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SALT TOLERANCE IN MAIZE WITH MELATONIN PRIMING TO ACHIEVE SUSTAINABILITY IN YIELD IN SALT AFFECTED SOILS

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Abstract

Salt stress has a significant impact on agricultural output, affecting seed germination and seedling growth. The uncontrolled production of oxidative species (ROS) induces a range of biochemical, physiological, and metabolic changes, resulting in reduced crop yields. Under such circumstances, seed priming may be a feasible and practicable approach for achieving rapid, uniform emergence, vigorous seedlings, and higher crop yields. The present study was therefore executed to explore efficacious effects of various seed priming agents, such as hydro-priming (HP), $CaCl_2$ @ 2% (CaP), and melatonin @ 1000 M (MP) to cope with salt stress in salt-affected fields. A non-priming control treatment (WP) was also included in the trial to allow for a fair comparison of the treatments. Three promising maize genotypes, SB-9617 (V1), YH-1898 (V2), and NCEV-1530-9, were investigated with these priming treatments (V3). The study results depicted that all seed priming methods significantly attenuated the adverse effects of salt stress. However, seed priming with melatonin (MP), on the other hand, improved development and adaptability in maize seedlings under salt stress conditions. In our findings, melatonin priming (MP) significantly produced the higher total soluble sugar (34.6%), total phenolics contents (61.1%), proline contents (45.1 %), and total soluble protein contents (34.8 %), combined with enhanced antioxidant enzyme activity viz., SOD (32.5%), POD (18.2%) and CAT (17.1%) than un-primed (WP). The improved organic osmolytes coupled with activated enzymatic defense system in melatonin priming (MP) caused a significant reduction of malondialdehyde (MDA) levels (35.1%), H_2O_2 concentration (31.3%) and electrolyte leakage (16.9%) via improving the ROS scavenging activity (15.6%), membrane stability (22.7%) and relative water contents (29.3%) relative to un-primed treatment (WP). The melatonin primed plants also exhibited the highest crop growth and leaf area indices without causing substantial damage to the chlorophyll contents, resulting in increased radiation interception (PAR) and its usage efficiency with improved yield. Interestingly, SB- 9617 (V2) was proved the outperforming maize genotype in maintaining better crop growth and yield with improved physiological and biochemical characters under salt-affected field conditions. The current findings may serve as a chunk of scientific information for the researchers to disclose further the unexplored aspects of salt tolerance mechanism in maize crop to achieve sustainability in crop yield in salt-affected soils.

Key words: Physiological; Biochemical; Salt tolerance; Growth; yield; Antioxidants; RUE.

Introduction

According to estimates, salinity has harmed roughly 20% of global cultural land, and the area is rapidly rising due to irregular climate changes and anthropogenic activities (Arora & Bhatla, 2017). Salinity impacts 6.68 million hectares of land in Pakistan, with 40000 hectares degraded each year (Zafar *et al.*, 2019; Hassan *et al.*, 2021). In the present scenario, salinity has become a severe danger to agricultural productivity, causing 50 % reduction in the agricultural output of the world's arid and semi-arid regions (Rehman *et al.*, 2019; Kamran *et al.*, 2020; Saleem *et al.*, 2020 a, b). However, crop's sensitivity to salt stress may differ depending on its developmental stage. Seed germination and early seedling growth stages, on the other hand, are proven to be highly vulnerable to salt damage (Saleem *et al.*, 2019; Afzal *et al.*, 2021; Nawaz *et al.*, 2021). Salt stress limits seed germination and seedling growth by reducing the abundant supply of water and nutrients available to plants through osmotic and ionic effects (Mohamed *et al.*, 2020; Mumtaz *et al.*, 2021). Secondly, salinity-induced oxidative stress damages essential cellular structures, causing physiological, biochemical, and metabolic processes to be disrupted (Habib & Shahbaz, 2013; Ali *et al.*, 2020; Kong *et al.*, 2021).

Maize (*Zea mays* L.) is a sensitive crop to salinity ranks third in production after wheat and rice in Pakistan. It contributes 3.4% of agricultural value-added and 0.6% of GDP (Nawaz *et al.*, 2017). It is a versatile crop used for food, feed, and fodder. The maize was cultivated on 1,418 thousand hectares in 2020-21 with a total production of 8.47 million tons compared to the previous year's area (1404 thousand hectares) and production (7.40

million tons) (Shaukat *et al.*, 2020). The projected increase in the production of the country was majorly attributed to increased land under cultivation of the crop, availability of the improved variety seed, and the reasonable market price of the produce (Saleem *et al.*, 2020). Salt stress has adverse effects on seed germination and seedling growth by disrupting necessary plant physiological, biochemical, and metabolic processes (Parida and Das, 2005; Zafar *et al.*, 2019; Naz and Perveen, 2021). Furthermore, crop production success in salt-affected soils is determined by the continuation of these processes throughout the plant life cycle (Ijaz *et al.*, 2021; Zafar *et al.*, 2021).

Seed priming is a shotgun strategy that can effectively down regulate the yield limiting germination and growth rates under salt stress (Anjum *et al.*, 2020). The economic feasibility and ease in the use of this approach in agriculture have opened up many horizons for scientists and farmers to achieve salt tolerance and sustainability in crop production (Khan *et al.*, 2019). The most efficient approaches for alleviating the adverse effects of salt stress on germination and early seedling stage are hydro-priming and osmo-priming (Parihar *et al.*, 2015). Hyro-priming can help to reduce the severity of salt stress to some extent (Khan *et al.*, 2019). Similarly, the scientific literature has demonstrated the efficiency of $CaCl_2$ as an osmo-priming agent in reducing the negative effects of salts (Chen *et al.*, 2021). The physiological and biochemical grounds of $CaCl_2$ priming in the acquisition of salt tolerance, on the other hand, remain unknown and need to be investigated further. Recently melatonin has got much attention as stress reliever plant hormone because of its higher ability to scavenge free radicals (ROS), which otherwise cause significant damage to cellular structures and key cellular functions (Mohamed *et al.*, 2020; Sharma *et al.*, 2020). Melatonin has the great potential to enhance photosynthetic efficiency by stimulating the ROS scavenging mechanism in tandem with signallingphyto-hormone pathways (Khan *et al.*, 2019; Zafar *et al.*, 2019). In a very recent study Bai *et al.*, (Bai *et al.*, 2020) also investigated that melatonin has a substantial effect on cotton seed germination in drought-stressed conditions. Yu *et al.*, (Yu *et al.*, 2021) discovered the increased plant height, fresh weight, number of branches, improved crop growth and higher chlorophyll contents in salt-affected soils.

Furthermore, Shi *et al.*, (Shi *et al.*, 2015) also reported that stressed plants treated with melatonin showed decreased ROS burst, electrolyte leakage, and cell damage than untreated plants. Therefore, using melatonin to boost seedling resistance to salt stress has been demonstrated to be very promising for maize production on salinized land. It's also worth mentioning that most of the research has been carried out in greenhouses. Hence field testing is required to evaluate whether the salt tolerance induced by melatonin priming can be continued and even last to the lateral development. Therefore, the present study was executed to explore the beneficial effects of melatonin priming on maize seed germination, seedling growth, antioxidant enzymatic system, ROS scavenging activity, radiation interception and its usage efficiency to achieve sustainability in yield.

Materials and Methods

Experimental site detail: The experiment was conducted at Soil Salinity Research Institute's research farm in Pindi Bhattian (31.8966° N, 73.2824° E), Hafizabad, Pakistan, for two years, i.e., 2018 and 2019. A salt-affected field with pH_s of 8.2, EC_e-7.3 dSm^{-1}, and SAR-18.5 $(mmol\ L^{-1})^{1/2}$ was chosen after doing the needful laboratory analyses. The chosen field was thoroughly prepared, and ridges were constructed at 75 cm apart using a tractor-mounted ridge maker.

Plant material: The researchers looked at three maize genotypes: SB-9617, YH-1898, and NCEV-1530-9. Two genotypes, SB-9617 and YH-1898, were obtained from the Maize and Millets Research Institute Sahiwal, while the seed of one genotype i.e., NCEV-1530-9 was obtained from the National Agricultural Research Centre Islamabad.

Seed priming protocol: The healthy and robust seed material of the three maize genotypes was surface-sterilized for three minutes with 1 % sodium hypochlorite (v/v), rinsed with distilled water, and then air-dried at room temperature (25°C). After disinfecting, the seed material of each genotype were immersed for 24 hours in the aerated solutions of $CaCl_2$ (2%), melatonin (1000 µM), and distilled water (hydro-priming). After priming, seeds were air-dried in the shade for 12 hours before being sundried to restore their natural weight.

Layout and planting technique: The research plan was implemented in a split-plot design with three replications. Maize genotypes were planted in the main plot, while seed priming treatments in sub-plots. The seeds were manually planted on the ridges at a distance of 20 cm in each plot as per treatment plan. After the completion of seed germination, the seedlings were then thinned to ensure one healthy plant per hill. All the other agronomic measures were adopted uniformly.

Sampling procedure for growth and leaf area indices measurements: The germination was recorded on a daily basis up to the final germination count. After that, the plants in each plot were separated into three halves and out of which 2/3 part was utilized to record statistics on growth, leaf area indices and radiation interception. The remaining 1/3 part was kept for recording yield. Five plants from each treatment were collected after every fifteen-day interval starting from 30 days after planting. Initially, the plant samples included leaf & stem parts. Then plant samples were taken from all parts of the plant, including leaves, stems, flowers, and cobs, chaffed and thoroughly mixed. The chaffed composite plant samples (30g) were placed in an oven heated at 70°C for 72 hours. The dried mass of each plant sample was recorded by using an electrical weighing scale (AND-3000; Japan). Using dry masses of the plant samples for each sampling interval, CGR was computed using Hunt's (1978) formula: $CGR = (DW_2 - DW_1) / (T_2 - T_1)$, where DW_1 and DW_2 are the dry weights (m^{-2}) at T_1 and T_2, respectively. In a similar way, leaf area was measured by multiplying the length of leaf (l) with leaf width (w) and a correction factor of 0.75 as proposed by Watson (1952). Leaf area indices (LAI) were calculated using the formula LAI = LA / La. Where LA & La are the leaf area and land area. The lengths and widths of the leaves were measured with the help of a wooden graduated meter rod.

Computation of radiation interception and its usage efficiency: Fraction of radiation interception was computed by using an equation $\mathrm{Fi} = 1 - \exp(-K \times LAI)$ as proposed by Monteith and Elston (Monteith & Elston, 1983). Where, K is the extinction coefficient for total solar radiation. The value of K for maize is 0.70 (Lindquist *et al.*, 2005). The intercepted radiation (*Sa*) was then determined by multiplying the incident radiation (*Si*) and the fraction of intercepted radiation (Fi). Incident radiations (Si) were assumed 50% of the total photosynthetically active radiations (*IPAR*). After computing the value of *Si*, the amount of radiation intercepted by a crop canopy (*Sa*) was calculated by multiplying the *Fi* values $\mathrm{Sa} = Fi \times \mathrm{Si}$. Radiation use efficiencies for total dry matter (RUE_{TDM}) and grain yield (RUE_{GY}) were computed by the equations viz., $RUETDM = \frac{TDM}{\Sigma Sa}$ and $RUEGY = \frac{GY}{\Sigma Sa}$ (Monteith, 1977). The values of leaf area duration (*LAD*) were computed by using the formula as$\mathrm{LAD} = (LAI1 + \mathrm{LAI2}) \times \frac{\mathrm{T2-T1}}{2}$. Grain yield data were recorded at maturity of the crop.

Determinations of Physiological Attributes: The crop was taken 45 days after sowing in order to perform the following tests:

Electrolyte leakage (EL): The electrolyte leakage was computed using the formula as $EL = \frac{\mathrm{EC1}}{\mathrm{EC2}}$ (Lutts *et al.*, 1995). Where, EC_1 and EC_2 are the electrical conductivities of the initial and after heating leaf sample.

Membrane stability index (MSI): The membrane stability index (MSI) was obtained using the formula as $MSI = \frac{\mathrm{EC1}}{\mathrm{EC2}} \times 100$ (Sairam, 1994).

Relative water contents (RWC): The relative water contents (RWC) were determined using the formula as $RWC = \frac{\mathrm{FW-DW}}{\mathrm{TW-DW}} \times 100$ (Hayat *et al.*, 2007), where, FW, DW and TW are the fresh, dry and turgid weights repectively.

Quantification of biochemical attributes

MDA content: Malondialdehyde contents were tested using a spectrophotometer (PD-303S-APEL Co. Japan) (Cakmak & Horst, 1991).

H_2O_2 concentrations: Hydrogen peroxide (H_2O_2) concentrations were determined using a spectrophotometer (PD-303S-APEL Co. Japan) (Mishra & Agrawal, 1997).

DPPH-Free radical scavenging activity (percentage): The methanolic leaf extract's DPPH-free radical scavenging activity (percentage) was measured using DPPH (2, 2-diphenyl-1-picrylhydrazyl) (Choi *et al.*, 2002).

Free proline contents: Proline contents were quantified spectrophotometrically (PD-303S-APEL Co. Japan) in the young leaves (Bates *et al.*, 1973).

Total soluble sugar (TSS): The total soluble sugar concentration was determined using the anthrone method (Dubois *et al.*, 1956) using a spectrophotometer (PD-303S-APEL Co. Japan).

Total chlorophyll contents: Total chlorophyll contents in young leaves were extracted with 80% acetone and quantified using Arnon's method (Arnon, 1949).

Total phenolic contents: Total phenolics in maize extracts were measured using a spectrophotometer (PD-303S-APEL Co. Japan) (Nadernejad *et al.*, 2012).

Ratio of K^+ to Na^+: Perchloric acid and HNO_3 concentrated H_2SO_4 were used to finish the digestion of the weighted leaf samples (Gorham *et al.,* 1984). The digested samples were then diluted to provide the appropriate quantities for determining K^+ and Na^+ concentrations in the leaves tissues using a flame photometer (Digiflame, DV-710).

Estimation of antioxidant enzymes (SOD, CAT & POD) activity: In 5 mL of 50 mM cooled phosphate buffer (pH 7.8) placed in an ice bath; a fresh leaf sample of 0.5 g was ground using a tissue grinder. At 4°C, the homogenate was centrifuged for 20 minutes at a speed of 15000 g. Antioxidant enzymes were measured using the supernatant. The technique proposed by Chen and Pan (Chen & Pan, 1996) for determining SOD activity was used. The approach used by Sakharov and Ardila (Sakharov & Ardila, 1999) and Aebi(Aebi, 1984) to record POD and CAT activity.

Statistical analysis

The data were analysed statistically using the SPSS version 16 computer programme. The differences between the treatment means were examined using the least significant difference (LSD) test at a 5% probability level. A computer software R studio version 1.2.5033 was used to carry out PCA.

Results

Germination percentage: Different seed priming techniques and maize genotypes substantially impact seed germination (Fig. 1). The seed primed with melatonin (MP) germination rate was higher than the seed primed with $CaCl_2$ (CaP). The seed with the lowest germination % was the seed that had not been primed (WP). SB-9617 (V2) outperformed other maize genotypes in terms of germination percentage, such as YH-1898 (V3) and NCEV-1530-9 (V1). The interaction between maize genotypes and seed priming treatments for seed germination was statistically non-significant ($p>0.05$).

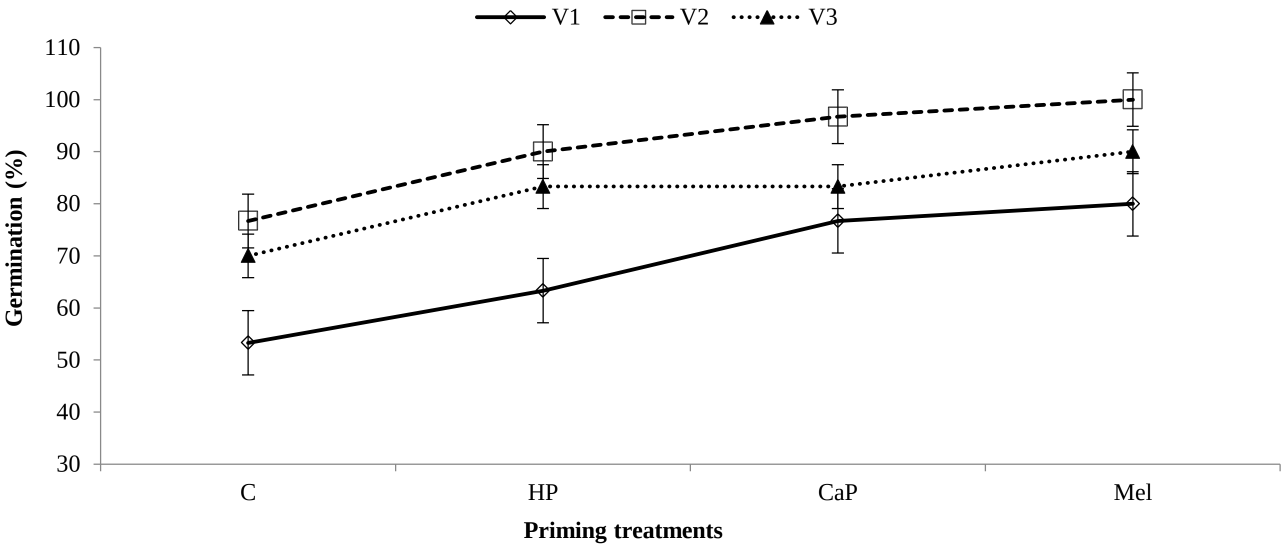

Fig. 1. Germination percentage as influenced by different maize genotypes and seed priming treatments.

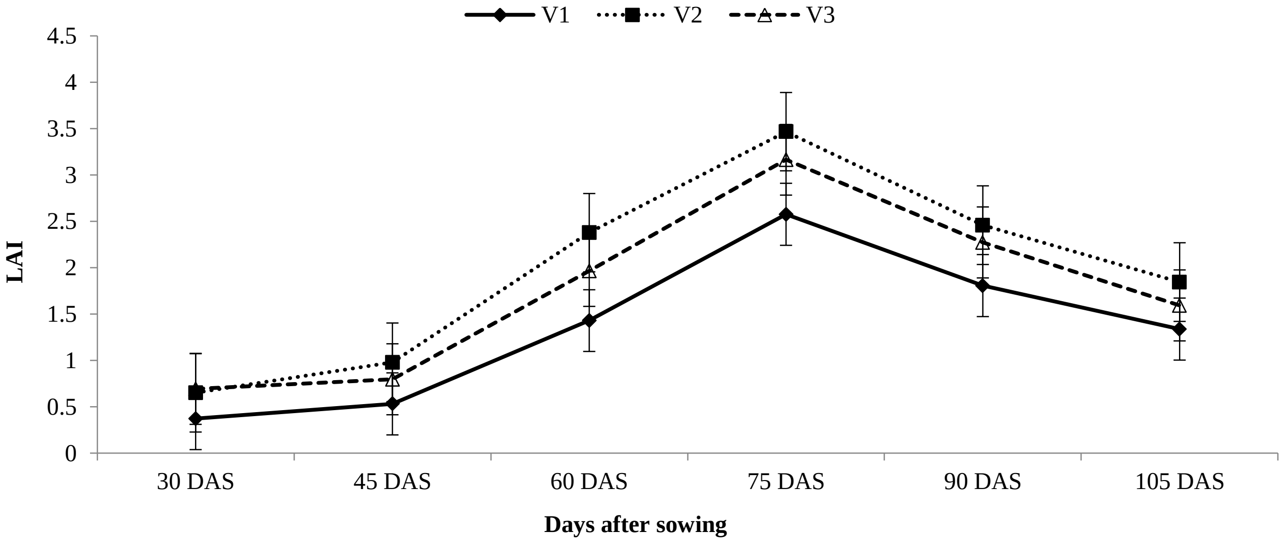

Fig. 2. Temporal changes in the leaf area indices as influenced by different maize genotypes.

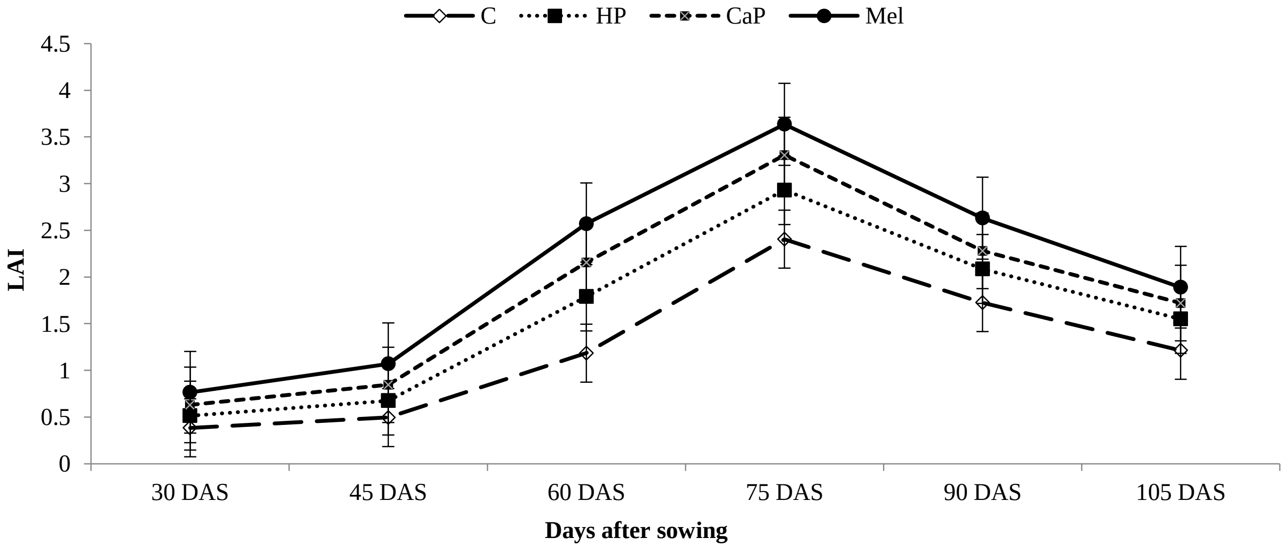

Fig. 3. Temporal changes in the leaf area indices as influenced by different maize and seed priming treatment.

Lipid per-oxidation and radical scavenging activity: Table 1 shows the findings on oxidative stress markers such as malondialdehyde (MDA) and hydrogen peroxide (H_2O_2). The results of this study revealed that plants grown from melatonin primed seed (MP) produced the least amount of H_2O_2 and MDA at 45 DAS. In contrast, plants grown from $CaCl_2$ priming (CaP) and hydro-priming (HP) produced the most DPPH-based free radical scavenging activity (Fig. 11). However, the plants grown from seed planted without priming (WP), which had the lowest free radical scavenging activity, had the highest amounts of H_2O_2 and MDA. As demonstrated in Fig. 11 and Table 1, the genotype SB-9617 (V2) had the highest DPPH-free radical scavenging activity while producing the least H_2O_2 and MDA. On the above-mentioned parameters, the combined effect of maize genotypes and priming of seed treatments (G x P) was statistically non-significant (p>0.05).

Physiological attributes: Table 2 shows the impact of maize genotypes on physiological variables like electrolyte leakage (EL), membrane stability index (MSI), and relative water content (RWC) as a result of different seed priming procedures. The current study's findings revealed that melatonin primed (MP) plants showed the greatest improvement in membrane stability index and relative water content, which was statistically equivalent to $CaCl_2$ priming (CaP). However, without priming (WP) treatment, the lowest membrane stability index (MSI) and relative water contents (RWC) were found. (Table 2) also showed the performance of maize genotypes in terms of physiological traits. SB-9617 achieved the highest membrane stability index (MSI) and relative water content (RWC) with the lowest electrolyte leakage percentage (V2). The interaction between maize genotypes (G) and seed priming treatments (P) was statistically insignificant (p>0.05).

Table 1. Effect of different seed priming treatments on the concentration of different biochemical parameters (Average of two years).

Treatments	H_2O_2	MDA	TPC	TSS	TSP	TCC	PC
	µmol g^{-1} FW	nmol g^{-1} FW	mg GAE g^{-1} FW	mg g^{-1} FW			µmol g^{-1} FW
V1	9.70 A	12.0 A	5.70 C	12.4 B	5.22 B	1.09C	6.76 B
V2	5.35 B	6.06 B	16.0 A	27.2 A	10.2 A	1.86A	14.8 A
V3	6.05 B	6.21 B	12.7 B	25.7 A	9.90 A	1.72B	14.0 A
LSD	**1.4797**	**1.3566**	**1.8098**	**5.1297**	**2.3511**	**0.0782**	**2.5116**
WP	8.40 A	9.95A	8.44D	18.5 D	6.96 C	1.25D	9.37 D
HP	7.24 B	8.34B	11.4 C	20.3 C	8.26 B	1.43C	11.7 C
CaP	6.74 B	7.64 C	12.5 B	23.3 B	9.10 A	1.72B	12.8 B
MP	5.77 C	6.46 D	13.6 A	24.9 A	9.38 A	1.82A	13.6 A
LSD	**0.7666**	**0.5725**	**1.0217**	**1.0039**	**0.3575**	**0.0590**	**0.8328**
			Significance level				
G	*	**	**	*	*	**	*
P	**	**	**	**	**	**	**
G x P	NS	NS	NS	NS	NS	NS	NS

1. H_2O_2 = Hydrogen peroxide, MDA = Malondialdehyde, TPC = Total phenolic contents, TSS = Total soluble sugars, TSP = Total soluble protein, TCC = Total chlorophyll contents, PC = Proline contents, V1 = NCEV-1530-9, V2 = SB-9617 and V3=YH-1898.
2. Mean values sharing the same letter (s) within the same column are non-significantly different at 5% probability level
3. NS = Non-significant,* = Significant & ** = Highly significant, G = Genotypes and P = Priming treatments.

Table 2. Physiological attributes, antioxidants defense system and grain yield as affected by different seed priming treatments and maize genotypes (Average of two years).

Treatments	Physiological attributes			Antioxidant enzyme defense system			Grain yield
	MSI	EL	RWC	SOD	POD	CAT	
	(%)			EU min^{-1} g^{-1} FW			(t ha^{-1})
V1	29.5 C	50.3 A	54.4 C	25.0 C	29.2 B	49.6 C	1.32 C
V2	46.1 A	29.7 B	68.9 A	44.3 A	49.7 A	81.1 A	2.56 A
V3	41.4 B	30.4 B	64.3 B	39.4 B	48.7A	72.1 B	1.93 B
LSD	4.7434	2.2918	3.8790	3.0248	4.4075	6.0389	0.0714
WP	34.8 C	40.7 A	54.2 C	31.4 C	38.9 C	62.1 C	1.62 D
HP	38.1 B	37.4 B	59.1 B	33.1 C	41.6 B	66.8 B	1.78 C
CaP	40.4 A	35.4 BC	66.8 A	38.9 B	43.5 B	68.5 B	1.97 B
MP	42.7 A	33.8 C	70.1 A	41.6 A	46.0 A	72.7 A	2.37A
LSD	2.3252	2.2746	4.9444	2.3668	1.9614	3.1653	0.1282
G	**	**	*	**	**	**	**
P	**	**	**	**	**	**	**
G x P	NS	NS	NS	NS	NS	NS	NS

1. WP-without priming, HP-hydro-priming, CaP-$CaCl_2$ priming, MP-melatonin priming, V1-NCEV-1530-9, V2-SB-9617 and V3-YH-1898
2. Mean values sharing the same letter (s) within the same column are non-significantly different at 5% probability level
3. NS = Non-significant,* = Significant & ** = Highly significant, G = Genotypes and P = Riming treatments

Biochemical attributes: The data in Table 1 demonstrated that different seed priming treatments had a substantial impact on the maintenance of several biochemical properties in three maize genotypes under salt-affected field conditions. SB-9617 (V2) differed considerably from YH-1898 (V3) in terms of total phenolic content accumulation but was statistically equal to YH-1898 (V3) in terms of total soluble sugars (TSS) and proline. Melatonin priming (MP) significantly differed from $CaCl_2$ priming (CaP), hydro-priming (HP), and no priming (WP) treatments in accumulating total phenolic contents (TPC), total soluble sugars (TSS), and proline contents, whereas $CaCl_2$ priming (CaP) was statistically non-significant for total soluble proteins (TSP). Without priming (WP) treatment, the lowest build-up of total phenolic contents (TPC), total soluble sugars (TSS), and proline contents (PC) was observed.

Chlorophyll and antioxidant defense system: Table 1 shows the overall chlorophyll concentration, while Table 2 shows the antioxidant enzymes (SOD, POD, and CAT). Melatonin priming (MP) produced the highest total chlorophyll concentrations (TCC) and antioxidant enzyme activity in SB-9617 (V2). The lowest total chlorophyll contents (TCC) and antioxidant enzyme activity (SOD, POD, & CAT) were recorded in the NCEV-1530-9 (V1) treatment without seed priming (WP).

The ratio of potassium to sodium (K^{+}/Na^{+}): Fig. 14 depicts the interactive effects of different seed priming treatments on the maintenance of K^{+}/Na^{+} ratios in three maize genotypes at 45 DAS under salt-affected field conditions. With melatonin priming (MP), the maize hybrid SB-9617 (V2) fared better in sustaining the highest K^{+}/Na^{+} ratio. Similarly, NCEV-1530-9 (V1) produced the lowest K^{+}/Na^{+} ratio without priming (WP).

Growth, leaf area index and leaf area duration: The effects of maize genotypes SB-9617 (V2), YH-1898 (V3), and NCEV-1530-9 (V1), as well as different seed priming treatments such as without priming (WP), hydro-priming (HP), and $CaCl_2$ priming (CaP), and melatonin priming (MP), on mean crop growth rate (CGR), leaf area index (LAI), and cumulative leaf area duration (LAD), are shown in Figures 1 (E & F). The highest mean CGR (Fig. 6), LAI (Figs. 2 & 3), and cumulative LAD (Fig. 7) were achieved in SB-9617 (V2) with melatonin priming (MP) administration in the current study. The treatment without priming (WP) had the lowest leaf area index (LAI) (Fig. 3).

Total dry matter and grain yield: Average statistics on total dry matter accumulation (Figs. 4 and 5) and grain yield (Table 2) as influenced by various seed priming treatments and maize genotypes were also shown to be significant (P 0.05). In comparison to other maize genotypes, SB-9617 (V2) had the largest TDM accumulation (Fig. 4). The optimum therapy for achieving the maximum TDM under salt-affected field conditions was found to be melatonin priming (MP), as shown in Figure 5. Table 2 shows that different seed priming treatments substantially impacted grain output in three maize genotypes. SB-9617 (V2) had the highest grain yield, with YH-1898 coming in second (V3). Similarly, seed priming with melatonin (MP) increased grain yield significantly in salt-affected soils (Table 2).

Radiation interception and its usage efficiency for TDM and grain yield: It was statistically significant (P 0.05) that maize genotypes and seed priming treatments impacted the PAR (Fig. 10). SB-9617 (V2) outperformed YH-1898 (V3) and NCEV-1530-9 (V1) in terms of intercepting higher photo-synthetically active radiation (PAR) and its usage efficiency for TDM (RUE_{TDM}) (Fig. 12) and grain yield (RUE_{GY}) (Fig. 13). Melatonin priming (MP) also produced the highest photo-synthetically active radiations (PAR) and radiation usage efficiencies (RUE_{TDM}& RUE_{GY}) when compared to other priming treatments. The interaction of maize genotypes and seed priming (G x P) revealed that seed priming with melatonin (MP) in SB-9617 (V2) significantly improved the interception of photo-synthetically active radiations (PAR, as shown in Figure 10) and radiation use efficiencies (RUE_{TDM}& RUE_{GY}) in salt-affected soils, as shown in Figs. 12 & 13 respectively. The regression analysis also revealed a strong relationship between TDM and grain yield and PAR, as evidenced by R^2 values of 0.794 and 0.810, respectively. Figs. 8 and 9 shows a positive and linear connection between PAR and TDM (r=0.892) and PAR and grain yield (r=0.900), respectively.

Correlation and PCA between several parameters: The presence of significant positive and negative correlations among various parameters was revealed by data analysis for Pearson correlation coefficient (Table 3). POD and DPPH, POD and PC, POD and TCC, TP and PC, TP and CAT, POD and CAT, POD and CAT, and PC and TSS, respectively, had the strongest positive correlations of r=0.964, 0.957, 0.956, 0.954, 0.952, 0.948, 0.947, and 0.947. EL and MDA, TSS and MDA, EL and TSS, H_2O_2 and TSP, and PC and MDA all had negative relationships (r = -0.942, -0.938, -0.934, -0.925, and -0.922, respectively). According to the main component analysis, the primary two axis components F1 and F2 contributed 97.9% of the overall variations (Fig. 16). F1, on the other hand, contributed the greatest (95.5%) when compared to F2 (2.4 percent).

Discussion

Salt stress significantly impacts seed germination and disrupts various physiological, biochemical, and metabolic processes at the lateral stages of plant growth, lessening crop yield (Avestan *et al.*, 2019; Kaya *et al.*, 2020; Zafar *et al.*, 2021). As a result, the continuation of these processes, as well as the accumulation of their resultant bio-products in seed and plant tissues, may provide a guarantee to sustain crop yield in salt-affected soils (Hussein & Abou-Baker, 2013; Ahanger *et al.*, 2020; Ali *et al.*, 2020). The current study was designed to emphasise the positivity of salt tolerance induced by different priming agents in general and melatonin priming in particular at germination and its persistence at the early seedling stage to carry out various physiological and biochemical processes. Maize is a moderately salt-sensitive crop (Kaya *et al.*, 2013; Kaya *et al.*, 2015), and salinity reduces plant growth and crop yield by delaying seed germination and disrupting various physiological and biochemical processes (Emam *et al.*, 2013; Kamran *et al.*, 2019; Yadav *et al.*, 2020). As a result, acquiring salt tolerance at the seed germination stage can provide a vigorous and healthy crop stand, which is required to sustain maize crop yield in salt-affected soils. In the current study, disturbed and scanty germination and inhibited plant growth were observed in salt-affected soil. SB-9617 may have better germination than YH-1898 and NCEV-1530-9 because it has a higher potential for reducing toxic free radical concentrations. However, improved germination associated with melatonin may cause increased reactive oxygen species (ROS) scavenging activity in salt-stressed conditions (Mohamed *et al.*, 2020; Sharma *et al.*, 2020). Similar studies were published by Castanares and Bouzo (Castañares & Bouzo, 2019) and Khan *et al.*, (Khan *et al.*, 2019), in which the efficacy of melatonin as an effective ROS scavenger in the protection of vital bio-molecules from oxidative damage was discussed. The findings of Chen *et al.*, (Chen *et al.*, 2020) showed melatonin had a high potential to promote seed germination in cotton under salt stress backed up the conclusions of this investigation. Similarly, excessive generation of reactive oxygen species (ROS) causes severe damage to cellular structures, disrupting essential physiological and biochemical processes necessary for healthy growth in salt-stressed conditions (Saleem *et al.*, 2020; Saleem *et al.*, 2021; Ghafar *et al.*, 2021; Hameed *et al.*, 2021; Perveen *et al.*, 2021).

The largest cellular generation of reactive oxygen species (ROS), mainly hydrogen peroxide (H_2O_2), promoted lipid peroxidation of cellular membranes (Kazemi *et al.*, 2019; Saleem *et al.*, 2020b), resulting in the synthesis of higher MDA in plant tissues in the current study. Under salt stress, melatonin priming (MP) dramatically reduced hydrogen peroxide (H_2O_2) and MDA production. Similar reductions in the synthesis of H_2O_2 and MDA with exogeneous application of melatonin in maize during salt stress were noticed by Chen *et al.*, (2021). The current results were also in agreement with the findings as reported by Zafar *et al.*, (2019), who observed significant reductions in H_2O_2 and MDA bio-synthetics in wheat plants under saline stress. SB-9617 with melatonin priming (MP) showed increased DPPH-based free radical scavenging activity, confirming the protective impact of melatonin against oxidative damage. SB-9617 had the lowest quantities of H_2O_2 and MDA, and the highest DPPH radical scavenging activity, indicating its ability to prevent potentially lethal levels of ROS from accumulating in biological tissues.

Increased salt accumulations induce irreversible damage to cellular membranes, resulting in increased electrolyte leakage at times of extreme stress (Chantre Nongpiur *et al.*, 2016). SB-9617 (V2) with melatonin priming (MP) had the best membrane stability and electrolyte leakage in this investigation, which could be due to its increased free radical scavenging activity under salt stress. When cotton and tomato crops were subjected to salt stress, Yin *et al.*, (2019) and Chen *et al.*, (2021) found that melatonin priming reduced electrolyte leakage, making cellular membranes more robust. The current study's findings were consistent with those of Cen *et al.*, (2020), who demonstrated that melatonin priming improved salt tolerance in alfalfa plants by reducing electrolyte leakage under salt stress conditions.

In order to survive under salt-stressed conditions, the plant body must retain a sufficient amount of water to maintain numerous physiological and biochemical activities (Yaseen *et al.*, 2020; Ghafar *et al.*, 2021; Yasmin *et al.*, 2021). The accumulation of dangerous concentrations of salts in the plant rhizosphere has significant impacts on the supply of water from soil to plant and within the plant, according to AbdElgawad *et al.*, (2016) and Yin *et al.*, (2019). Seed priming with melatonin (MP) enhanced water balance significantly in all maize genotypes. However, the effects were more evident in SB-9617, possibly because salt stress produced less damage to the cellular membranes than the other maize genotypes evaluated. Cen *et al.*, (2020) found that maintaining the required amount of water in melatonin-treated salt challenged plants is directly related to cellular membrane healing.

Table 3. Pearson correlation coefficients for different parameters as influenced by different priming treatments and maize genotypes in salt affected soil.

Parameters	CAT	TCC	DPPH	EL	GY	H_2O_2	MDA	MSI	PAR	POD	TP	PC	RWC	SOD	TSS	TDM
TCC	0.881															
DPPH	0.932	0.926														
EL	-0.917	-0.916	-0.968													
GY	0.887	0.903	0.864	-0.814												
H_2O_2	-0.854	-0.904	-0.901	-0.894	-0.847											
MDA	-0.889	-0.910	-0.920	-0.942	-0.826	0.893										
MSI	0.915	0.922	0.929	-0.893	0.907	-0.906	-0.874									
PAR	0.866	0.930	0.869	-0.860	0.900	-0.889	-0.889	0.882								
POD	0.947	0.956	0.964	-0.967	0.829	-0.874	-0.902	0.885	0.844							
TP	0.948	0.937	0.938	-0.897	0.923	-0.860	-0.893	0.906	0.913	0.920						
PC	0.940	0.924	0.938	-0.929	0.863	-0.876	-0.922	0.894	0.884	0.957	0.952					
RWC	0.699	0.857	0.748	-0.712	0.822	-0.794	-0.754	0.786	0.873	0.717	0.801	0.891				
SOD	0.906	0.954	0.921	-0.918	0.896	-0.879	-0.923	0.910	0.915	0.896	0.915	0.910	0.814			
TSS	0.894	0.931	0.943	-0.934	0.859	-0.904	-0.938	0.888	0.883	0.938	0.939	0.947	0.784	0.913		
TDM	0.868	0.917	0.875	-0.829	0.946	-0.859	-0.813	0.923	0.921	0.825	0.934	0.855	0.848	0.886	0.867	
TSP	0.843	0.919	0.935	-0.916	0.823	-0.925	-0.910	0.916	0.857	0.886	0.877	0.886	0.799	0.894	0.911	0.866

TCC-total chlorophyll contents, DPPH-Diphenylpicrylhydrazyl, EL-electrolyte leakage, GY-grain yield, H_2O_2-hydrogen peroxide, MDA-malondialdehyde, MSI-membrane stability index, PAR-photo-synthetically active radiations, POD-Peroxidase, TP-total phenolics, PC-proline contents, RWC-relative water contents, SOD-superoxide dismutase, TSS-total soluble sugars, TDM-total dry matter, TSP-total soluble proteins

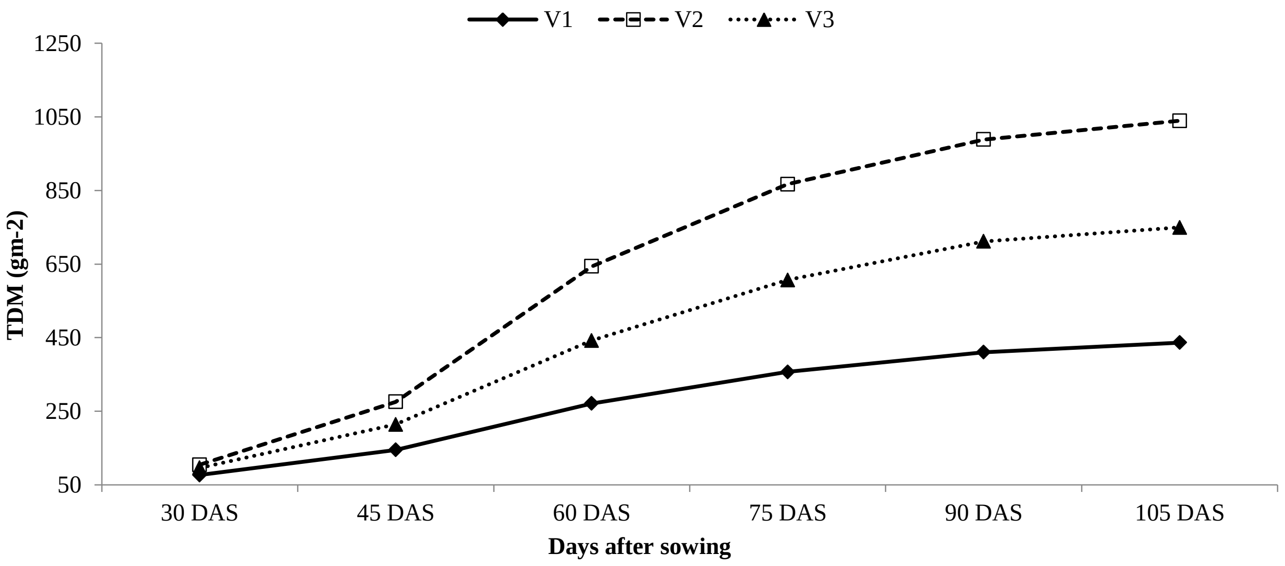

Fig. 4. Temporal changes in the TDM accumulation as influenced by different maize.

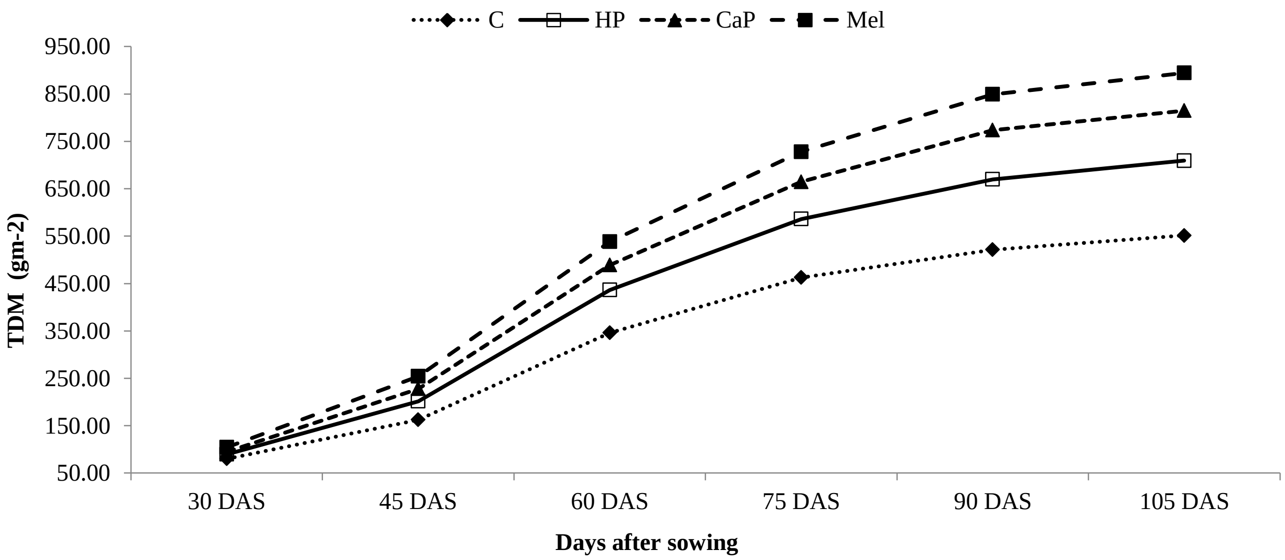

Fig. 5. Temporal changes in the TDM accumulation as influenced by different seed priming treatmen.

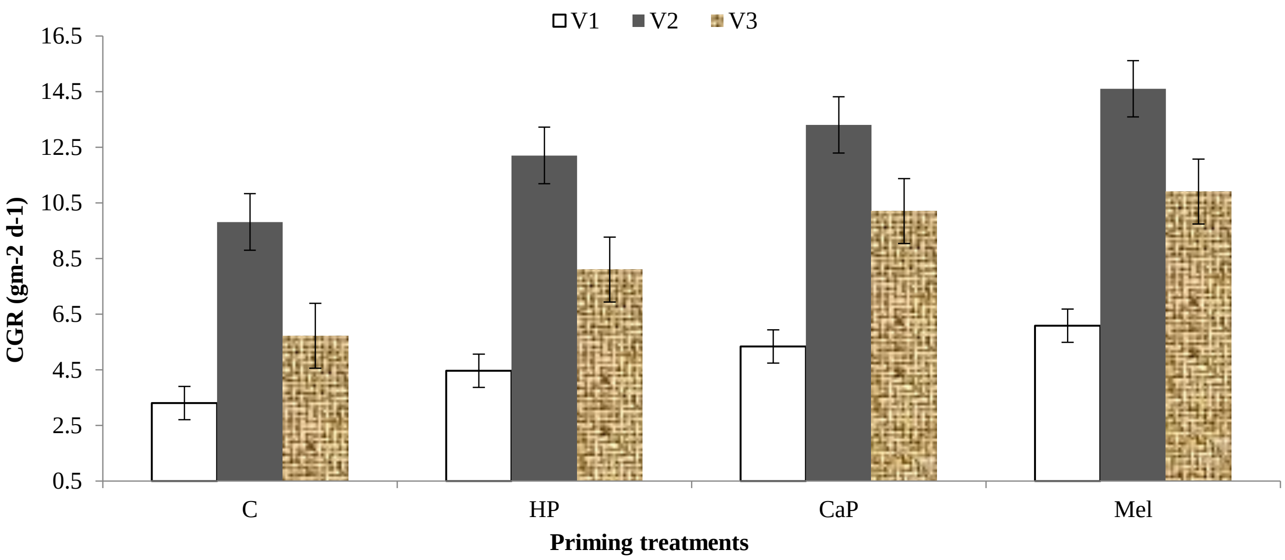

Fig. 6. Crop growth rate as influenced by different maize genotypes and seed priming treatments.

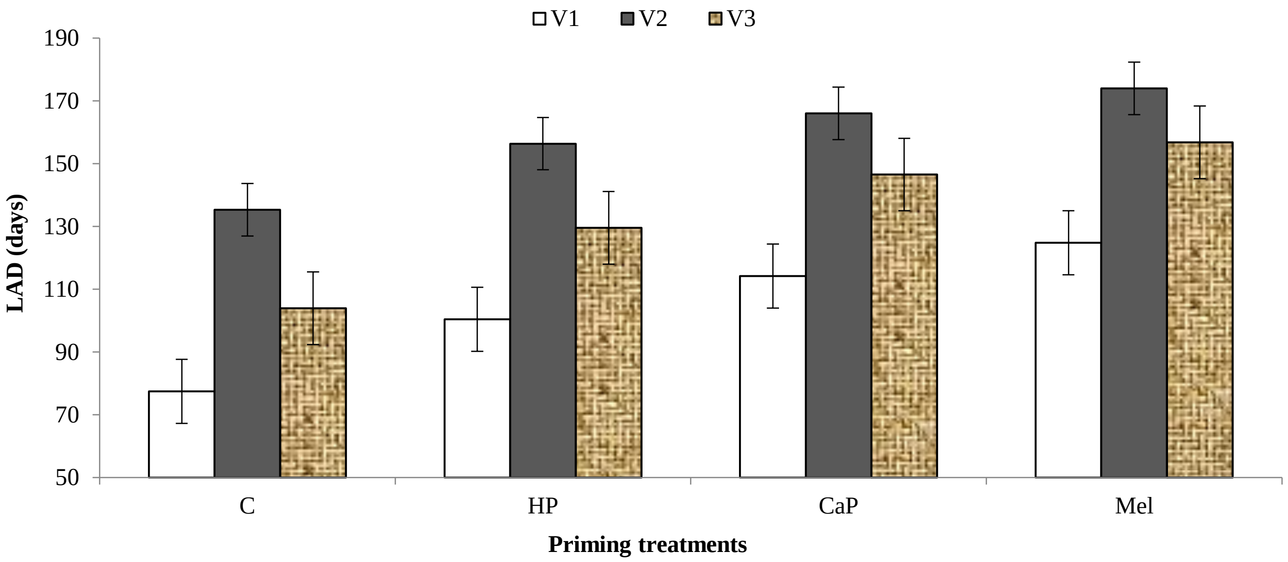

Fig. 7. Leaf area duration as influenced by different maize genotypes and seed priming treatments.

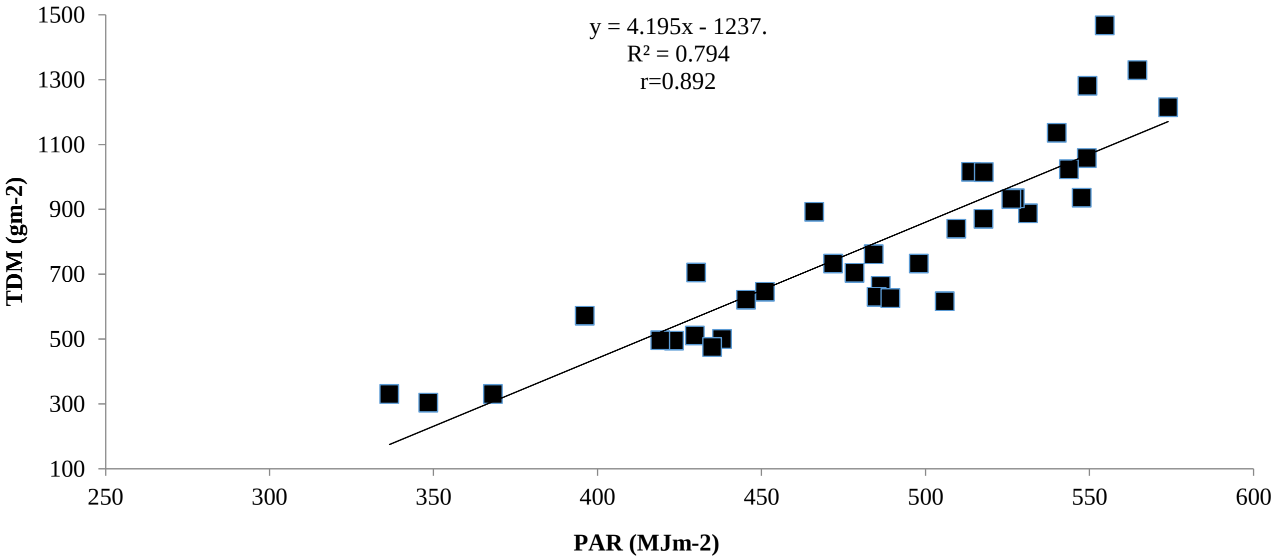

Fig. 8. Relationship between TDM and PAR as influenced by different maize genotypes and seed priming treatments.

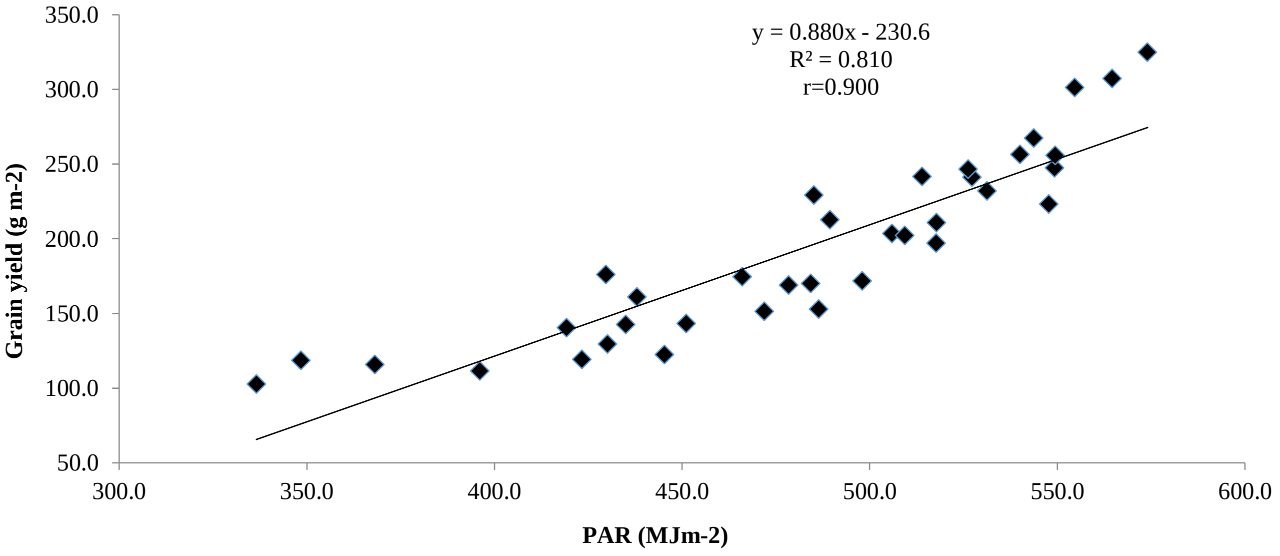

Fig. 9. Relationship between grain yield and PAR as influenced by different maize genotypes and seed priming treatments.

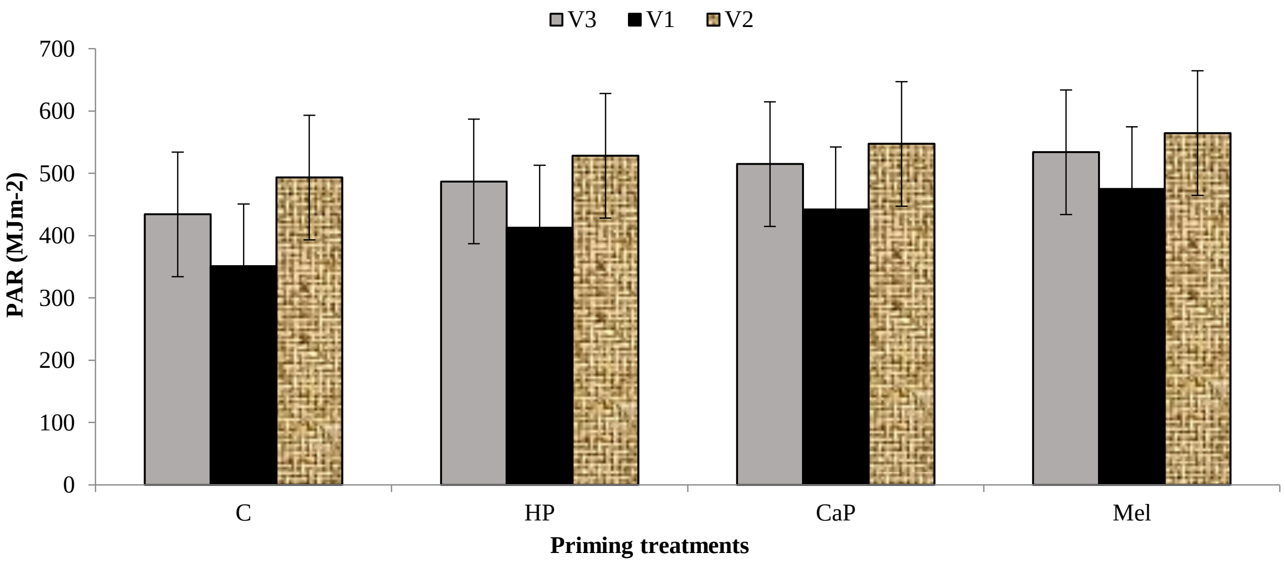

Fig. 10. Photosynthetic active radiations as influenced by different maize genotypes and seed priming treatments.

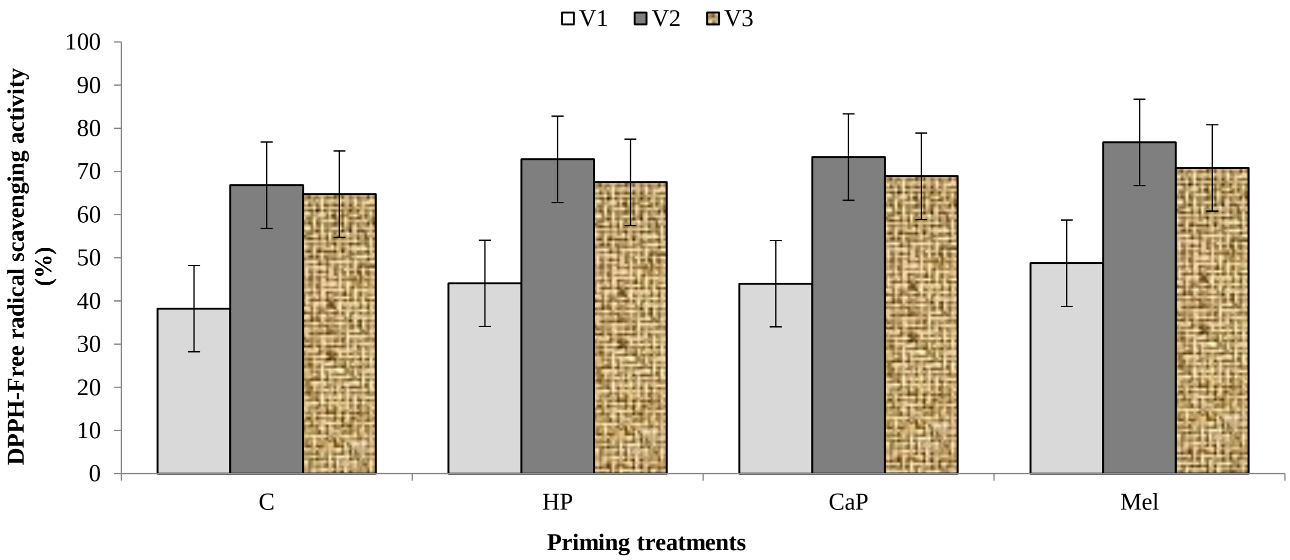

Fig. 11. DPPH-Free radical scavenging activity as influenced by maize genotypes and seed priming treatments.

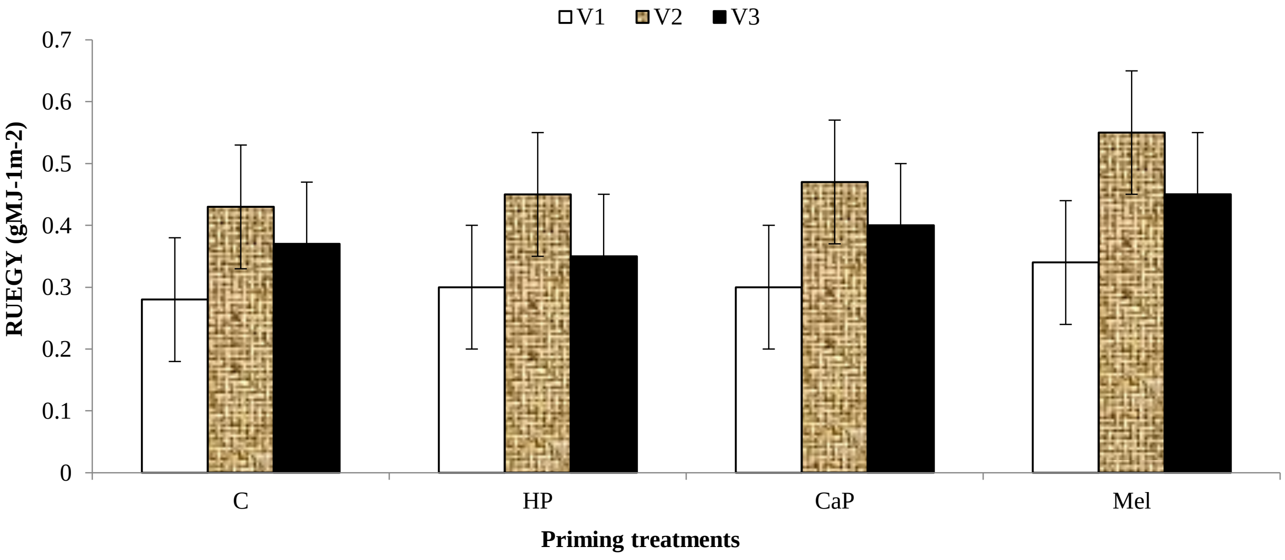

Fig. 12. RUE for grain yield as influenced by different maize genotypes and seed priming treatments.

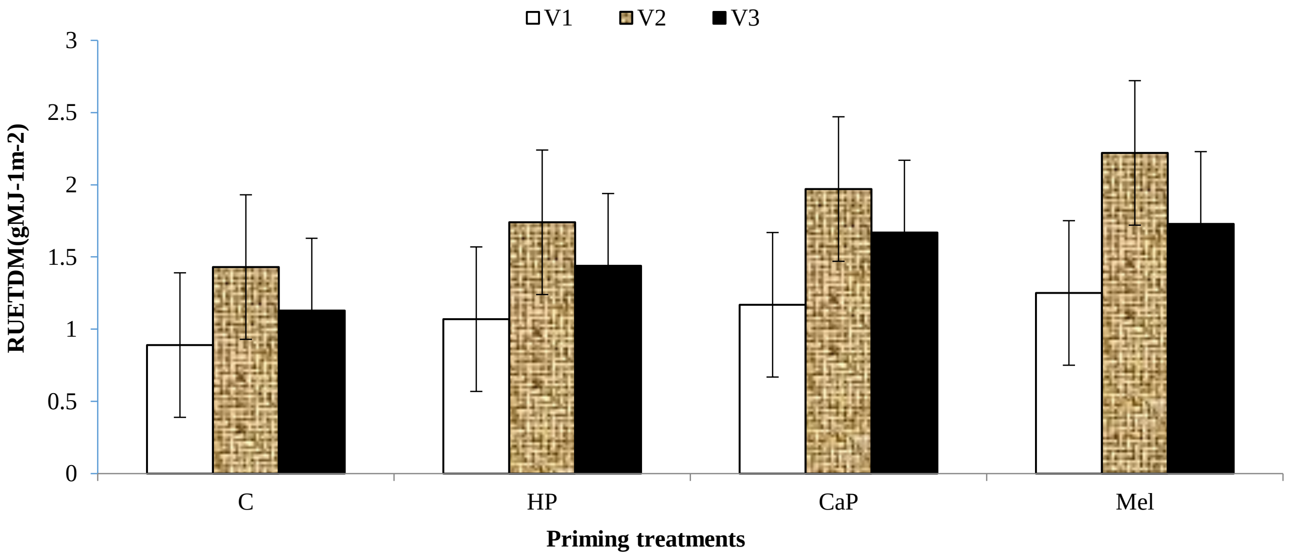

Fig. 13. RUE for TDM as influenced by different maize genotypes and seed priming treatments.

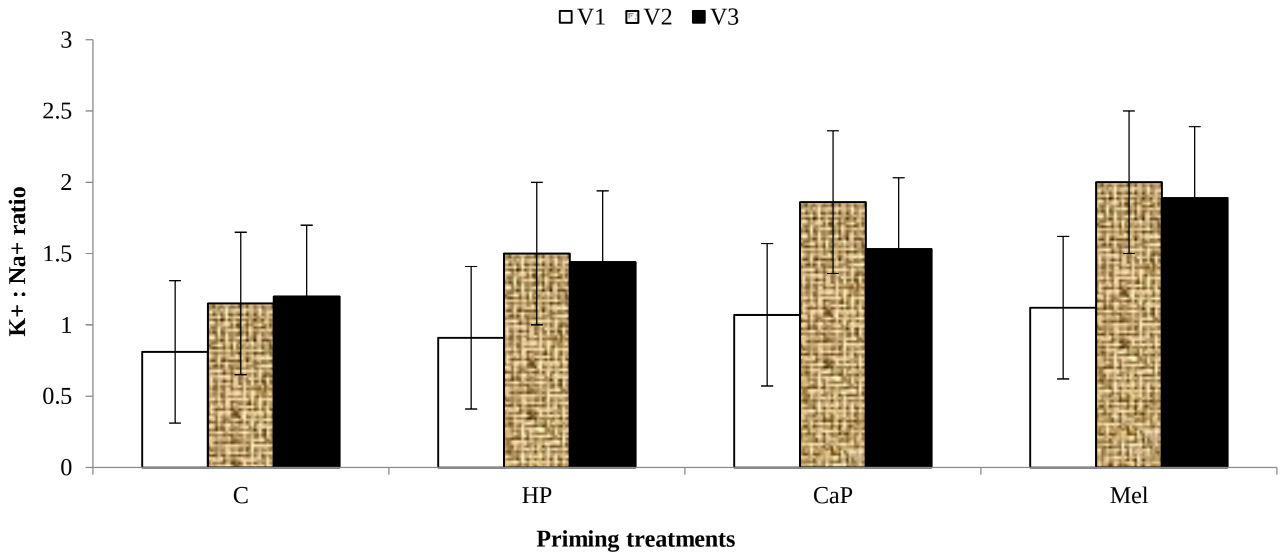

Fig. 14. K^+: Na^+ ratio as influenced by different maize genotypes and seed priming treatments.

Under the effect of salt stress, stressed plants began to collect appropriate solutes that may reduce salt stress's oxidative damage. When it comes to minimising the adverse effects of salt damage on plants, proline, total soluble sugars, soluble proteins, and phenolics are critical (Afzal *et al.*, 2020; Javed *et al.*, 2020). The present investigation also discovered a considerable rise in the accumulations of the osmolytes as mentioned above in salt-stressed field circumstances. Higher levels of proline and soluble carbohydrates may act as osmoprotectants, altering osmotic potential and allowing for improved water uptake to keep plants growing and developing under salt stress (Agami, 2014; Mumtaz *et al.*, 2021).

Similarly, the accumulation of soluble protein may provide a storage form of nitrogen when required by salt-stressed plants (Mumtaz *et al.*, 2021) and effectively prepares plants to cope with stressful environments (Chen *et al.*, 2021), which were very consistent with the current findings. During oxidative stress, phenolics also play an important role as free radical scavengers (Mobeen *et al.*, 2021). Melatonin priming (MP) was similarly found to cause a considerable increase in phenolic content in the current investigation. It was also discovered that melatonin priming increased the phenolic content of salt-stressed maize (Jiang *et al.*, 2016).

The level of salt tolerance in plants is determined by the improved and positive activities of antioxidant enzymes (Farhangi-Abriz & Torabian, 2018; Cen *et al.*, 2020). With melatonin priming (MP), the maximum antioxidant enzyme activity such as SOD, POD, and CAT was attained in SB-9617 (V2). SB-9617's antioxidant enzyme activities may have reduced potentially hazardous free radical concentrations more effectively than YH-1898 (V3) and NCEV-1530-9 (V1). Cai *et al.*, (2019), who found that salt tolerant cultivars have higher enzyme activity and related gene expression than moderately tolerant and salt-sensitive plants, support the findings of the current study. However, in our findings, increased antioxidant enzyme activities (SOD, POD, and CAT) with melatonin priming (MP) were linked to improved salt tolerance. The up-regulation of antioxidants enzymes (SOD, CAT, and POD) defence system with melatonin priming was also found by Zafar *et al.*, (2019), implying that melatonin priming is effective in the acquisition of salt tolerance in various crops. Furthermore, Zhang *et al.,* (2014) discovered that melatonin priming increased the expression of genes encoding antioxidant enzymes during salt stress. The results were also inline with the findings of Saleem *et al.,* (2020a) where it was invested that the exogenous application of gibbrelic acid significantly alleviated the oxidative stress in jute by enhancing the anti-oxidant enzymes activities.

Plants' ability to maintain an optimal K^+: Na^+ ratio in the cytosol impacts their ability to survive in saline circumstances (Mobeen *et al.*, 2021; Saleem *et al.*, 2021). The studied maize genotypes revealed a wide range of K^+: Na^+ ratios under salt stress in the current investigation (Fig. 7). Maqbool *et al.*, (2020) found that salt-tolerant maize hybrids retained greater K^+: Na^+ ratios than salt-sensitive maize hybrids, which is consistent with previous research. Furthermore, melatonin's promotive role in the acquisition of salt tolerance by plants when exposed to salt stress could explain why larger K^+: Na^+ ratios are retained with it. These findings were backed up by Chen *et al.*, (2018) and Chen et al. (Chen *et al.*, 2020), who found that melatonin priming in salt challenged maize seedlings resulted in the greatest K^+: Na^+ ratios.

To promote better crop development for increased biomass and agricultural output, oxidative damage in response to salt stress causes severe damage to chlorophyll, which can significantly impact radiation interception and use efficiency by the crop canopy (Ali & Ashraf, 2008; Ali *et al.*, 2020). The present study also discovered a considerable rise in chlorophyll content, leaf area development and related features, crop growth, photosynthetically active radiation interception, and radiation usage efficiency for TDM and grain yield. SB-9617 had the highest chlorophyll content, which could be related to its superior capacity to reduce hazardous free radical concentrations when compared to YH-1898 and NCEV, according to our data.

Melatonin's probable effect in slowing down the process of chlorophyll breakdown by delaying leaf senescence could explain the increased chlorophyll content (Ali *et al.*, 2020; Chen *et al.*, 2020). Pre-soaking seed with melatonin raised chlorophyll content by postponing leaf senescence in wheat crops under salt exposed situations, according to Yu *et al.*, (2021) and Zafar *et al.*, (2019). The lowest leaf area related measures, such as the leaf area index (LAI), leaf area duration (LAD), radiation interception (PAR), and its usage efficiency, were seen in plants under salt stress, indicating that crop growth was suppressed. However, the maize genotype SB-9617 (V2) outperformed the other maize genotypes in crop growth, LAI, radiation interception, TDM accumulation, and grain yield. This could be due to its inherent potential to prevent free radical concentrations from reaching dangerous levels. Cai *et al.*, (2019) found that salt tolerant cultivars have stronger free radical scavenging enzyme activity and gene expression than moderately tolerant and salt-sensitive plants. However, the increased photosynthetic and antioxidant enzymatic activities associated with melatonin priming were responsible for the improved crop growth rate, LAI, radiation interception (PAR), and usage efficiencies for TDM and grain production (MP). Another rationale for the highest values for these metrics with melatonin priming (MP) could be the delay in the initiation of leaf senescence in salt-stressed plants. According to Waqas *et al.*, (2017), priming treatments dramatically increased crop growth performance by increasing photosynthetic activity.

The crop yield is determined by solar energy, which is a driving force for photosynthesis (Rehman *et al.*, 2019; Nazar *et al.*, 2020). Salinization stimulates the onset of leaf senescence by shortening the plant life cycle and reducing PAR interception (Ali *et al.*, 2021). Radiation utilisation efficiency for TDM and grain yield is the connection between total dry matter build up and grain yield to intercepted radiation. With melatonin priming (MP), higher values of radiation interception and use efficiencies (RUE_{TDM} & RUE_{GY}) were discovered in SB-9617 (V2). These findings are similar to those published by Ye *et al.*, (Ye *et al.*, 2020), who found that melatonin administration significantly delayed leaf senescence. Melatonin priming likely increased radiation interception because of its probable function in keeping photo-pigments (chlorophylls) from being destroyed by reactive oxygen species (ROS). These results backed up by the Chen *et al.*, (2018) findings that melatonin profoundly influenced chlorophyll improvement under salt stress. Our findings are inconsistent with Zhang *et al.*, (2021), who found that seed priming with melatonin improved photosynthetic efficiency and radical scavenging activity in cotton under salt stress. The R^2 value of 0.794 revealed that the accumulation TDM was significantly dependent on the interception of PAR in the regression model (Fig. 8). A strong and positive connection between these parameters was discovered, with $r = 0.892$ (Fig. 8). On the other hand, the grain was found to be strongly reliant on PAR, with an R^2 of 0.810 and a linear and positive correlation for both parameters, indicating a value of $r=0.900$ (Fig. 9). Similar correlations between different traits were assessed in copper-sensitive and copper-resistant varieties of jute (Saleem *et al.,* 2019). Under salt stress, the overload of reactive oxygen species to hazardous levels makes it hard for plants to continue key physiological and biochemical processes that are required for optimum development and agricultural output. Salt stress damages chlorophylls (photo-pigments) and the photosynthetic process (Li *et al.*, 2020; Chen *et al.*, 2021), potentially disrupting the daily passage of photo-assimilate from leaves to growing grains and resulting in a significant loss of grain yield (Aref & Rad, 2012). Under salt stress, however, melatonin priming (MP) dramatically boosted grain yield. These results were also in line with those published by Zafar *et al.*, (2019) and Ye *et al.*, (2020), who discovered that pre-soaking seed with melatonin had a high potential for protecting grain production from salt damage.

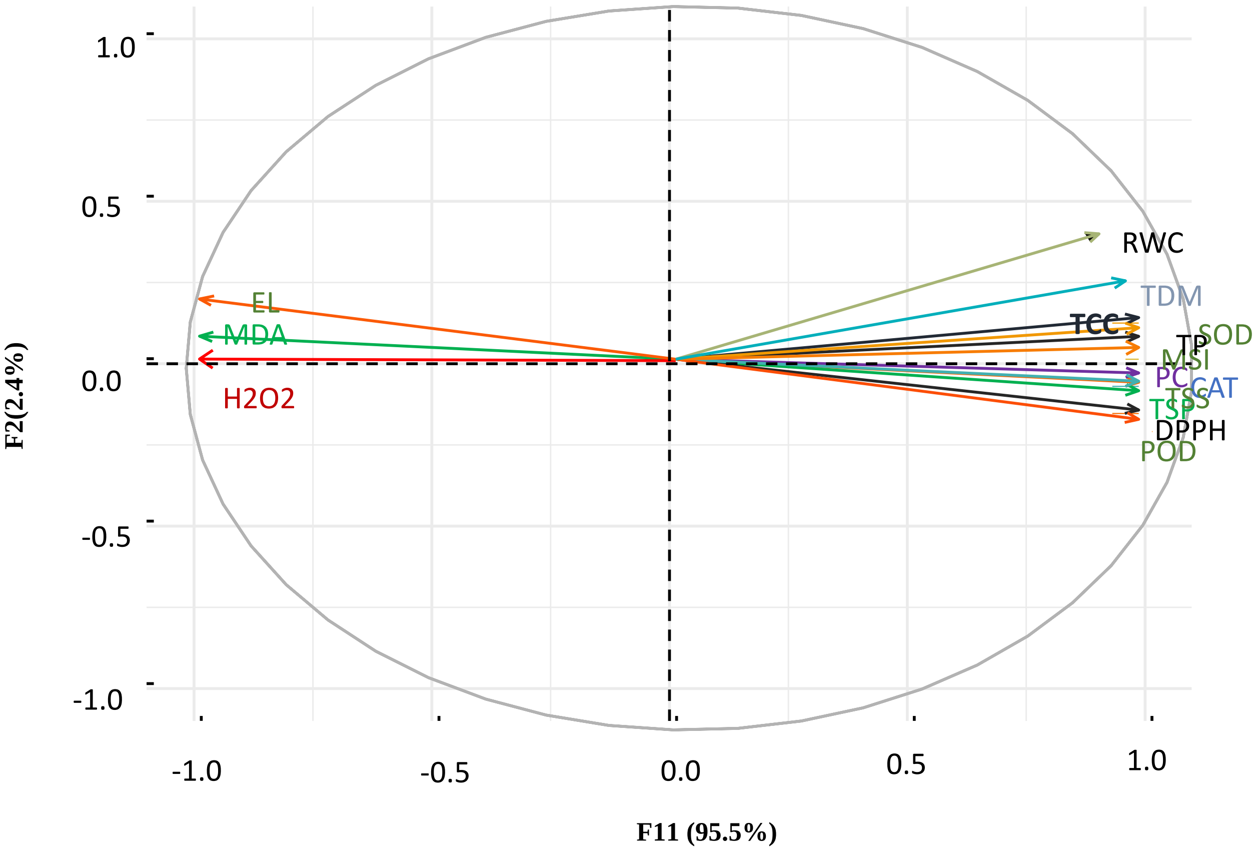

Fig. 15. Variables PCA.

Bi-Plot

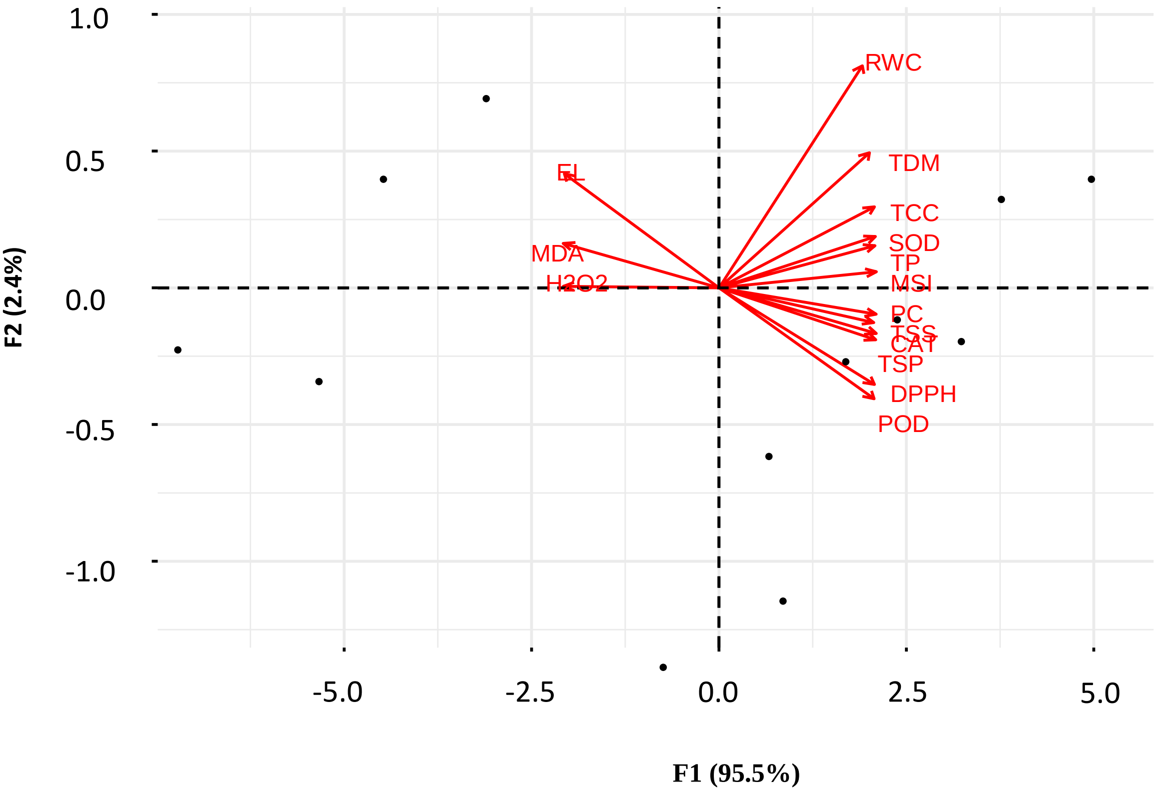

Fig. 15. Bi-plot analysis indicating growth, physiological and bio-chemical attributes as influenced by maize genotypes and priming treatments.

Conclusions

According to the findings of this research, seed priming may confer salinity tolerance in maize by enhancing nutrient uptake and, most likely, by increasing antioxidant defence mechanisms. SB-9617 with melatonin priming, on the other hand, performed better in terms of membrane stability, relative water contents, DPPH-free radical scavenging activity, total soluble sugars, total soluble proteins, total phenolics, total chlorophyll contents, and K^+: Na^+ ratio, as well as a significant decrease in H_2O_2, MDA, electrolyte leakage, and Na^+ contents. Furthermore, our findings revealed that seed priming with melatonin improved physiological and biochemical parameters in maize under salt-affected field conditions, such as LAI, LAD, PAR, radiation interception and use efficiencies (RUE_{TDM}& RUE_{GY}) and grain yield. It was also concluded that seed priming with melatonin was found to lower physical barriers during germination, improving salt tolerance in maize seedlings and resulting in higher crop yields in salt-affected soils. It was also discovered that seed priming with melatonin reduced physical barriers during germination, boosting maize seedling salt tolerance and yields in salt-affected soils.

Author contributions: Conceptualization, Syed Saqlain Hussain and Ghulam Jilani; Data curation, Muhammad Rasheed, Zammurad Iqbal Ahmed Mohamed Hashem, and Ghulam Jilani; Formal analysis, Syed Saqlain Hussain and Ghulam Jilani; Funding acquisition, Saad Alamri, Mohamed Hashem, Syed Saqlain Hussain, Muhammad Rasheed, Ghulam Jilani and Xiukang Wang; Investigation, Muhammad Rasheed; Methodology, Syed Saqlain Hussain and Xiukang Wang; Project administration, Zammurad Iqbal Ahmed, Saad Alamri, Mohamed Hashem, and Xiukang Wang; Software, Muhammad Hamzah Saleem, Saad Alamri, Mohamed Hashem, Zammurad Iqbal Ahmed and Aishah Alatawi; Supervision, Shafaqat Ali; Validation, Aishah Alatawi; Visualization, Muhammad Rasheed, Muhammad Hamzah Saleem and Shafaqat Ali; Writing – original draft, Muhammad Hamzah Saleem, Zammurad Iqbal Ahmed, Xiukang Wang, Aishah Alatawi and Shafaqat Ali; Writing – review & editing, Muhammad Hamzah Saleem, Saad Alamri, Mohamed Hashem, Aishah Alatawi and Shafaqat Ali. All authors have read and agreed to the published version of the manuscript.

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