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Original Research

Characterization of Salt-Tolerant Cultivars of Date Palm Based on Morphological and Biochemical Responses Under Salinity Stress

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Abstract

Abiotic stress, especially salinization, is considered a major soil problem in arid and semiarid regions. To combat salinization, halophytes such as date palms are grown in these areas. However, less information is available on the morphological and biochemical responses of different date palm cultivars under high salinity. In this regard, eight cultivars of date palm were selected and treated with different salinity levels to check the adaptive capabilities of these cultivars against salt stress in terms of morphological and biochemical attributes. The objective of the current study was to screen these cultivars for tolerance or susceptibility to salt stress (0, 50, 100, and 200 mM NaCl). The results of the morphological parameters revealed the negative impact of salt stress on the morphology. Higher concentrations of salt reduced the plant height (Haleemi, Dahakki, Sanduri, Saghoi, Tarwali, and Hamanwali), the number of leaves (Haleemi, Basrawali, Dhakki, Sanduri, Saghoi, and Gajjar),

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leaf length (Haleemi, Dhakki, Sanduri, Saghoi, and Hamanwali), leaf width (Sanduri), leaf area (Haleemi, Sanduri, Saghoi, Tarwali, and Hamanwali), fresh weight (Halimi, Dhakki, Sanduri, Saghoi, and Tarwali), dry weight % (Halimi, Basrawali, Dhakki, Sanduri, Saghoi, Tarwali, Hamanwali, and Gajjar) and root length (Haleemi, Basrawali, Dhakki, Sanduri, and Gajjar) in most of the cultivars. Generally, it was observed that leaf tissues showed a significant ($p \leq 0.05$) increase in the superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) activities with the increasing salt concentrations. Current analysis showed that salinity significantly ($p \leq 0.05$) decreased the accumulation of total phenolic contents (TPC) in the leaf tissues of Haleemi, Basrawali, Dhakki, Sanduri, Saghoi, Tarwali, Hamanwali, and Gajjar. However, a reduction in the carotenoid contents in cultivars (Haleemi, Basrawali, Dhakki, Sanduri, Saghoi, Tarwali, Hamanwali, and Gajjar) was noted with an increase in the salt concentration. Salt stress significantly reduced the anthocyanin contents in some cultivars (Haleemi, Basrawali, Sanduri, Saghoi, Tarwali, Hamanwali, and Gajjar). This species is highly adapted to salt stress conditions by the evolution of an osmoregulation mechanism. These results suggest that although date palm is tolerant of high salinity, there is variation in tolerance among different cultivars. Based on the recorded parameters, it is concluded that Saghoi was the most salt-tolerant cultivar out of the test ones, followed by Sanduri, Tarwali, and Hamanwali, respectively.

Keywords: CAT, date palm, POD, salt stress, SOD

Introduction

Different types of environmental degradation, such as climate change resulting from population explosions and anthropogenic activities, lead to land degradation, such as soil salinity. Soil salinity has become a major global issue, currently found in different geographical regions of the world [1, 2]. Soil salinity produces a massive disturbance in the soil structure (osmotic and ionic stresses) and agricultural production and is responsible for the unstoppable reduction in arable land [3, 4]. Most of the area in Pakistan is situated in arid and semi-arid conditions, which are mostly bare, due to many stress factors accentuating the salinity stress, which include drought and harsh temperatures. In such areas, various kinds of halophytic plants are grown. These plants are well known for their capability to deal with high salt concentrations (>400 mM) due to their mechanisms of salt accumulation and exclusion, limitation of transpiration, ionic adjustment, antioxidant mechanism activation, and expression of secondary metabolites, which are necessary for the mitigation of salinity stress [5, 6]. Salinity stress has resulted from the desertification of huge agricultural zones, where the overharvesting of water has led to an infiltration of seawater into the groundwater [7]. The higher amount of salt in the soil is responsible for land damage, resulting in a reduction in plant development due to the toxicity of Na^+ and other ions [8].

Plants have developed different morphological, biochemical, and physiological adaptations to cope with high salinity stress by maintaining the ion influx and efflux in the plasma membrane, ion compartmentation in vacuoles, and regulating the osmotic balance [9]. Plants exposed to salt stress are engaged in the production of suitable solutes such as sugars and sugar alcohols, amines, and amino acids [10]. Plants can adapt themselves to soil salinity mainly through various mechanisms, such as (i) osmotic tolerance, (ii) Na^+ or Cl^- exclusion and secretion, and (iii) buildup of Na^+ or Cl^- in the tissues [11]. The enzymatic and non-enzymatic antioxidant systems are only a few of the astonishing array of defensive mechanisms that plants have developed to combat oxidative stress. The antioxidant enzymes include superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR), while the non-enzymatic antioxidants include water-soluble (ascorbate, glutathione, phenolic compounds, and flavonoids) and lipid-soluble (a-tocopherol, b-carotene, and lycopene) metabolites. Higher plants create a large range of secondary metabolites from primary metabolites (e.g., carbohydrates, lipids, and amino acids). These are essential for the protection of plants against different kinds of pathogenic and herbivorous activities. These metabolites frequently protect against environmental stress [12]. During stressful conditions, plants can secrete a variety of chemicals, such as calcium, abscisic acid (ABA), salicylic acid (SA), polyamines, and jasmonates (JA) [13]. Stressed plants frequently accumulate metabolites, such as different elicitors or signal molecules.

On the other hand, halophytes are regarded as one of the best plants that can adapt themselves to high salinity levels and can grow well in such conditions [14]. These plants can modify salt stress through their defensive mechanism, which is comprised of a reduction in Na^+ uptake, accumulation of Na+ in their vacuoles, retrieval of Na^+ from xylem arteries into xylem parenchyma cells, and recirculation of Na+ from shoot to root and back to the soil through the phloem [11, 15, 16]. There is a need to develop and screen certain plant cultivars that can tolerate high salinity stress. Moreover, the responsible mechanisms behind their salt tolerance are also required to be elucidated to get a clear picture behind the scenes. Based on the above discussion, eight cultivars of date palms were selected and treated with different salinity levels to check the adaptive capabilities of different cultivars against salt stress in terms of morphological and biochemical attributes. The objective of the current study was to screen out the available date palm germplasm against salinity stress to enhance date production in the country in general and in salt-affected areas. The screening was done based on morphological and biochemical parameters.

Experimental

Site Description

A three-year study was conducted during 2020- 2023 at the experimental area of the Institute of Forest Sciences, a demonstration site of the Islamia University of Bahawalpur, Punjab, Pakistan, located at latitude 29.378304° or 29°22’42”N and longitude 71.764706° or 71°45’53”E. The experimental site has a continental monsoon climate with a mean annual air temperature of 28.1°C and annual precipitation of 143 mm.

Plants, Seed Germination, and Growth Conditions

Date palm fruits were collected at the ripening stage from the date palm section of the Regional Agricultural Research Institute (RARI) in Bahawalpur during June and July. Seeds were removed from fruits and washed thoroughly with tap water. The washed seeds were treated with 5% (v/v) sodium hypochlorite solution for 10 minutes to remove surface microbes, followed by rinsing 2-3 times with distilled water and air drying in the laboratory at room temperature. This experiment was conducted in the nursery of the Institute of Forest Sciences, Islamia University, Bahawalpur. From these collections, eight cultivars were chosen to study the effects of various salt levels on their morphological and biochemical parameters. The seeds were sown in pots filled with salinized soil, and light irrigation was applied as per the requirement to ensure germination, as explained by the method of [17]. Five weeks after germination, the seedlings were fertilized by using NPK (20:5:10, including micro-nutrients) fertilizer. The pots were placed in the greenhouse. Then the pots were arranged in a completely randomized experimental design and subjected to 0 (control), 50, 100, and 200 mM NaCl (salt stress). Four different concentrations of salt solutions were applied to eight cultivars of date palms, while each cultivar comprises three replications. In the end, measurements were carried out, and samples were collected for various morphological and biochemical analyses.

Collection Site

Different cultivars of date palms were collected from the Regional Agriculture Research Institute in Bahawalpur. These cultivars are Haleemi (HA), Basrawali (BA), Dhakki (DH), Sanduri (SA), Saghoi (SH), Tarwali (TA), Hamanwali (HW) and Gajjar (GA).

Measurement of Morphological Attributes

Measurements before Harvesting

The plant height of two randomly selected seedlings from each replicate was measured in centimeters (cm) from the base of the hypocotyls to the tip of the shoot with the help of a meter rod. The average of each replication was calculated following the method of Muhammad et al. [18]. The number of leaves on each plant was counted very carefully, and the average was calculated by following the method explained by Hamid et al. [19]. After counting the number of leaves, plants were selected randomly for each treatment to measure the length of the leaf by using measuring tape [20]. Leaf width was measured by using a measuring tape to evaluate the qualitative character of date palms [21]. The leaf area of all plants under all treatments was measured by multiplying leaf length by leaf width, as described in [22].

Measurements after Harvesting

After the sixth month of growth, the seedlings were uprooted and washed with distilled water to remove the foreign particles of sand. After washing these plants, they were wrapped in filter paper to remove any drop of water present on their leaves and shoots. Then these were placed on the digital balance for the calculation of plant fresh weight. The average fresh weight of each variety consists of three replicates that were recorded in grams. After measuring fresh weight, plant materials were placed in a drying oven (Memmert-110, Schawabach) for drying at 72ºC for a week. After one week of drying, the dried biomass percentage was weighted on a digital balance, and the average dry weight percentage of each replicate was recorded and described by [23]. After that, the roots were separated from the whole plant. Roots were washed with tap water, followed by distilled water, and lengths were measured using measuring tape, followed by the method of [24].

Measurement of Biochemical Attributes

The activity of SOD was analyzed according to the protocol of [25] by calculating its potential to hinder the photoreduction of nitroblue tetrazolium (NBT). Catalase (CAT) activities were measured by the procedure of [26] with some alteration. Peroxidase (POD) activity was measured using the guaiacol test by following the change in absorbance at 470 nm. The activity was assayed for 3 min. in a reaction solution containing 100 mM potassium phosphate buffer (pH 7.0), 20 mM guaiacol, 10 mM H_2O_2, and 0.1 mL enzyme extract in a 3 mL volume [27]. The Folin-Ciocalteu reagent method was used for the determination of phenol quantity in plant extract (sample) [22]. For carotenoid extraction, 2 g of plant tissue was placed in a falcon tube, and after adding 18 ml of hexane, acetone, and ethanol (at a ratio of 1:1:2), it was shaken for 20 min. The mixture was centrifuged at 5000 rpm for 15 min. The absorbance of the hexane layer was read at 450 nm, and the concentration of carotenes was calculated by comparison against a calibration curve prepared with the 0-24 μg/mL standard solutions of β-carotene [28]. The Anthocyanins Assay Kit (Cosmo Bio, Carlsbad, CA, USA) was used to measure total anthocyanin content (TAC) in accordance with the procedure outlined by [29], with a small modification to allow for the reaction in 96-well microplates (Genesee Scientific, San Diego, CA, USA).

Statistical Analysis

The data were statistically analyzed using one-way ANOVA using SPSS (V.8.1). The mean comparison was carried out using the least significant difference (LSD) at a 5% probability level.

Results

Morphological Parameters

The plant height of all selected cultivars was measured against different concentrations of NaCl (0, 50, 100, and 200 mM). The statistical analysis showed that there are highly significant differences in shoot length between different salt concentrations recorded in Haleemi, Dahakki, Sanduri, Saghoi, Tarwali, and Hamanwali (Fig. 1). Salt stress did not affect cultivars Haleemi and Gajjar concerning plant height.

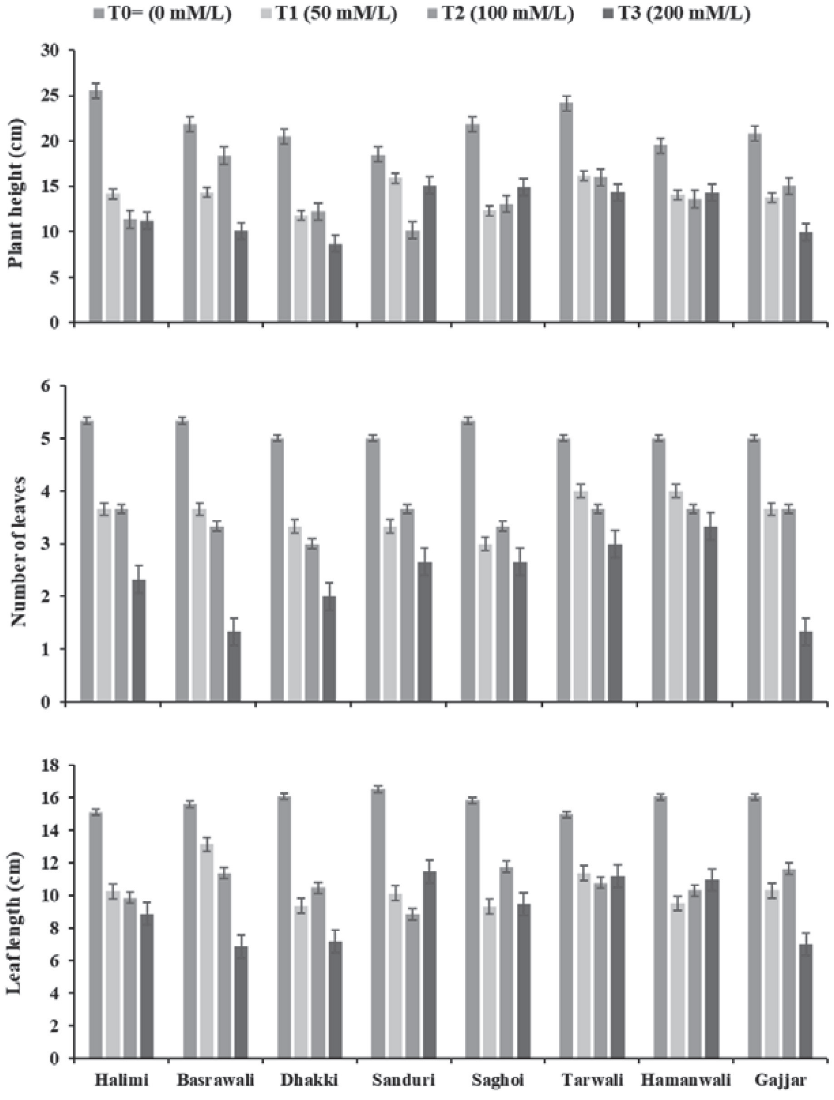

Fig. 1. Impact of different salinity levels on plant height (cm), number of leaves, and leaf length (cm) of different cultivars of date palm.

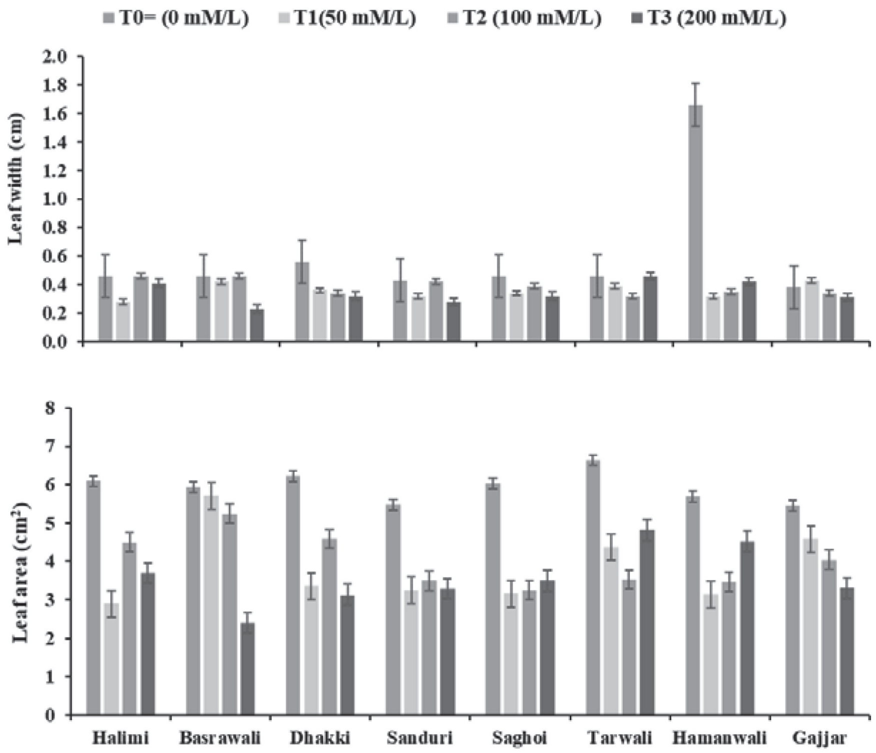

Fig. 2. Impact of different salinity levels on leaf width (cm) and leaf area (cm2) of different cultivars of date palm.

The number of leaves of all selected cultivars was measured against different concentrations of NaCl (0, 50, 100, and 200 mM). The statistical analysis showed that there are highly significant differences in the number of leaves between different salt concentrations recorded in Haleemi, Basrawali, Dhakki, Sanduri, Saghoi, and Gajjar. Salt stress did not affect the cultivars Tarwali and Hamanwali. The leaf length of all selected cultivars was measured against different concentrations of NaCl (0, 50, 100, and 200 mM). The statistical analysis showed that there are highly significant differences in leaf length between different salt concentrations recorded in Haleemi, Dhakki, Sanduri, Saghoi, and Hamanwali. No significant effect was seen in the cultivars Basrawali, Tarwali, and Gajjar regarding leaf length.

The leaf width of all selected cultivars was measured against different concentrations of NaCl (0, 50, 100, and 200 mM). The statistical analysis showed that there are highly significant differences in leaf width between different salt concentrations recorded in Sanduri (Fig. 2). On the other hand, no significant differences were recorded for cultivars (Haleemi, Basrawali, Dhakki, Saghoi, Tarwali, Hamanwali, and Gajjar) regarding leaf width. The leaf area of all selected cultivars was measured against different concentrations of NaCl (0, 50, 100, and 200 mM). The statistical analysis showed that there are highly significant differences in leaf area between different salt concentrations recorded in Haleemi, Sanduri, Saghoi, Tarwali, and Hamanwali. No significant difference in leaf width between different salt concentrations was recorded for Basrawali, Dhakki, and Gajjar.

The plant fresh weight of all selected cultivars was measured against different concentrations of NaCl (0, 50, 100, and 200 mM). The statistical analysis showed that there are highly significant differences in plant fresh weight between different salt concentrations recorded in Halimi, Dhakki, Sanduri, Saghoi, and Tarwali (Fig. 3). No significant difference in plant fresh weight between different salt concentrations was recorded for Basrawali, Hamanwali, and Gajjar. The plant dry weight % of all selected cultivars was measured against different concentrations of NaCl (0, 50, 100, and 200 mM). The statistical analysis revealed that salt stress significantly reduced the plant dry weight % in all cultivars as compared to the control. The cultivars (Halimi, Basrawali, Dhakki, Sanduri, Saghoi, Tarwali, Hamanwali, and Gajjar) were sensitive to salt stress. The root length of all selected cultivars was measured against different concentrations of NaCl (0, 50, 100, and 200 mM). Salt stress significantly reduces the root length against different concentrations of salt in the cultivars Haleemi, Basrawali, Dhakki, Sanduri, and Gajjar. No significant difference in plant root length between different salt concentrations was recorded for Saghoi, Tarwali, and Hamanwali.

Biochemical Parameters

The antioxidant enzyme activity of SOD, CAT, and POD was evaluated in eight different cultivar seedlings,

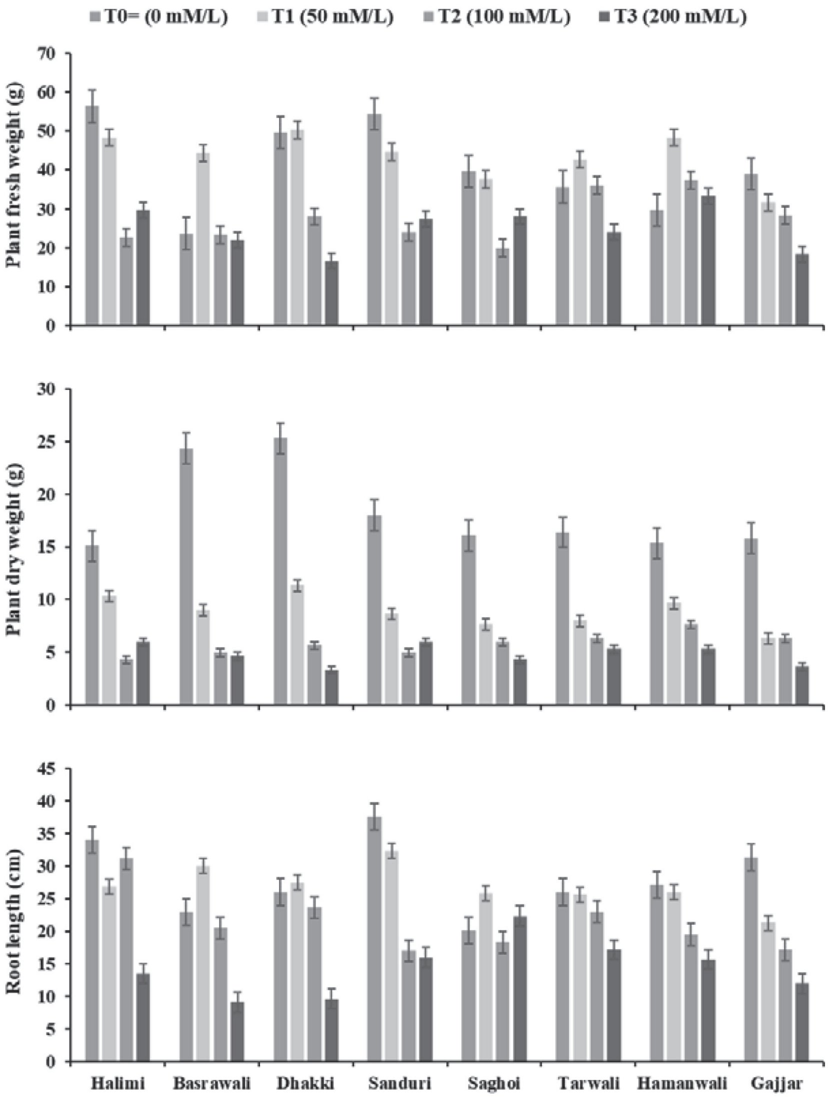

Fig. 3. Impact of different salinity levels on plant fresh and dry weight (g) and root length (cm) of different cultivars of date palm.

and non-enzymatic activity was also measured, such as phenolic contents, carotenoid contents, and anthocyanin contents (Fig. 4). The SOD activity of all selected date palm cultivars at the seedling stage was measured against different concentrations of NaCl (0, 50, 100, and 200 mM).Under normal conditions, SOD activity was significantly ($p \leq 0.05$) higher in the leaves of different cultivars of date palm seedlings. This statistical analysis revealed that SOD activity was significantly ($p \leq 0.05$) increased in the leaves of Haleemi, Sanduri, Saghoi, Tarwali, and Hamanwali, respectively, at different salt concentrations. The POD activity of all selected date palm cultivars at the seedling stage was measured against different concentrations of NaCl (0, 50, 100, and 200 mM). In the POD assay of different date palm cultivar seedlings under normal conditions, POD activity was significantly ($p \leq 0.05$) higher in the leaves of different cultivars of date palm seedlings. This statistical analysis showed that POD activity was significantly ($p \leq 0.05$) increased in the leaves of Haleemi, Sanduri, Saghoi, Tarwali, and Hamanwali at different salt concentrations. The CAT assay of all selected date palm cultivars at the seedling stage was measured against different concentrations of NaCl (0, 50, 100, and 200 mM). These statistics showed that CAT activity was significantly ($p \leq 0.05$) increased in the leaves of Haleemi, Sanduri, Saghoi, Tarwali, and Hamanwali, respectively, at different salt concentrations.

The total phenolic content (TPC) of all selected date palm cultivars at the seedling stage was measured against different concentrations of NaCl (0, 50, 100, and 200 mM). This statistical analysis showed that salinity significantly ($p \leq 0.05$) decreased the accumulation of TPC in the leaf tissue of Haleemi, Basrawali, Dhakki, Sanduri, Saghoi, Tarwali, Hamanwali, and Gajjar with increasing concentrations of salt (Fig. 5). The carotenoid contents of all selected cultivars were measured against different concentrations of NaCl (0, 50, 100, and 200 mM). This statistical analysis showed that there is a significant difference ($p \leq 0.05$) in carotenoid contents

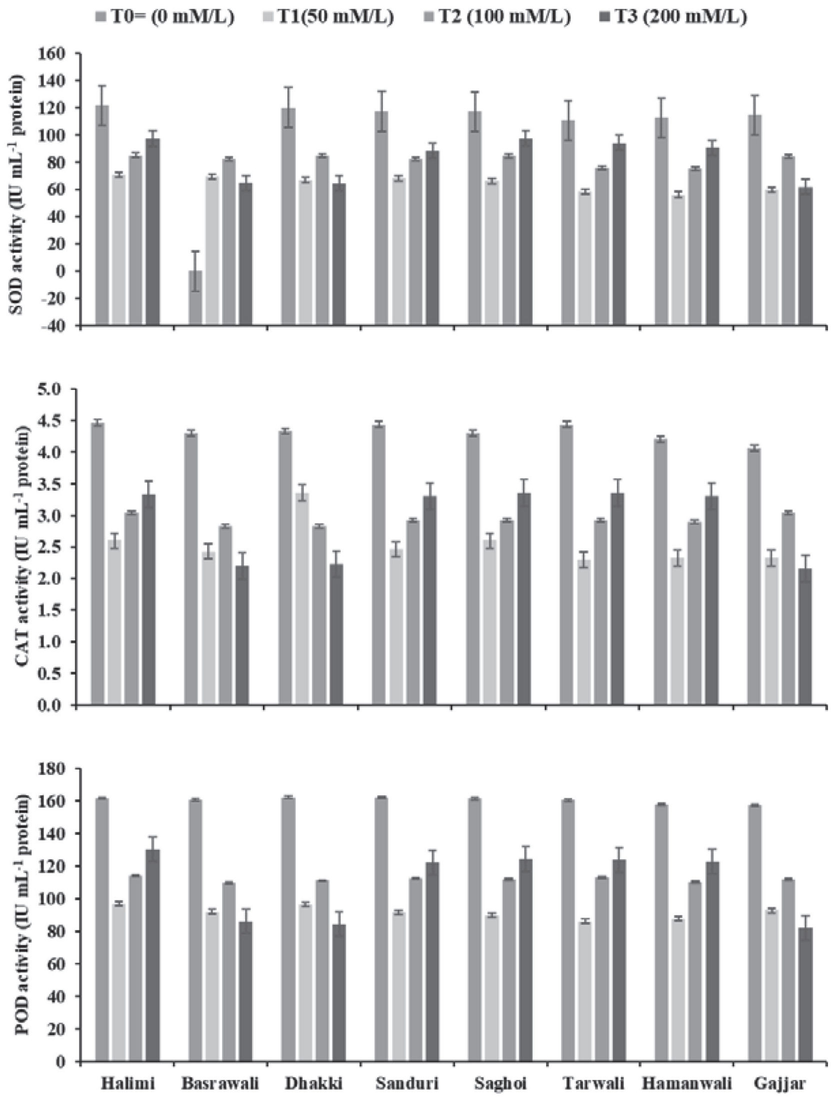

Fig. 4. Impact of different salinity levels on the superoxide dismutase (SOD), and catalase (CAT), and peroxidase (POD) activities of different cultivars of date palm.

between different salt concentrations recorded in Haleemi, Basrawali, Dhakki, Sanduri, Saghoi, Tarwali, Hamanwali, and Gajjar. The amount of anthocyanin content of all selected cultivars was measured against different concentrations of NaCl (0, 50, 100, and 200 mM). This statistical analysis showed that salinity had no significant ($p \leq 0.05$) impact on the anthocyanin content in Dhakki. On the other hand, significant differences were recorded in Haleemi, Basrawali, Sanduri, Saghoi, Tarwali, Hamanwali, and Gajjar at different concentrations of salt.

Discussion

The present study sought to screen eight date palm cultivars for salt stress tolerance and to determine the mechanisms underlying their tolerance or susceptibility. In a preliminary screening under different NaCl concentrations (0, 50, 100, and 200 mM), we observed significant differences in growth among the cultivars under different concentrations of salinity levels. Salt stress significantly reduced plant height in Haleemi, Dahakki, Sanduri, Saghoi, Tarwali, and Hamanwali.

Whereas increasing the amount of salt did not affect the Basrawali and Gajjar regarding plant height. The current findings are consistent with previous studies [30]. Salt stress significantly reduced the number of leaves in Haleemi, Basrawali, Dhakki, Sanduri, Saghoi, and Gajjar. Increasing the amount of salt did not affect the cultivars with respect to the number of leaves in Tarwali and Hamanwali. The present finding is similar to that of the previous study [31]. Salt stress significantly reduces the leaf length in the cultivars Haleemi, Dhakki, Sanduri, Saghoi, and Hamanwali. Whereas increasing amounts of salt had no effect on the cultivars regarding leaf length in Basrawali, Tarwali, and Gajjar. This present study is in line with previous research work [32].

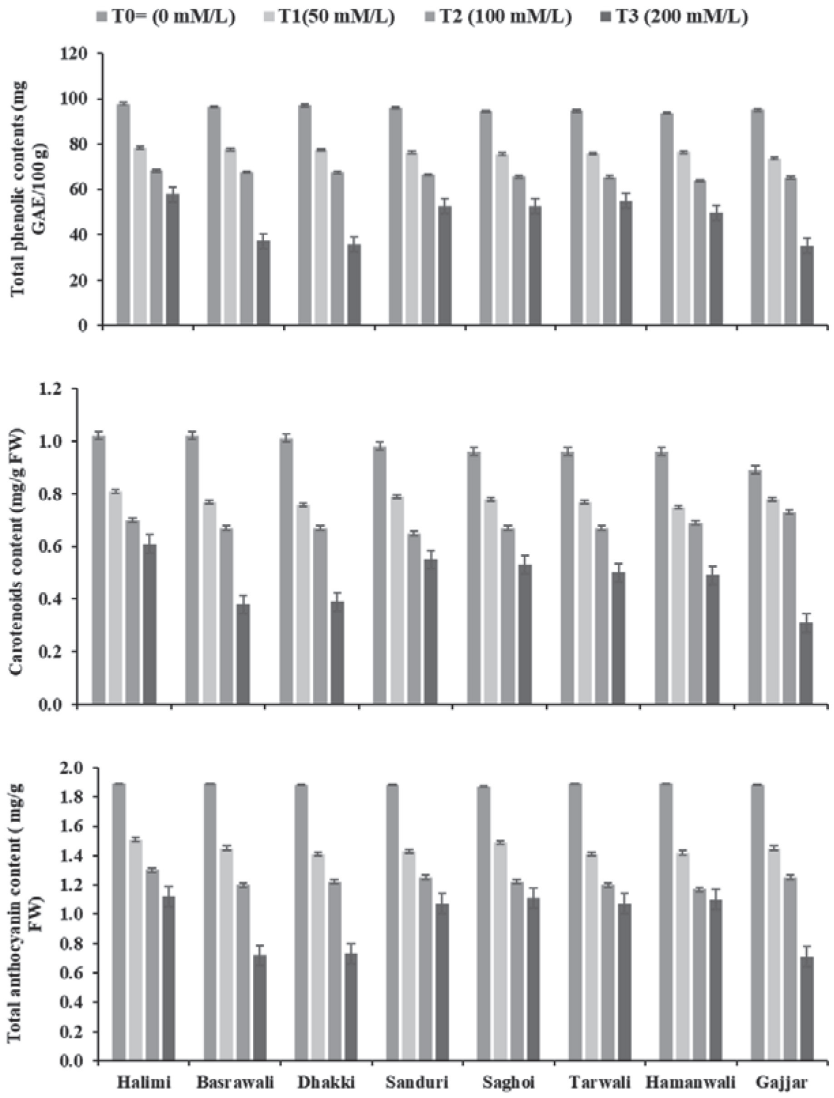

Fig. 5. Impact of different salinity levels on total phenolic, carotenoid, and anthocyanin contents of different cultivars of date palm.

Different salt concentrations significantly reduce the leaf width in Sanduri. Cultivars such as Haleemi, Basrawali, Dhakki, Saghoi, Tarwali, Hamanwali, and Gajjar were salt tolerant in terms of leaf width. The present research is matched with the earlier research work [33].

Different concentrations of salt stress significantly reduce leaf area in Haleemi, Sanduri, Saghoi, Tarwali, and Hamanwali. These findings are similar to the results obtained in Zabad cultivars under salt stress during a previous study [34]. The leaf area is directly correlated with the photosynthetic rate [35]. Salt stress significantly reduces the fresh weight in Halimi, Dhakki, Sanduri, Saghoi, and Tarwali. No significant difference in plant fresh weight between different salt concentrations was recorded for Basrawali, Hamanwali, and Gajjar. The present study is in line with previous research work [32]. Statistical analysis showed a significant interaction effect between cultivars and salt treatments on dry weight percentage. Results for relative dry weight % of different date palm cultivars in response to increasing salinity, dry weight % reduction was maximum for the plantlets of Halimi, Basrawali, Dhakki, Sanduri, Saghoi, Tarwali, Hamanwali, and Gajjar. Current findings on dry weight percentage are consistent with an earlier study by [23]. However, linear relationships between the decreases in dry weight of different tissues with an increase in salt concentration suggest that osmoregulation is effective for salt tolerance only to a limited range in the continuum of salt stress. Salt stress significantly reduced the root length in the cultivars Haleemi, Basrawali, Dhakki, Sanduri, and Gajjar. The results of the present study are in line with the earlier scientific work of Hewitt [36].

Plants tolerate oxidative stress caused by salinity through antioxidant mechanisms [37, 38]. These are subdivided into enzymatic and non-enzymatic antioxidant mechanisms. Several non-enzymatic antioxidants have been well characterized; these enzymes catalyze redox reactions and rely on electron donation via the reduction of low-molecular-weight antioxidants, such as glutathione, flavonoids, phenols, and free proline [38-40]. Enzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), have been previously reported as the major antioxidant enzymes that serve as an antioxidant defense system in many plants under salt stress [41-43]. The enzymatic antioxidant activities of SOD, CAT, and POD were measured in eight cultivar seedlings to investigate the contribution of these enzymes to the salinity tolerance mechanism of this plant. In general, it was observed that leaf tissues showed a significantly ($p \leq 0.05$) higher concentration of SOD, CAT, and POD activity in response to different salt concentrations. The current findings are in line with previous studies [44].

Current analysis showed that salinity significantly ($p \leq 0.05$) decreased the accumulation of TPC in the leaf tissues of Haleemi, Basrawali, Dhakki, Sanduri, Saghoi, Tarwali, Hamanwali, and Gajjar. These results are consistent with the previous study by Al Kharusi et al. [45]. However, the carotenoid contents in cultivars (Haleemi, Basrawali, Dhakki, Sanduri, Saghoi, Tarwali, Hamanwali, and Gajjar) showed a significant effect against different salinity levels. Salt stress significantly reduces the anthocyanin contents in some cultivars (Haleemi, Basrawali, Sanduri, Saghoi, Tarwali, Hamanwali, and Gajjar). These present findings are in line with the previous research work of Al Kharusi et al. [44].

Conclusions

Overall, higher salt concentrations significantly affected the morphology of the different date palm cultivars. A higher concentration of salt reduces plant height, number of leaves, leaf length, leaf width, leaf area, root length, fresh weight, and plant dry weight % in these cultivars. Generally, it was observed that leaf tissues showed a significantly ($p \leq 0.05$) higher concentration of SOD, CAT, and POD activity in response to different salt concentrations. In this research, salinity significantly ($p \leq 0.05$) decreased the accumulation of TPC, carotenoid contents, and anthocyanin contents in the leaf tissue of different cultivars. In conclusion, Saghoi was the most salt-tolerant cultivar based on the observed parameters among the test date palm cultivars. It was followed by Sanduri, Tarwali, and Hamanwali, respectively. The screened cultivars could enhance date production in the country in general and in salt-affected areas. However, rigorous field experiments are required to authenticate these claims under natural field conditions.

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Conflict of Interest

The authors declare no conflict of interest.

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