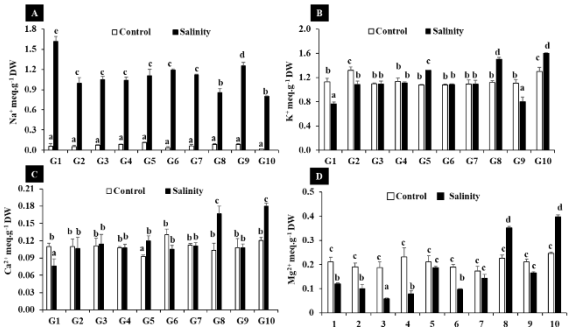

Figure 2. Effect of 6000 ppm seawater concentration on shoot Na^+ **(A)**, K^+ **(B)**, Ca^{2+} **(C)** and Mg^{2+} **(D)** concentration of ten wheat genotypes cultivated under control and salt treatment during 70 days of treatment. Data are means of 5 replicates ± standard errors. Means with similar letters are not different at $P < 0.05$ according to Duncan's multiple range test at 95% confidence interval.

3.3. Chlorophyll concentration

Under control condition (tap water), all genotypes showed no significant difference in Chla, Chlb, Chlb/Chla ratio and total Chl (Figures 4 and Table 1). Under salt treatment, Chla, Chlb and total Chl concentrations decreased significantly in all genotypes as compared to control. Regarding G8 and G10, both exhibited the highest level of Chla, Chlb and total Chl as compared with the other genotypes. Interestingly, both genotypes showed an increase of Chla, Chlb and

total Chl under 6000 ppm seawater concentration as compared with control (Figures 4A, B and C), no significant changes in Chlb/Chla ratio with respect G5 that shows a significant decrease as compared with others genotypes under salinity.

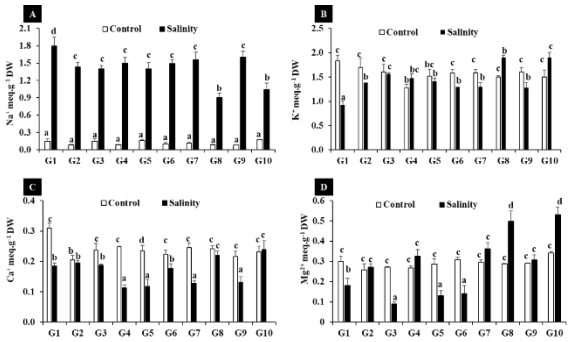

Figure 3. Effect of 6000 ppm seawater concentration on root Na^+ **(A)**, K^+ **(B)**, Ca^{2+} **(C)** and Mg^{2+} **(D)** concentration of ten wheat genotypes cultivated under control and salt treatment during 70 days of treatment. Data are means of 5 replicates ± standard errors. Means with similar letters are not different at P < 0.05 according to Duncan's multiple range test at 95% confidence interval.

3.4. Lipid peroxidation

Salt treatment, gradually increased the accumulation of MDA with respect to genotypes (Figure 5). Under salt treatment (6000-ppm seawater), G8 and G10 were qualified as the most resistant varieties showing the lowest accumulation of MDA as compared to the other genotypes. Moreover, under salt treatment, G1 and G4 exhibited the highest increase of MDA content as compared to other genotypes (G1, G2 and G3) which showed an increase about 92 and 93% respectively, as compared with control. However, an increase about 73, 26, 33 and 52% respectively for G2, G3, G6, G7 and G9 (Figure 5).

3.5. Proline concentration and total sugars

Proline concentration, significantly, increased in shoot as compared with control with increasing salt concentration (6000 ppm). This increase was about 40, 33, 78 and 72% respectively for G4, G5, G8 and G10, with G8 and G10 showing the highest proline concentration. (Figure 6A). In the same way, the root showed the highest amount of proline under (6000-ppm seawater) in G8 and G10 as compared with the other genotypes with a significant increase about 101 and 144%, respectively as compared with control (Figure 6B).

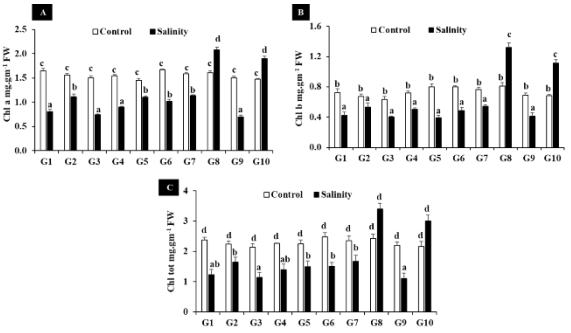

Figure 4. Variation in Chla **(A),** Chlb **(B)** and total Chl **(C)** concentration in ten wheat genotypes cultivated under control and 6000 ppm seawater concentration during 70 days of treatment. Data are means of 5 replicates ± standard errors. Means with similar letters are not different at P < 0.05 according to Duncan's multiple range test at 95% confidence interval.

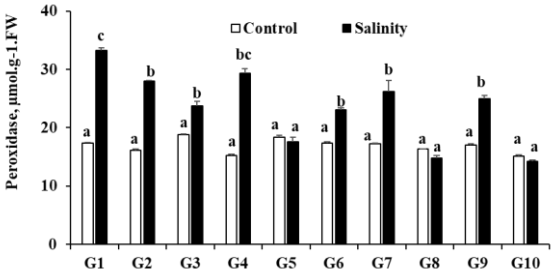

Figure 5. Varietal difference in shoot MDA of ten wheat genotypes cultivated under control and 6000 ppm seawater concentration during 70 days of treatment. Data are means of 5 replicates ± standard error. Means with similar letters are not different at P < 0.05 according to Duncan's multiple range test at 95% confidence interval.

An increase was also detected in G5 and G7, about 52 and 47%, respectively as compared with control (Figure 6B). In the same way, our results showed that the highest values of total sugars were presented under (6000-ppm seawater) in the shoots of G3, G4, G5, G8 and G10 and in the roots of G5, G8 and G10, as compared with control (Figures 5C, D). It should be noted that no remarkable changes were observed in the other genotypes.

Table 1. Variation in Chl b/a ratio in ten wheat genotypes cultivated under control and 6000 ppm seawater concentration during 70 days of treatment. Data are means of 5 replicates ± standard errors. Means with similar letters are not different at P < 0.05 according to Duncan's multiple range test at 95% confidence interval.

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
Control	0.44 ±0.11 a	0.43 ±0.02 a	0.42 ±0.04 a	0.46 ±0.02 a	0.55 ±0.005 a	0.47 ±0.01 a	0.48 ±0.09 a	0.503 ±0.04 a	0.455 ±0.02 a	0.46 ±0.01 a
Salinity (6000-ppm)	0.51 ±0.03 a	0.47 ±0.05 a	0.54 ±0.04 a	0.55 ±0.01 a	0.35 ±0.004 b	0.47 ±0.02 a	0.47 ±0.04 a	0.634 ±0.01 a	0.591 ±0.01 a	0.583 ±0.07 a

3.6. Total phenolics

Total phenol concentration in shoot was significantly increased at (6000-ppm seawater), depending on genotypes (Figure 7C). Under salinity, total phenolics was significantly increased and was more obvious, especially, in G2, G4, G5, G7, G8, G9 and G10 (Figure 7C). It should be noted that, total phenolics was much higher in G10 under salt treatment than other genotypes (Figure 7C).

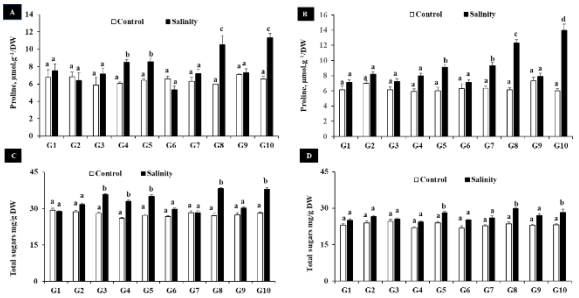

Figure 6. Changes in proline concentration (µmol/g. DW) in shoot **(A)** root **(B)** and total sugars (µmol/g. DW) in shoot **(C)** and in root **(D)** of ten wheat genotypes cultivated under control and 6000 ppm seawater concentration during 70 days of treatment. Data are means of 5 replicates ± standard error. Means with similar letters are not different at $P < 0.05$ according to Duncan's multiple range test at 95% confidence interval.

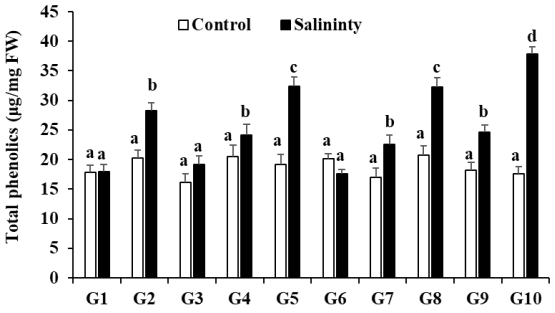

Figure 7. Changes in shoot total phenolics (µg/mg. FW) of ten wheat genotypes cultivated under control and 6000 ppm seawater concentration during 70 days of treatment. Data are means of 5 replicates ± standard error. Means with similar letters are not different at $P < 0.05$ according to Duncan's multiple range test at 95% confidence interval.

4. Discussion

The aim of this experiment was to uncover prospective of the Egyptian wheat genotypes tolerant to salinity. Ten wheat genotypes were tested for salt tolerance based on morphological, physiological and biochemical parameters. Phenotypic screening at seedling stage of plants genotypes for stress tolerance increase the ability to select resistance genotypes, which demonstrated in maize [30], sorghum [31], wheat [32], cotton [33] and rice [34]. In fact, plant growth and biomass yield parameters are generally used to assess plant salt tolerance, as previously described in several investigations [35, 36]. Our study showed that salt stress imposition for 70 days caused damage to the plants, particularly to the sensitive genotypes. However, G1, G2, G3, G4 and G9 showed a significant decrease in whole plant dry weight under salt treatment (Figure 1A).

Here, we qualified G8 and G10 as the most resistant varieties due to their ability to keep the same biomass production, even at salt stress (Figure 1A). These genotypes also showed a developed length and flag leaf area under salinity stress (Figure 1). However, the effects of salt stress on plant growth parameters have been suggested to be an important criterion for evaluating salt tolerance in crop plants [37]. Even so, stress burden for 70 days followed by observations on dry matter yield, plant length and flag leaf area, (Figure 1) provided an indication about salt tolerance ability. Changes in leaf flag area and dry matter yield due to the stress validated the above observations on salt tolerance ability of the wheat genotypes. Moreover, the tested genotypes in the present study showed a different response under salt concentration. Therefore, limited success in growing wheat on salt-affected soils has been achieved because only a few salt-tolerant wheat genotypes have been identified [38-40].

Screening for genotypes tolerance to salinity requires other appropriate physiological traits such as: Na^+ accumulation and its relationship with the distribution of the others essential ions (K^+, Mg^{2+} and Ca^{2+}) [41]. In our study, Na^+ accumulation was recorded under salt treatment with respect to genotype and organs considered (Figures 2 and 3). Due to roots are in the direct contact with soil and absorb nutrients, a higher accumulation of Na^+ was observed in root under salt stress compared to root under control condition, same increase was observed also in shoot which consistence with results by [18]. However, under the stress genotypes G8 and G10 showed a high regulation of Na^+ uptake and accumulation to maintain its optimum level as compared with the others genotypes (Figure 2 and 3) which indicated that the regulation of Na^+ uptake and accumulation is one of the most important salt resistance mechanisms [42].

Generally, the reason for Na^+ being toxic that Na^+ inhibits enzyme activity, and this is particularly the case for the many enzymes that require K^+ to be active [43]. For example, the K^+ dependent pyruvate kinase has a Km (for K^+ binding) of around 5 mM concentration [44]. Nevertheless, excessive accumulation of Na^+ leads to a nutritional imbalance, usually associated with the restriction of nutrients (K^+, Mg^{2+} and Ca^{2+}) uptake. Such mechanism was previously demonstrated for several halophyte species: *Limonium delicatulum* [45], *Cakile. maritima* [46], *Atriplex lentiformis* [47] and *Crithmum maritimum* [48].

We found also in our work the same Na^+ effect caused a significant decrease in leaf K^+, Mg^{2+} and Ca^{2+} under salt treatment, depending on genotype (Figures 2 and 3). Consequently, we suggested that these varieties remain selective for K^+ as a sign of osmotic adjustment [48, 49]. The adverse effect of salt stress

on K^+ uptake could be seen in the wheat genotypes with the maximum reduction in absorption/transport was observed in case of G1 and G9 in shoot and significant decrease in all genotypes in roots with respect to G8 and G10 (Figures 2B, 3B). Our findings suggest that salt-tolerant genotypes G8 and G10 retain selectivity for K^+ over Na^+ under the stress, while the salt-sensitive genotypes failed (Figures 2B and 3B).

Previous study conducted on quinoa demonstrates that in both young and old leaves the full osmotic adjustment can be achieved only through the inorganic osmolyte accumulation (Na^+ and K^+) [50]. Our data also demonstrated that these two most resistant genotypes (G8 and G10) showed a high Ca^{2+} and Mg^{2+} efficiency under salt stress that showed an increase higher than control (Figures 2C and D and Figures 3C and D). Besides, our findings demonstrated that a high capacity of G8 and G10 maintain mineral ion homeostasis with low damage that could be a suitable marker for wheat salt tolerance. The other genotypes encountered the maximum damage (Figures 2 and 3), which is in agreement with [51] which demonstrated that salt stress is known to decrease Mg^{2+} and Ca^{2+} concentration in plant tissues, which correlated with the lower level of chlorophyll content in the salt-affected plants.

Chla, Chlb and total Chl concentrations together with cell growth are among the primary process affected by ionic and osmotic stress [18, 52]. No significant reduction in Chla, Chlb and Chl total content in G8 and G10 and both showed an increase of these parameters under salinity stress, with maximum reduction in Chla, Chlb and Chl total concentrations of other genotypes emerged as salt-sensitive (Figure 3A), supported better salt tolerance ability of G8 and G10 (Figures 4A, B and C). The observed effect of salt stress on chlorophyll content is in agreement with those reported earlier in different species of wheat [53]. Accumulation of Na^+ was reported to adversely affect chlorophyll biosynthesis and photosynthesis process (particularly photosystem-II) in plants [54]. The salt stimulation of chla content was previously reported in an extreme halophyte like *Arthrocnemum macrostachyum* cultivated under high salinity [55], and also observed in *Sarcocornia fruticosa* and *Atriplex portulacoides* cultivated under low salinity [56, 57].

Plants under salt stress have developed complex mechanisms for adaptation to osmotic stress. These mechanisms include osmotic adjustment by accumulating organic solutes/osmolytes such as proline [58]. The compatible solutes, particularly total sugars and proline, play significant role in osmotic adjustment/structural stability during abiotic stress [59]. Nonetheless, the role of proline and total sugars in screening of genotypes for salt tolerance, remains highly controversial in the literature as investigators have obtained contrasting results [60, 61]. In the present study, salt treatment induced a significant accumulation of proline with respect to genotypes and organ tissue considered (Figures 6A and B). Eventually, proline, likewise other osmolytes, modulate redox potential by conferring osmotic adjustment, protecting cellular membranes, and stabilizing enzymes under abiotic stress [62]. Our finding is in agreement with Maghsoudi *et al.*, [63] suggested that higher expression of the gene responsible for proline biosynthesis (P5CS) in *Ae. cylindrica* may correlate with salt tolerance also is in agreement with Kumar *et al.*, [59] in wheat. In fact, increased accumulation of total sugars and proline (in shoot and root), especially in G5, G8 and G10 considerate as resistance genotypes (Figure 6) was in agreement with the earlier observations of Romero-Aranda *et al.*, [62]. Total sugars have been suggested to enhance salt tolerance by protecting/stabilizing cellular membranes/enzymes which substantiate the observation of [64]. On the other hand, proline contributes to stabilizing subcellular structures (e.g. membranes and proteins), scavenging free radicals, and buffering cellular redox potential under stress conditions [65].

This is the case of the most resistant genotypes G5, G8 and G10 which showed the highest proline accumulation paralleled by the lowest MDA content as a sign of low oxidative stress (Figures 5 and 6x). Our data are in agreement with previous study conducted on that salinity stress induced the oxidative damage by increasing lipid peroxidation level in roots and more specifically in leaf tissues [35]. Another reports revealed that lipid peroxidation, known as the sign of membrane damage, should be regarded as a sign of oxidative stress and could be used to discriminate between salt-tolerant and salt-sensitive genotypes [21].

Phenolic compounds like flavonoids are not only the most abundant secondary metabolites in the plant kingdom but also are the most crucial antioxidants for scavenging the excessive ROS that is generated by the majority of stressors [66]. Our result showed a significant increase in total phenolic in response to salt stress which agreed with Hichem and Mounir [67], who reported the significant effect of salt stress on the total phenolic in two maize (Zea mays L.) cultivars. Increase in total polyphenol content in different tissues under increasing salinity has been reported in number of plants [68, 69].

In the present study, seawater treatment led to enhance total polyphenol especially in G5, G8 and G10 resistance genotypes showed a highly increased polyphenol accumulation under salt stress in shoot, in agreement with [70] who proposed that the main sites of polyphenol accumulation in plants are the mesophyll, epidermis and sub epidermis of photosynthetic tissues. Taken together, these observations lead to the proposal of a fundamental role of polyphenols in the protection of the photosynthetic apparatus under severe stress.

5. Conclusion

In order to develop promising useful strategies for selecting salt tolerant genotypes of wheat, we focused on the relevance of Genotype x Environment interaction effects. Our comprehensive analysis based on biochemical and physiological traits resulted into identification of the most contrasting salt-responsive wheat genotypes. In summary, we qualified G8 and G10 as the most tolerant genotypes according to: (i) High growth performance and biomass production (ii) Low level of sodium toxicity accompanied with high K^+, Ca^{2+} and Mg^{2+} efficiency under salinity. (iii) Limitation of membrane lipid peroxidation, oxidative stress and photorespiration. (vi) The important role of proline, polyphenol and total carbohydrate in ROS defense, protein protection and cytoplasmic osmotic adjustment. For better understanding about the functional and regulatory control of salt tolerance mechanism and to identify, potential candidate genes responsible for wheat salt-tolerance transcriptomic and proteomic approaches will be needed for investigation.

6. Conflicts of interest

The authors declare no conflict of interest.

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