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Morphological and biochemical characterization of Date palm genotypes under salinity stress in arid regions

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Abstract

Salinization is a physical process in arid and semi-arid soils that causes serious issues for crop production. Date palm is an economically and nutritionally important crop, revealing a significant variability in salinity tolerance among cultivars. This study investigated the morphological and biochemical responses of selected date palm varieties subjected to different NaCl concentrations. Among the cultivars and treatments, significant differences were found. Results revealed that different salinity stresses have significant effects on plant growth, chlorophyll, antioxidants, photosynthetic activity and Total Phenolic Content. Maximum plant height and leaf numbers were observed at T3 (17.56 A) in khupra and Amber at T2 (3.66 AB), respectively and leaf area was maximum in Zahidi (4.84 AB) at T3. Plant fresh weight and dry weight were maximum in Zahidi (39 A) and Amber (39 AB). Root length was maximum reported in Halwain cultivar (29.50 A). At T1, the highest photosynthetic activity and chlorophyll content were noted in Zahidi and Ajwa. Superoxide Dismutase (SOD) activity was maximum in leaves of Khupra, at T3. The Kurr had notably higher levels of CAT activity (4.46 A) in T3. Barhee, cultivar exhibited substantial improvements in Peroxidase activity at T3. Total phenolic contents were highest in Zahidi at T1. Carotenoid content was maximum in Zahidi and Zirin cultivars. The anthocyanin content was maximum in Aseel and Kurr cultivars in T1. The findings of this study have significant implications for the selection of a particular variety that exhibits tolerance to salinity and an ability to thrive in saline environments.

1 Introduction

Date palm, a member of the Arecaceae family, is a versatile tree that has significant nutritional, medicinal, and decorative value. Its global production, use and industrialization are persistently growing. Production of date palm plantlets is conducted on an extensive scale to meet the growing demand. Geoclimatic variations have a significant impact on agricultural systems globally (Goharrizi *et al*., 2021). The effects of environmental stress factors on plant growth, development and productivity are too many, but salinity is well understood to be the most crucial abiotic stress factor. It

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also leads to an increase in salinity and the formation of salts in the root zone and thus causes plant disintegration and a lower standard of the plants (Verma *et al.,* 2012; Ahmed and Khalid, 2023).

At high salinity of the irrigation water, which cause sodium buildup and the generation of ROS in the plant tissues are rapid oxidative and ionic stresses, and the homeostasis of basic elements and water intake are altered, which causes the damaging of the membranes and decreases the levels of chlorophylls and carotenoids and negatively impacts growth and development (Golezani and Abdoli, 2022; Ahmed *et al*., 2023). In addition, the salty water in the irrigation process hinders the formation of proteins, phenols, flavonoids, lipid metabolism, sugar accumulation and several other natural product metabolites (Khalid and Silva, 2010). The impact of high concentrations of salt in irrigation water varies according to specific plant types (Khalid and da Silva 2010; Khalid and Ahmed 2017). For instance, plants show the most effective antioxidant defense mechanisms, which might be helpful in neutralizing ROS (Sharma *et al*., 2012; Sumra *et al*., 2023). Superoxide dismutase, catalase, peroxidase, ascorbate peroxidase, and glutathione reductase are the biochemical antioxidants that play a significant role of antioxidant protection system in plants based on salt stress conditions in some works (Latef and Chaoxing, 2014; Foyer 2018; Evelin *et al*., 2019). By virtue of their adaptability to saltwater environments, halophytic plants show unique morphological, biochemical, anatomical, and functional characteristics in comparison to non-halophytic plants. The removal of salt ions or their sequestration in vacuoles or specific cellular compartments is a vital mechanism for salt tolerance in halophytes. The aforementioned mechanisms function by means of alterations in the proteome, metabolomic, transcriptomic, or genomic levels (Elsahookie *et al*., 2014).

Date palms are celebrated for their exceptional hardiness, flourishing even in extremely hot and water-scarce environments. However, these crucial plants continue to confront considerable challenges from non-biological environmental factors. Such abiotic stresses detrimentally affect various internal functions, leading to manifestations like reduced leaf dimensions, inhibited stem elongation, impaired root development, and disrupted water absorption and movement within the plant (Mohanty *et al*., 2022; Yasin *et al*., 2024). To cope with specific adversities such as high salinity, plants activate a multifaceted defense system, involving sophisticated adaptations at the physiological, biochemical, and molecular levels. Date palm cultivars display varying degrees of resilience to elevated salt levels. While the date palm species generally possesses a notable capacity to endure irrigation with increasingly saline water, its ability to withstand these challenging conditions diminishes proportionally with rising salt concentrations. High salinity in the growth medium directly impedes root elongation and restricts overall plant growth. Additionally, this increased salt content negatively impacts the plant's ability to absorb nutrients, thereby affecting its internal mineral composition (Keltoum, 2022; Afzal *et al*., 2020). Therefore, a group of nine different cultivated varieties of date palm was chosen and exposed to different degrees of salinity in order to evaluate their ability to adjust in terms of physical and chemical characteristics when faced with stress caused by salt. This work aims to assess the current pool of date palm genetic resources in relation to their ability to withstand high levels of soil salt and to enhance the national-level production of dates, particularly in areas where soil salinity is a significant problem. The screening approach included the evaluation of morphological and biochemical traits to examine the adaptive responses under salt-induced stress.

2. Materials and Methods:

2.1 Study area description

The present work was conducted in the nursery and laboratory of the Institute of Forest Sciences, Islamia University Bahawalpur. The total duration of the experiment was two years, during which plants were grown and treated very carefully in the nursery. After that, the plants were harvested and shifted into the laboratory for further analysis. The area has a continental monsoon climate. The region's climate is defined by a continental monsoon flow, with an average yearly rainfall of about 150 millimeters. The Cholistan climate features an average annual temperature of 27.34 °C. The scorching peak of its climate occurs in June, when the mean temperature reaches 37.4 °C. Date palm cultivars were collected from various parts of Bahawalpur city. These cultivars are Zahidi (ZA), Aseel (AS) Kurr (KU), Barhee (BA), Ajwa (AJ), Halwain (HA), Khupra (KH), Amber (AM) and Zirin (ZI)

2.2 Experimental layout and Growth Conditions

Fruits from the date palm plantation in Bahawalpur were gathered in June and July. The collected seeds were rinsed with running tap water and then subjected to three consecutive surface disinfections using a laboratory-optimized procedure. Initially, we immersed the seeds in 100% commercial bleach for 5 minutes while vigorously agitating them. Subsequently, we immersed them in 50% (v/v) commercial bleach for 10 minutes, and finally, in 25% (v/v) commercial bleach for 10 minutes. Next, the seeds were meticulously washed with sterile distilled water. Following disinfection, date palm seedlings were subsequently grown within polythene bags. According to Bouhouch *et al*. (2021), 2 to 3 date palm fruit seeds are placed in a single polythene bag. Following a five-week period of germination, the seedling was exposed to an NPK fertilizer

(15:5:10) including micronutrients. After that, the pots were transferred to the greenhouse and arranged according to the total random arrangement. Four distinct degrees of salt stress were applied to the date palm cultivars. The concentrations of (control, 50, 100, and 200 mM) of NaCl were used to compare the salt levels. Three replicates were used for each cultivar in this experiment.

2.3 Measurement of Morphological Parameters

The technique described by Al-Shoaibi *et al*. (2020) was used to conduct a measurement of plant height using a metric ruler. Using the approach proposed by (Aldhebiani *et al*., 2018), the number of leaves was quantifiably counted at regular intervals. Measurements of the leaf length and leaf breadth were made using digital calipers. To fulfill this goal, three juvenile leaves were picked randomly from each plant and then the mean figures for each individual plant were calculated using the method put forth (Al-Shoaibi *et al*., 2020). The leaf area was worked out by multiplying the leaf length by its breadth, following the strategies presented by Riaz *et al*. (2021). After concluding the study experiment, the plants were carefully extracted and their roots were thoroughly cleaned to eliminate clumps of dirt. The entire plant was quickly measured using an automated scale. To that end, the plants were dried in an oven at 70°C for 48 hours until they achieved an even weight. Additionally, the dry weights recorded for the plants were calculated using the technique outlined (Al-Shoaibi *et al*., 2020). The root systems had a detailed washing process to eliminate all dirt. A ruler was used to measure the roots' elongation.

2.4 Measurement of Physiological and Biochemical Characters

Date palm leaves' photosynthetic productivity was assessed using a portable fluorometer (Opti-Sciences 0SI 30P) (Baker 2008). The chlorophyll meter (SPAD) offers a straightforward, rapid, non-invasive technique for assessing leaf chlorophyll content (LCC) (Watanabe, 1980). A portable photosynthesis system (LI-6400 (LI-COR, USA)) was used to quantify the transpiration rate (Farouk *et al*., 2011). Utilizing the established standard method, the activity of SOD was determined. This depends on the suppression of the photochemical reduction of nitro blue, as described by Fridovich (1971) tetrazolium (NBT). Plewa et al. (1991) describe the activity of peroxidase (EC1.11.1.7), which was examined by keeping track of the production of tetraguaiacol and calculating the absorbance at 470 nm. Catalase (CAT) was quantified from the samples using an approach established by a past developer. Aebi (1983). Foliage samples were analyzed for total phenolic content (TPC) concentration using a well-established method (Singleton *et al*., 1999). Carotenoids and anthocyanins were quantified by measuring the absorbance using spectrophotometry at wavelengths of 646, 480, and 567 nm, respectively, following the procedure described by Porra *et al*., (1989).

2.5 Statistical Analysis

Analysis of the data was conducted using one-way analysis of variance with the SPSS statistical software version 8.1. Significance tests were conducted by using the LSD) test at a significance value of p = 0. 05.

3. Results:

3.1 Growth measurements

Significant heterogeneity in plant height measurements was observed across numerous cultivars of date palm under varying amounts of sodium chloride salt concentration. The height of plants is significantly reduced in different cultivars when subjected to different levels of salt exposure. Maximum plant height was observed in T3 (17.56 A) in khupra cultivars (Figure 1B). Altogether, the leaf number decreases constantly in cultivars (Zahidi, Aseel, Kurr, Barhee, Ajwa, Halwain, Khupra, Amber and Zirin) under different levels of salinity stress. The number of leaves was maximum observed in cultivar Amber at T2 (3.66 AB) (Figure 1A). Various types of date palm plants were measured in terms of leaf length and the results, as tested by different concentrations of sodium chloride salt, showed some significant variations. The cultivars such as Zahidi, Aseel, Kurr, Barhee, Halwain, Khupra and Zirin had their leaf length affected by changes in salt concentrations. In the two cultivars Ajwa and Amber, no reduction in the length of the leaves was observed in response to salt stress. The leaf length was maximum recorded in cultivar Halwain, in T3 (12.85 A) (Figure 2C). The minor differences in the width of leaves were observed against different concentrations of salt. When exposed to salt chloride, only the Zahidi, Barhee, and Zirin cultivars demonstrated notable variation in leaf width. No variation has been observed in varieties and cultivars of dates (Aseel, Kurr, Ajwa, Halwain, Khupra, and Amber) under different concentrations of salinity. Leaf width was

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maximum noted in the Zahidi cultivar (0.45 AB) at T3 (Figure 1D). An assay of leaf area in many date palm (Phoenix dactylifera) cultivars was performed with slight variations, in which different concentrations of sodium chloride were applied. A marked variation in the leaf area was observed between the cultivars Zahidi, Aseel and Barhee depending on the level of salt chloride concentration. There was no discernible difference seen in leaf area among the cultivars (Kurr, Ajwa, Halwain, Khupra, Amber, and Zirin). Plant leaf area was maximum in cultivar Zahidi (4.84 AB) at T3 (Figure 1C).

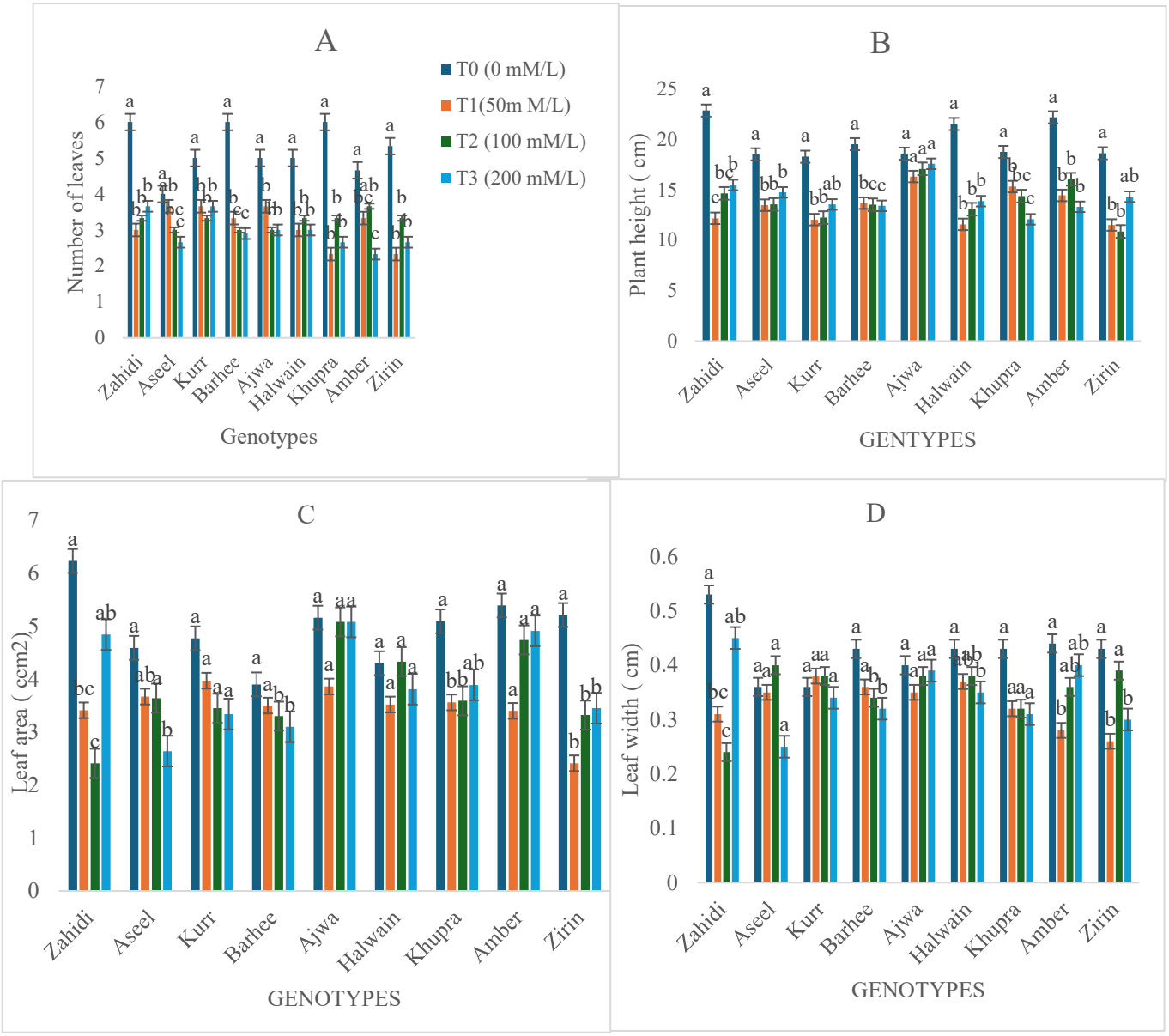


Fig 3.1. Morphological parameters (A= Number of leaves, B= Plant height, C=Leaf area and D=Leaf width)

Significant differences were seen in the fresh weight of several cultivars of date palm (*Phoenix dactylifera*) when exposed to different amounts of sodium chloride salt. The plants of the cultivars Zahidi, Aseel, Kurr, Barhee, Ajwa, and Khupra exhibited a significant reduction in fresh weight. Upon exposure to varying degrees of salt stress, the fresh weight of plants in the Halwain, Amber, and Zirin cultivars did not exhibit any significant changes. Plant fresh weight was maximum in cultivar Zahidi (39 A) and Amber (39 AB) at T3. Plant dry weight % was highest in cultivar Amber in T1 (7.66 B). Dry weight percentage of the plant in every date palm genotype was significantly influenced by different levels of sodium chloride salt stress compared to the control conditions. Specifically, the Zahidi, Aseel, Kurr, Barhee, Ajwa, Halwain, Khupra, Amber, and Zirin cultivars had a notable reduction in the proportion of dry weight % in plants when subjected to

varying amounts of sodium chloride. Plant dry weight % was highest in cultivar Amber in T1 (7.66 B). Various cultivars of date palm (*Phoenix dactylifera*) exhibited notable differences in root length measurements when exposed to varying concentrations of salt chloride. Elevated concentrations of sodium chloride significantly reduced the root length of the Zahidi, Aseel, Barhee, Ajwa, Halwain, and Zirin cultivars. Conversely, the cultivars Kurr, Khupra, and Amber exhibited resistance to salt stress as seen by their root length expansion. Root length was maximum reported in Halwain cultivar (29.50 A).

Table-1: Photosynthetic activity of various cultivars of *Phoenix dactylifera* under different salt conditions

Varieties	T0 (0 mM/L)	T1(50 mM/L)	T2 (100 mM/L)	T3 (200 mM/L)	Significant
Zahidi	19.00 A	17.40 B	14.20 C	8.93 D	**
Aseel	21.00 A	16.83 B	13.60 C	10.20 D	**
Kurr	20.00 A	16.30 B	13.90 C	7.20 D	**
Barhee	18.00 A	16.86 B	0.00 C	0.00 C	**
Ajwa	20.00 A	16.16 B	14.20 C	11.20 D	**
Halwain	19.33 A	15.30 B	13.53 C	10.83 D	**
Khupra	18.66 A	16.96 B	13.40 C	10.86 D	**
Amber	21.33 A	17.23 B	13.96 C	9.20 D	**
Zirin	21.33 A	15.86 B	13.26 C	9.36 D	**

Table2: Chlorophyll content of various cultivars of *Phoenix dactylifera* under different salt conditions.

Varieties	T0 (0 mM/L)	T1(50 mM/L)	T2 (100 mM/L)	T3 (200 mM/L)	Significant
Zahidi	31.00 A	25.66 B	22.76 C	18.76 D	**
Aseel	32.00 A	26.16 B	22.60 C	18.63 D	**
Kurr	33.00 A	24.73 B	22.70 C	18.66 D	**
Barhee	31.66 A	24.66 B	0.00 C	0.00 C	**
Ajwa	31.00 A	26.26 B	22.20 C	18.23 D	**
Halwain	30.00 A	26.16 B	22.43 C	17.46 D	**
Khupra	29.00 A	25.86 B	22.66 C	17.60 D	**
Amber	30.00 A	25.86 B	23.00 C	17.76 D	**
Zirin	32.00 A	25.73 B	23.10 C	18.50 D	**

Table-3: Rate of transpiration of various cultivars of *Phoenix dactylifera* under different salt conditions.

Varieties	TO (0 mM/L)	T1(50mM/L)	T2(100 mM/L)	T3(200 mM/L)	Significant
Zahidi	17.76 A	3.37 A	2.64 A	2.30 A	NS
Aseel	4.23 A	3.15 B	2.64 C	2.21 D	**
Kurr	4.36 A	3.42 B	2.89 C	2.2O D	**
Barhee	4.43 A	3.20 B	0.00 C	0.00 C	**
Ajwa	4.43 A	3.20 B	2.72 C	2.25 D	**
Halwain	4.36 A	3.60 B	2.56 C	2.45 D	**
Khupra	4.33 A	3.40 B	2.81 C	2.44 D	**
Amber	4.20 A	3.15 B	2.81 C	2.40 D	**
Zirin	4.40 A	3.28 B	2.67 C	2.25 D	**

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3.2 Biochemical and Physiological parameters

3.2.1 Physiological parameters

In different cultivars of date palm, photosynthetic activity significantly decreased under different treatments of salt stress. Zahidi was the best cultivar against salt stress in T_1 (17.40 B) with respect to photosynthetic activity, as shown in Table 1. Chlorophyll content was significantly reduced at different levels of salt stress in various date palm cultivars. Ajwa was the best cultivar against salt stress in T_1 (26.26 B) with respect to Chlorophyll content, shown in Table 2. Rate of transpiration was significantly decreased against different salinity levels in various date palm cultivars. Zahidi was best salt-tolerant cultivar with respect to rate of transpiration (3.37 A), as shown in Table 3.

3.2.2 Antioxidant enzymes activity

Elevated activity levels of Superoxide Dismutase (SOD) were detected in every date palm genotype leaf tissue cultivated under controlled conditions. SOD activity was highest in Khupra leaves in T3 (121.33 A) with increasing saline levels (Figure 3.2 A). Similarly, the activity of catalase (CAT) in leaf tissues showed an elevation when subjected to controlled settings. Notably, the cultivar Kurr had notably higher levels of CAT activity in T3 (4.46 A) across different concentrations of sodium chloride (Figure 3.2 B). The Peroxidase activity in leaf tissues also showed an increase when subjected to controlled settings. Notably, Barhee, cultivar exhibited substantial improvements in POD activity at various sodium chloride concentrations in T3 (162.33 A) (Figure 3.3 B). Variations in salt stress levels significantly impacted total phenolic contents (TPC) in each genotype compared to controls. Specifically, Zahidi showed maximum total phenolic content in T1 (73.73 B) against various sodium chloride concentrations (Figure 3.3 A). Furthermore, the carotenoid content in these cultivars was markedly decreased when subjected to various degrees of salt stress, as compared to the control group (Figure 3.3 C). Carotenoid content was maximum in T1 (0.79 B) with respect to Zahidi and Zirin cultivars. In addition, the anthocyanin content decreased significantly at all salt sodium chloride values under investigation (Figure 3.3 D). The anthocyanin content was maximum in Aseel and Kurr cultivars in T1 (1.47 B) against various salt concentrations.

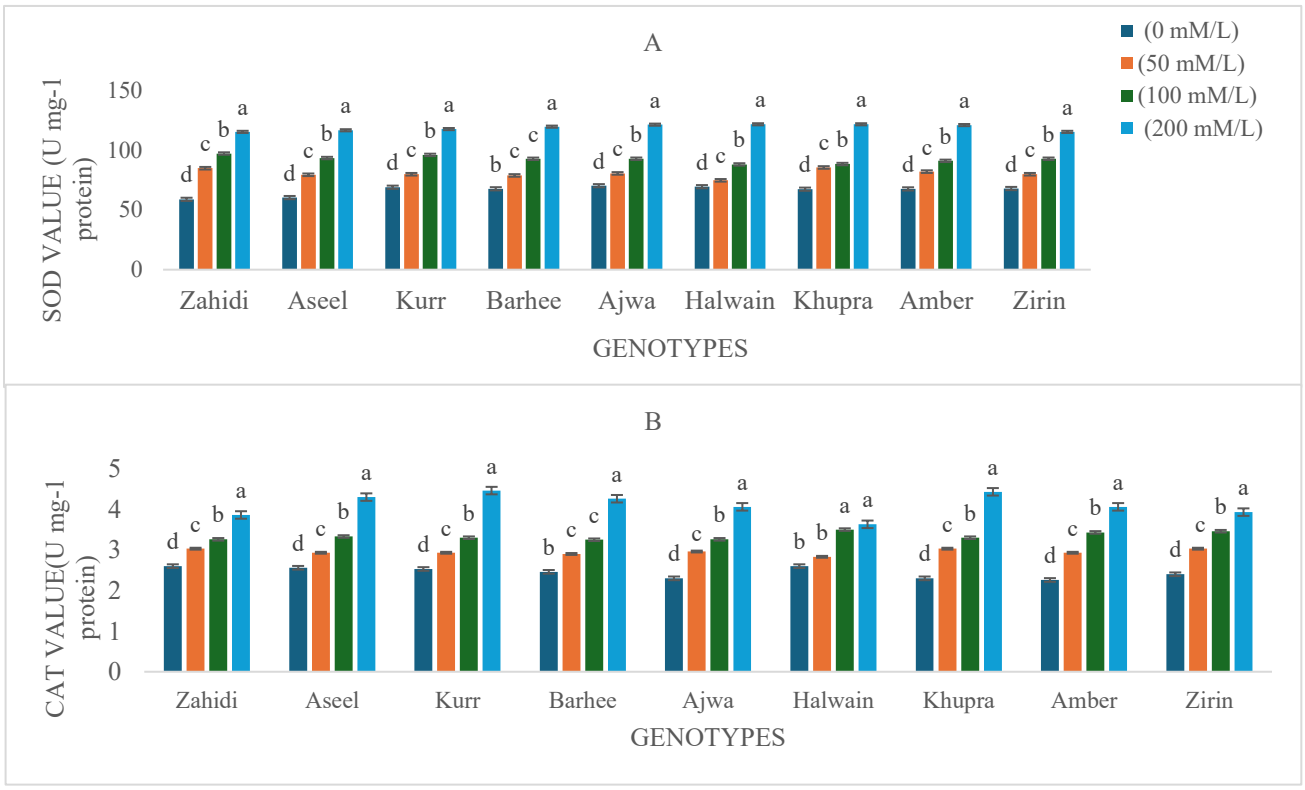


Fig. 3.2. Shows biochemical activities (A=SOD Value, B=CAT Value.

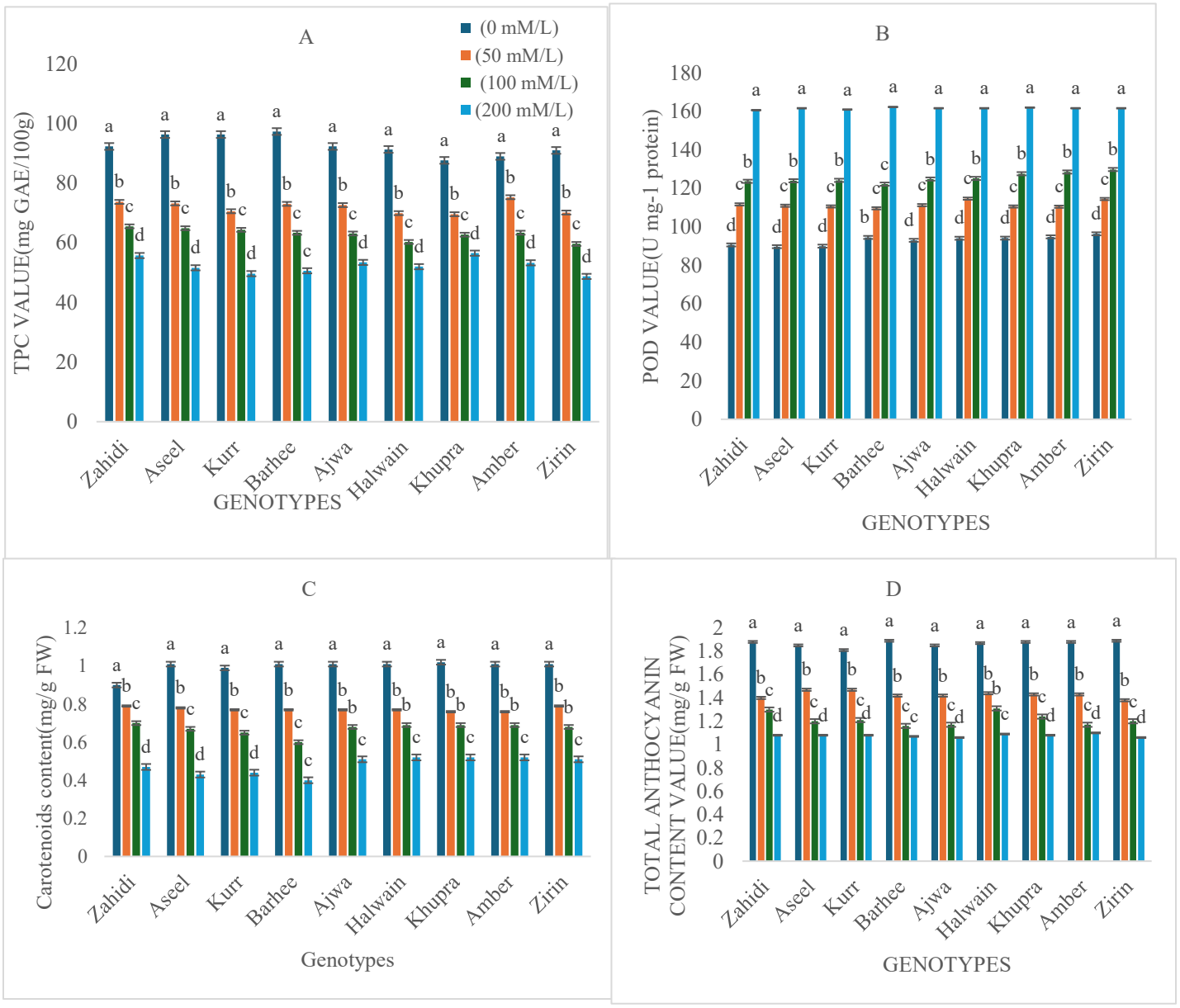


Fig. 3.3. Biochemical parameters (A=TPC Value, B= POD Value, C= Carotenoids content, D=Total anthocyanin content)

4 Discussion

The aim of this present study was to determine the salt stress tolerance in nine cultivars of date palm (*Phoenix dactylifera* L.) and to explore the possible physiological aspects behind the same. When tested with preliminary screening using different concentrations of NaCl salt, it was observed that there was a marked difference in the growth response elicited from different cultivars. The reduction in plant height due to salt stress was observed significantly in Zahidi, Aseel, Barhee, Halwain, Khupra, Amber and Zirin cultivars. Nonetheless, the plant height in Kurr and Ajwa cultivars was not affected significantly as salinity decreased, which is supported by an earlier study (Al-Absi, 2023). In an earlier study by Saad *et al*. (2021), some of the cultivars that were investigated include the Zahidi, Aseel, Kurr, Barhee, Ajwa, Halwain, Khupra, Amber, and Zirin; all genotypes showed a decreased number of leaves when under salt stress. Our findings are in line with Shamim *et al*. (2022), where the impact of salt stress on leaf length was significant for Zahidi Aseel Kurr Barhee

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Halwain Khupra and Zirin cultivars; a significant reduction in the size of the leaf was observed with increasing salt concentration. In another study done by Akensus *et al*. (2022), a decrease in the leaf size was observed for Ajwa and Amber cultivars. While a decrease in the leaf width was noted under varied salt concentrations in Zahidi, Barhee, and Zirin cultivars; however, Aseel, Kurr, Ajwa, Halwain, Khupra, and Amber cultivars exhibited moderate salt tolerance in terms of mean leaf width. Moreover, different levels of salt stress reduced the area of the leaf in Zahidi, Aseel, and Barhee, similar to the condition observed in Zabad cultivars of coconut (Al Kharusi *et al*., 2019).

Recent evidence shows that a reduction in the size of the leaves means a reduction in the rate of photosynthesis (Banavath *et al*., 2018; Rahman *et al*., 2025). The cultivars Zahidi, Aseel, Kurr, Barhee, Ajwa and Khupra also exhibited a reduced trend in their fresh weight under salt stress. On the other hand, Halwain, Amber, and Zirin cultivars showed no significant alteration in their fresh weight upon treatment with different salt concentrations. The statistical analysis of date palm cultivars revealed a significant effect of different types of date palm and salt treatments on the percentage of dry weight %. Interestingly, the plantlets of Zahidi, Aseel, Kurr, Barhee, Ajwa, Halwain, Khupra, Amber, and Zirin cultivars reduced their dry weight % most significantly as the saline levels went up. The findings align with the prior research done by Xu et al. (2022). Significant inhibition of root extension was seen in cultivars (Zahidi, Aseel, Barhee, Ajwa, Halwain, and Zirin) under high salt conditions. The results presented here are consistent with the research conducted by Karlova *et al*. (2021).

Photosynthetic activity significantly decreased against different treatments of salt stress (Abreu *et al*., 2013). Elevated salinity demonstrably impairs photosynthetic processes, which consequently hinders overall plant vitality and maturation. For instance, when plants encounter salt-induced stress, a critical disequilibrium develops between the photosynthetic electron transport chain and the Calvin cycle reactions. This maladjustment culminates in the over-reduction of components and an accumulation of excess energy within the thylakoid membranes (Cerqueira *et al*., 2019; Mir *et al*., 2024). Chlorophyll content was significantly reduced at different levels of salt stress in various date palm cultivars. By analyzing chlorophyll content, researchers gained a more profound understanding of how date palms adapt to and tolerate saline conditions, specifically concerning the operational characteristics of Photosystem II (PSII) and Photosystem I (PSI). When these plants were exposed to 150 mM NaCl, several observed fluorescence parameters increased. Notably, the rise in P700ox levels suggests enhanced activity within PSI, indicating an effective strategy for photoprotection against excess light energy. This is significant because P700ox plays an essential part in reducing the generation of reactive oxygen species (ROS) and preventing the over-reduction of PSI (Wada *et al*., 2019; Sabir *et al*., 2023). Consequently, it is regarded as a critical regulatory process for shielding PSI from photoinhibition (Farhat *et al.,* 2021). The rate of transpiration was significantly decreased against different salinity levels in various date palm cultivars. Recent findings are similar to earlier research of (Kaveh *et al*., 2011). High salt stress caused a notable increase in the antioxidant enzyme activity (SOD, POD, APX, and CAT) of the date palm with an increasing trend in salinity level. Our results are in line with the previous work of scientists Hashem *et al*. (2015) and Laouane *et al.* (2019). High concentration of salt caused a notable reduction in the overall phenolic content in the leaf tissues of cultivars (Zahidi, Aseel, Kurr, Barhee, Ajwa, Halwain, Khupra, Amber, and Zirin).

This current research is similar to the results published by Al Kharusi *et al.* (2019). Carotenoid content significantly reduced with increasing trends in salinity stress in cultivars (Zahidi, Aseel, Kurr, Barhee, Ajwa, Halwain, Khupra, Amber, and Zirin). Furthermore, high salinity remarkably reduces anthocyanin content in cultivars (Zahidi, Aseel, Kurr, Barhee, Ajwa, Halwain, Khupra, Amber, and Zirin). These present results are consistent with the previous work of (Wungrampha et al. 2018). The current results of this research have noteworthy consequences for the selection of a particular variety that exhibits tolerance to salinity and an ability to thrive in saline environments. The Aseel is a significant cultivar that can thrive in salty environments and is particularly valued in arid and semi-arid geographical areas affected by soil salinity. Implementing this approach can effectively improve date palm productivity and so mitigate the negative impact of salt stress on agricultural cultivation regions.

5 Conclusions

High salt stress caused a notable decrease in morphological attributes. Morphological parameters are significantly reduced with increasing concentration of salt. In general, increased salt concentrations led to a significant increase in the activity levels of (superoxide dismutase, catalase and peroxidase) in the leaf tissues. Conversely, the examination revealed a significant reduction in TPC, carotenoid contents and levels of anthocyanins decreased with increasing Nacl salt. The findings of this study have significant implications for the selection of a particular variety that exhibits tolerance to salinity

and an ability to thrive in saline environments. The Zahidi is a significant cultivar that can thrive in salty environments and is particularly valued in arid and semi-arid geographical areas affected by soil salinity. Implementing this approach can effectively improve date palm productivity and mitigate the adverse effects of salt on agricultural cultivation regions.

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