Research Article

Melatonin-Mediated Induction of Salt Tolerance in Maize at Germination and Seedling Stage

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Abstract | Salt stress is more detrimental at germination, and seedling establishment stages in a plant life. Moreover, the escape of crop from salt injury at these stages is direly needed. Melatonin is known as N-acetyl-5-methoxytryptamine with chemical formula melatonin as $C_{13}H_{16}N_2O_2$. It is a versatile molecule with the speciality to lessen salt stress on the plant. However, mechanism of melatonin which is involved in salt stress mitigation is still under inspection of plant scientists. For the purpose, this research was carried out and the effects of melatonin priming was searched out viz., hydro-priming (HP), 500, 1000 and1500 µM on seed germination and early seedling establishment of six maize genotypes viz., MMRI-Yellow, NCEV-1530-9, YH-1898, Composite, SB-9617 and FH-949 under salinity-induced stress. Seed priming with 1000µM melatonin efficiently lowered the deleterious effects of salt stress on germination and early seedling stages in all the studied genotypes. Moreover, SB-9617 exhibited comparatively the greater salt tolerance at these stages. Finally, it was concluded that SB-9617could survive and thrive best in saline soils. Furthermore, seed priming with 1000µM of melatonin proved to be a good choice to alleviate the adversities of salts at germination and early seedling establishment stage in maize.

Received | February 08, 2023; **Accepted** | April 08, 2024; **Published** | May 27, 2024
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Citation | Hussain, S.S., M. Rasheed, Z.I. Ahmed, G. Jilani, M. Irfan, M.K. Aziz, F. Hussain, M.A. Mudassar and Zuhair, H. 2024. Melatonin-mediated induction of salt tolerance in maize at germination and seedling stage. *Sarhad Journal of Agriculture,* 40(2): 490-501.
DOI | https://dx.doi.org/10.17582/journal.sja/2024/40.2.490.501
Keywords | Genotypes, Attributes, PCA, Growth, Saline stress, Promptness

Introduction

Approximately 90 percent of the total world food consumption rely on 30 different crops; belongs to the salt-sensitive group of plants, the glycophytes (Zorb *et al.*, 2019). Maize (*Zea mays* L.), being the member of this group, is highly sensitive to salt stress even at moderate salinity levels i.e., EC 4–8 dSm^{-1} (Koyro *et al.*, 2008). Interestingly, the severity of saltiness is reported to be more pronounced at germination, where salty conditions cause significant reductions of seed germination (Ucarli, 2020). The excessive concentrations of salts in the soil restricts water availability to seed, thereby leading to inhibited seed germination (Welbaum *et al.*,1990). The salinity-induced ill effects on crop establishment can be withheld if salt tolerance is achieved especially at germination stage. Under such circumstances, priming of seed with melatonin proved to be a best option to impart salt tolerance in maize crop. Melatonin is known as N-acetyl-5-methoxytryptamine. The chemical formula of melatonin is $C_{13}H_{16}N_2O_2$ (Molar mass, 232.281 g). It is synthesized both in animals and plants. However, the biosynthesis of melatonin in plants begins with the production of tryptophan, a compound that plants can synthesize de novo via the shikimate pathway (Mannino *et al*., 2021). Tryptophan is then decarboxylated to tryptamine by *tryptophan decarboxylase (TDC)*, followed by serotonin biosynthesis catalyzed by *tryptamine 5-hydroxylase (T5H)* in plants. Serotonin is converted into 5-methoxytryptamine by *N*-acetyl *serotonin O-methyltransferase (ASMT)* and finally into melatonin (Byeon *et al*., 2014; Lee *et al*., 2014; Back *et al*., 2016). Mitochondria and chloroplast are the main sites of melatonin synthesis in plants (Kanwar *et al*., 2018). Moreover, the potentiality of melatonin for inducing salt tolerance at germination and early seedling stage in different crops, is well established (Castanares and Bouzo, 2019; Jiang *et al*., 2016; Jiang *et al*., 2020; Marthandan *et al*., 2020; Chen *et al*., 2020; Arun *et al*., 2022).

Li *et al*. (2019) and Eisa *et al*. (2023) reported that an ample quantity of melatonin in the seed may enhance salt tolerance at germination stage by utilizing better nutrient resources and protein formation. Secondly, the exogeneous application of melatonin safeguards the photosynthetic machinery (Chloroplast) and causes significant resumption of the lost activities of the enzymes e.g. SOD, POD, CAT etc. under salt stress (Jiang *et al*., 2020; Marthandan *et al*., 2020; Alharbi *et al*., 2021; Arun *et al*., 2022). Under salt stress, the reactive oxygen species (ROS) e.g. $^1O_2$, O2•–, H_2O_2,•OH. O_2 etc. in the mitochondria, chloroplast, plasma membranes, peroxisome etc. in plants. They cause lipid-peroxidation resulting in the generation of malondialdehyde (MDA) by lipoxygenase activity, leading to the perforation of cellular membranes (Kesawat *et al*., 2023). The application of melatonin causes downregulation of lipoxygenase activity by melatonin protects the cells from the hydro-peroxidation of polyunsaturated fatty acids of the membranes during stress (Kopustinskiene *et al*., 2021). In this connection, a melatonin concentration @ 300 μM L^{-1} was found effective in wheat crop to alleviate water stress during germination phase (Zhang *et al*., 2022). In another study, Jiang *et al*. (2016) reported the effectiveness of melatonin to mitigate the saline stress in maize when used @800 μM L^{-1}. In the similar way, the exogenous application of melatonin@ 100 μML^{-1} significantly reduces the cellular membrane damage and safeguards the photosynthetic machinery in maize crop under salt stress (Chen *et al*., 2018). Furthermore, Wang *et al*. (2022) also reported the effective concentration of melatonin in the rage of 50-300 μML^{-1} to mitigate the salt stress in wheat during germination and early seedling phase.

In view of the discussion above, it has been summed up that melatonin possesses a great potential to ameliorate salinity-induced ill effects on germination and early seedling stage in maize crop, in a dose dependent way. Hence, the present study was done to assess the efficacy of graded doses of melatonin to induce salt tolerance in maize with the special emphasis on seed germination and early seedling stage.

Materials and Methods

The study was carried out by performing two experiments with the purpose to test the efficacy of three doses of melatonin (500, 1000 and 1500 μM) to impart salt tolerance in six maize genotypes (MMRI-Yellow, NCEV-1530-9, YH-1898, Composite, SB-9617 and FH-949). Hydro-priming (HP) treatment was included as control for comparison. The seed material for this study was collected from Maize and Millets Research Institute (Sahiwal), Maize Research Station (Faisalabad), and National Agricultural Research Center (Islamabad). The study was executed in the form of two experiments. In the first part of the study, the impacts of different doses of melatonin on the germination indices were noticed. For this purpose, sixty (60) healthy seeds from each genotype were selected and sterilized in 1 % sodium hypochlorite solution to avoid any infection. The sterilized seeds of each genotype (60 seeds) were divided into four parts i.e., 15 seeds for each of the four-priming treatments viz., hydropriming (HP), 500, 1000 and 1500 μM. Accordingly, the solutions of melatonin with requisite concentrations were prepared by dissolving 0.029, 0.058 and 0.087 g of melatonin powder (Sigma-Aldrich, 98% purity) in 250 ml of distilled water separately to achieve the above said concentrations viz., 500, 1000 and 1500 μM, respectively. The seeds of each genotype were soaked in the aerated solutions of melatonin for 24 hours in beakers. For aeration, an air pump was installed in the experimental set up to avoid anxious conditions for seed. Moreover, the quantities of melatonin were computed by employing the formula as: weight of melatonin required (g) = Molarity required (M) x Molar mass of melatonin (i.e., 232.28 g/mole) x Volume of water used (L). Five (5) out of fifteen (15) primed seed of each genotype from each priming treatment were planted equidistantly in each petri dish well fitted inside with filter paper. The petri dishes were placed according to complete randomized design (CRD) with factorial arrangement replicated thricely in the laboratory of Agronomy Department, PMAS-Arid Agriculture University Rawalpindi. These petri dishes were watered daily with saline water of EC 8 dSm^{-1} (developed by adding weighed quantities i.e., 1.831, 2.441, 1.220 and 0.061 g of NaCl, Na_2SO_4, $CaCl_2$ and $MgSO_4$ in one litre of distilled water in the ratio of 3:4:2:1, respectively). This experiment was left up to 14 days from the date of planting in petri dishes (Figure 1). Finally, the data regarding germination indices such as germination percentage (GP), mean germination time (MGT), seedling vigour index (SVI) and promptness index (PI) were recorded using the following formulae:

Germination percentage was computed using the formula as described by Cokkizgin and Colkesen (2012) as GP(%)= (Germinated seeds)/(Total seeds)×100. The Mean germination time was determined by the formula Fuller *et al*. (2012) as MGT=Σ(NiTi)/ΣNi. Where Ni represents the number of seed germinations on the i^{th} day and T_i is the order of days (number of days observed since the start of germination). Similarly, the seedling vigour index was assessed by adopting the procedure of Elis and Robert (1981) employing the formula as SVI= Germination (%) × Seedling length (cm). Finally, the promptness index was computed using the following formula as PI=nd1(1.00) + nd2 (0.75) + nd3(0.5) + nd4(0.25). Where, nd1, nd2, nd3 and nd4 were the number of seeds germinated on the first, second, third, and fourth day (Ashraf *et al*.,2008). Furthermore, the promptness indicates the number of the seeds germinated at once or without delay.

In the second experiment of the study, the impact of above said doses of melatonin was evaluated on the early seedlings' traits of maize genotypes under saline stress. The trial was executed in the green house of the Soil Salinity Research Institute Pindi Bhattian. Before starting the experiment, a soil with EC 3.2 dS m^{-1} was taken from the research farm of the institute. The soil was sandy loam (Rasulpur soil series). The soil was sun dried and sieved properly. Meanwhile, four salts of NaCl=0.278, Na_2SO_4=0.450, $CaCl_2$=0.176 and $MgSO_4$ =0.095 g were weighed on per kg of soil basis to maintain the salt ratios as 3:4:2:1, respectively (Haider and Ghafoor, 1992). In after making a very careful calculation, the weighed quantities of above four salts were dissolved in fit water and then applied to soil. The application of salts to the soil followed the continuous drying and wetting cycles to achieve the requisite level of EC i.e., 8 dSm^{-1}. Finally, the salty soil was filled into the pots of 21 cm diameter and 25cm height. After filling the pots, a total of five primed seed from each genotype were sown in each pot. Moreover, the priming procedure was like that as adopted in the first experiment. The pots were arranged according to complete randomized design (CRD) with factorial arrangements replicated thricely. Water was sprinkled daily to keep the soil moist. Hoagland's nutrient solution was applied to ensure ample supply of nutrients (Hoagland and Arnon, 1950). At the end of the experiment (45 days after sowing), the early seedlings traits were recorded using standard scientific procedures as under:

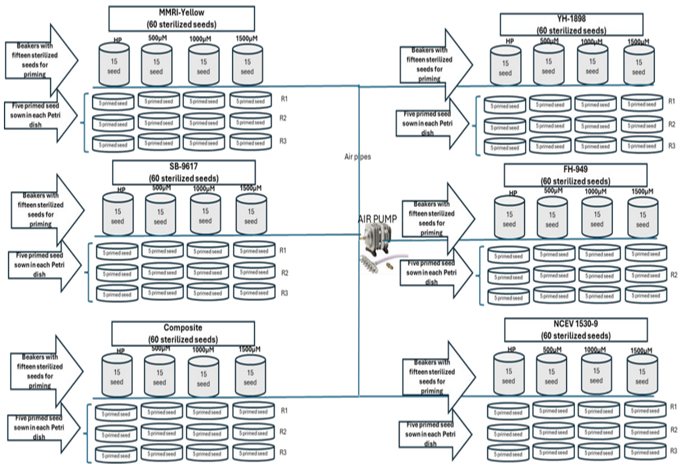

Figure 1: *Schematic diagram of the first experiment showing priming of seed with different melatonin concentrations including hydropriming. Each beakers contain requisite melatonin solution and fifteen (15) seeds out of sixty sterilized seeds (60) from each genotype and connected with air pump (only beakers). Each petri dish contains five (5) primed seed out of fifteen (15) seeds from each beaker and replicated thrice.*

At 45 days after sowing, shoot and root lengths were recorded with help of scale. The fresh weights of shoot and root were recorded with digital balance. After recording the fresh weights of shoots and roots, they were dried at 70°C for 48h in an oven and their dry weights were estimated on electrical digital balance. Stem diameter was measured with the help of digital vernier caliper. The number of leaves per plant were also counted. The diameter of shoots was measured using digital vernier calliper. Moreover, the leaf area per plant was measured by the formula of Carleton and Foote (1965) as Leaf area = Length x Breadth x 0.75.

Statistical analysis

Analysis of variance (ANOVA) and Pearson correlations were calculated with the latest version of SPSS statistical software. Further, the means of the treatments were equated using the single-level Tukey/HSD test (5% probability (Tukey, 1949). PCA was performed to select the most appropriate dose using the computer software R version 1.2.5033 (Blighe and Lun, 2019).

Results and Discussion

Germination indices

Germination percentage (GP): The final germination count of un-treated seed (HP-control) was 60% (Table 1). Interestingly, the treated seed with melatonin caused significant improvement in the germination count. However, adding melatonin @ 1500 µM increased the count (76.1 %) compared to melatonin @ 1000 µM (75.0 %). Similarly, among maize genotypes, SB-9617 recorded the highest germination count (83.3%) compared with the lowest count in composite (50.0%).

Salt stress causes a decrease in growth (Khatami *et al*., 2017; Khan *et al*., 2023). However, the evolution of melatonin-regulated genes can be attributed to its important role in improving nutrient utilization and the biosynthesis of new proteins responsible for talk of salt tolerance during saline stress (Li *et al*., 2019; Rosinska *et al*., 2023). The results were parallel with those of Chen *et al*. (2020) and Yu *et al*. (2021) where a significant improvement in seed yield of cotton and alfalfa was observed, when melatonin primed seeds were exposed to salt stress. Furthermore, inter-specific genotypic variations in maize genotypes were possibly the existence of salt tolerance (Zahra *et al*.,2018).

Mean germination time (MGT)

In terms of MGT (Table 1), Composite (5.53 days) and NCEV-1530-9 (5.45 days) record the highest average germination time among all maize genotypes. However, SB-9617 showed the largest reduction of germination time (5.02 days). Similarly, addition of melatonin cause shortening of the germination time. In our results, the application of melatonin @ 1000µM (5.19 days) and 1500µM (5.12 days) reduced the germination time viz., 5.19 and 5.12, respectively compared to the un-treated seed (HP), the control treatment (5.43 days).

Khatemi *et al*. (2017) reported that salt stress prolongs the time required for seed germination. In this context, melatonin/priming leads to a significant reduction in the time required for seed germination of corn and cotton crops in the saline soil (Yu *et al*., 202; Chen *et al*., 2020).

Seedling vigour index (SVI)

Table 1 showed a significant response of melatonin and maize genotypes on seedling vigour index. Results clearly depicted that SB-9617 exhibited the better performance for seedling vigour index (763.4) among the other studied maize genotypes. However, the least performer was Composite indicating the lowest seedling vigour index (247.8). Among the melatonin doses, the melatonin dose when applied @ 1000µM significantly produced the maize seedlings with higher seedling vigour index (602.9) compared with the lowest index in hydropriming (HP) as control treatment (310.7).

Table 1: *Germination indices as affected by melatonin priming and maize genotypes under saline stress conditions.*

Treatments	GP (%)	MGT (Days)	SVI	PI
Genotypes (G)				
MMRI-yellow	70.0 bc	5.22b	413.5 c	4.93 bc
NCEV-1530-9	62.5 c	5.45 a	336.4 cd	4.05cd
YH-1898	74.2 abc	5.18bc	558.9 b	5.26 b
Composite	50.0d	5.53 a	247.8d	3.18 d
SB-9617	83.3 a	5.02 c	763.4a	6.25 a
FH-949	80.0ab	5.13 bc	656.4 ab	5.73ab
LSD	12.474	0.1734	113.70	0.9506
Melatonin doses (M)				
HP-control	60.0 b	5.43 a	310.7 c	3.96 c
Mel@500µM	68.9 ab	5.28 b	478.1 b	4.76 b
Mel@1000µM	75.0 a	5.19 bc	602.9 a	5.33 ab
Mel@1500µM	76.1 a	5.12 c	592.7 a	5.56 a
LSD	9.132	0.127	83.239	0.696
Significance				
G	**	**	**	**
M	**	**	**	**
G x M	ns	ns	ns	ns

Dissimilar letters with treatment mean indicated the significant difference among treatment means; ns=non-significant; G=genotypes; M=melatonin levels, GP-germination percentage, MGT-mean germination time, SVI-seedling vigour index, PI-promptness index.

Akram *et al*. (2020) reported that salinity is injurious to the vigour of maize seedlings. While the addition of melatonin causes improvement in the said parameter in maize crop during saline stress (Jiang *et al*., 2016), which is very consistent with our findings.

Promptness index (PI)

In terms of promptness index, SB-9617 recorded the highest promptness index (6.25). Moreover, the composite had the PI value (3.18). However, the other maize genotypes were ranked places in the middle between them. Similarly, in melatonin priming treatment, the maximum PI (5.56) was calculated for melatonin at a dose of 1500µM that was non-significant with melatonin @ 1000µM treatment (5.33). Finally, the lowest value (3.96) in terms of PI was recorded in the HP control treatment.

The results of this study show similarity with the Ashraf *et al*. (2008) reported a decrease in the promptness index due to salinity. However, the seed prepared with phytohormones before planting have better PI values than seed without treatment (Al-Tabbal, 2017). From the similar findings as reported by Ali *et al*. (2019), co-application of jasmonoid and humic acids are found helpful in producing robust plant seedling even in the saline soil.

Early seedling traits

Length of shoot (LS): In the Table 2, the length of shoot was influenced significantly by melatonin and maize genotypes. The highest length of shoot was ofSB-9617(46.28 cm) and the lowest was measured in Composite (36.49 cm). All the other genotypes were found in-between them. Likewise, the maximum length of shoot was measured in the treatment with melatonin application @1000 µM (45.00 cm) and the minimum was recorded in control (37.20 cm).

Abdelgawad *et al*. (2019) also inferred that salt stress causes shortening of shoot length by limiting leaf expansion rate. The findings are consistent with Batool *et al*. (2020); Chen *et al*. (2020); Shafique *et al*. (2021) and Wang *et al*. (2021) observed stunted growth in various crops due to accelerated leaf abscission/senescence during saline stress. Furthermore, the protective potential of melatonin in controlling salt-induced damage to photosynthetic machinery in different crops has also been reported (Ahmad *et al*., 2021; Jiang *et al*., 2021; Eisa *et al*. 2023; Hussain *et al*., 2023; Karumannil *et al*., 2023), which provides a strong support to our data in the present study.

Table 2: *Early seedling traits as affected by melatonin priming and maize genotypes under saline stress conditions.*

Treatments	LS	FSW	DSW	LR	FRW	DRW	SD	NL	LA
	cm	g per plant		cm	g per plant		cm	per plant	cm^2 per plant
Maize genotypes									
MMRI-yellow	42.6 b	4.74 c	1.44 c	43.2 c	4.90 b	0.86 c	0.54 c	4.82 c	196.7 d
NCEV-1530-9	41.2 b	4.52 c	1.13 d	41.0 c	4.23 c	0.73 d	0.49 d	4.26 d	170.4 e
YH-1898	43.0 ab	5.68 b	1.57 c	47.5 b	5.31 ab	1.00 b	0.62 ab	5.20 b	233.8 c
Composite	36.5 c	3.27 d	0.71 e	36.1 d	2.89 d	0.54 e	0.43 e	3.78 e	121.3 f
SB-9617	46.3 a	7.41 a	2.09 a	51.9 a	5.64 a	1.12 a	0.63 a	5.60 a	283.1 a
FH-949	44.3 ab	6.63 a	1.86 b	49.6 ab	5.45 ab	1.02 ab	0.60 b	5.46 ab	254.0 b
LSD	3.5390	0.8440	0.1697	3.6202	0.5464	0.1037	0.0340	0.3317	16.634
Melatonin priming									
HP (Control)	37.2 c	3.08 c	0.71 c	39.1 c	3.67 c	0.65 c	0.47 c	4.29 c	172.9 c
Mel@500µM	42.3 b	5.65 b	1.57 b	44.5 b	4.65 b	0.86 b	0.55 b	4.80 b	203.6 b
Mel@1000µM	45.0 a	6.50 a	1.82 a	47.4 a	5.27 a	1.01 a	0.58 a	5.19 a	230.2 a
Mel@1500µM	44.9 ab	6.27 a	1.76 a	48.7 a	5.35 a	0.99 a	0.60 a	5.13 a	232.8 a
LSD	2.5909	0.6179	0.1242	2.6504	0.4000	0.0759	0.0249	0.2428	12.178
Significance									
G	**	**	**	**	**	**	**	**	**
M	**	**	**	**	**	**	**	**	**
G x M	ns	ns	ns	ns	ns	ns	ns	ns	ns

Dissimilar letters with treatment mean indicated the significant difference among treatment means at 5% probability level according to Tukey's HSD Test. s; ns=non-significant; G=genotypes; M=melatonin priming, LS-length of shoot, LR-length of roots, FSW-fresh shoot weight, DSW-dry shoot weight, FRW-fresh root weight, DRW-dry root weight, SD-stem diameter, LA-leaf area and NL-number of leaves.

Fresh shoot weight (FSW)

Fresh shoot weight (Table 2) of SB-9617(7.41 g plant^{-1}) produced the highest FSW followed by FH-949 (6.63g plant^{-1}). Additionally, the lowest fresh shoot weight was attained in Composite (3.27g plant^{-1}). When comparing the melatonin treatments, the seedling produced from seeds primed with melatonin at 1000μM (6.50 g plant $^{-1}$) produced more FSW than the control treatment (HP) (3.08 g plant $^{-1}$). The results were alike with Jiang *et al.* (2016); Ahmad *et al.* (2021); Rajora *et al.* (2022) and Jiang *et al.* (2021), reported that growth and biomass accumulation is enhanced by the melatonin seed priming.

Dry shoot weight (DSW)

As far as the dry shoot weight is concerned, SB-9617 (2.09 g plant^{-1}) had the highest DSW among maize genotypes while composite (0.71 g plant^{-1}) had the lowest DSW. When comparing priming treatments, the maximum DSW was attained by the seedlings where melatonin was applied at 1000 μM (1.82 g plant^{-1}) followed by treatment with the melatonin applied at 1500 μM (1.75 g plant^{-1}). Despite this, control treatment (0.71 g plant^{-1}) showed the lowest DSW.

Yamazaki *et al.* (2020) and Li *et al.* (2023) found that the toxic build-up of salts negatively impacted the photosynthesis process with the severe reduction of biomass accumulation in plants. Moreover, the plants can be compensated for this reduction by inducing salt tolerance. According to Muhammad *et al.* (2022), melatonin significantly enhances biomass accumulation in maize under salt stress. Alharbi *et al.* (2021) and Ahmad *et al.* (2021) also found similar findings. They observe a noticeable increase of dry shoot weight was observed in soyabean, and maize were observed when melatonin priming was used to mitigate salinity-induced stress.

Length of roots (LR)

The length of roots as affected by melatonin priming and the genotypes is presented in the Table 2 showed considerable increase of LR in SB-9617(51.92 cm) when compared with the least performer maize genotype, the composite (36.12 cm). Similarly, adding melatonin at 1000 and 1500 μM produced the seedlings with maximum LR viz., 47.36 cm and 48.62 cm, respectively. However, the minimum LR was recorded in the control treatment (39.10 cm).

Plant growth and development is restricted by the osmotic and ionic effects if exposed to saline environment (Muns and Tester, 2008; Acosta *et al.*, 2017). It is worth mentioning that plant roots experience severe alterations of architecture under salt stressed conditions (Balasubramaniam *et al.*, 2023). The use of melatonin under such circumstances may be a good opportunity to mitigate the salinity-induced impairment on plant growth for a robust type of roots system (Rajora *et al.*, 2022; Jiang *et al.*, 2021).

Fresh root weight (FRW)

Table 2 indicated that SB-9617(5.64 g plant^{-1}) produced higher FRW with respect to composite (2.89 gplant^{-1}). Furthermore, all the other maize genotypes were ranked in-between them. By taking melatonin priming treatments, adding melatonin at 1500 μM (5.35 g plant^{-1}) produced the maximum FRW that was non-significant with melatonin at 1000 μM (5.27 g plant^{-1}). Moreover, the least FRW was achieved in control treatment (3.67g plant^{-1}). The results clearly indicated that the reduction of FRW may be compensated by adding melatonin under saline conditions. The findings of the present study are in accordance with Alharbi *et al.* (2021); Ahmad *et al.* (2021); Wang *et al.*, 2021 and Rajora *et al.* (2022) reported that melatonin application has a pivotal role in improving growth and biomass especially during saline conditions.

Dry root weight (DRW)

In terms of dry root weight (Table 2), SB-9617 (1.12 g plant^{-1}) attained the maximum Which was non-significant with FH-949 (1.02 g plant^{-1}). Additionally, the minimum DRW was recorded in composite (0.54 g plant^{-1}). In case of melatonin priming treatments, melatonin application at 1000 μM (1.01g plant^{-1}) gave higher DWR compared with control treatment (0.65g plant^{-1}).

Sandhu *et al.* (2020) and Dikobe *et al.* (2021) reported the drastic effects of salinity in plants (Sandhu *et al.*, 2020; Dikobe *et al.*, 2021). In other study, Muhammad *et al.* (2022) also reported the reduction of DRW under saline condition. Such reductions can be compensated in plants by the extrinsic application of melatonin in a stressful environment (Ahmad *et al.*, 2021; Rajora *et al.*, 2022).

Shoot diameter (SD)

Shoot diameter was also influenced significantly by different melatonin seed treatments and maize genotypes (Table 2). Among the maize genotypes, the highest SD was measured in SB-9617 (0.63 cm) while the lowest value was recorded in composite (0.43 cm). By comparing melatonin priming treatments, the use of melatonin at 1500µM (0.60 cm) produced the plants with higher SD which was non-significant with melatonin application at 1000µM (0.58 cm). However, the lowest SD was observed in control treatment (0.47 cm).

In this context, Othman *et al.* (2023) reported the significant alteration of stem thickness in salt stressed plants. Similarly, Jiang *et al.* (2021) investigated the appreciable potential of melatonin to mitigate the adversities of salt stress in plants. The current results agree with the findings as published by Park *et al.* (2021) and Jiang *et al.* (2021) reported that melatonin application had positive effects on stem diameter in *Brassica juncea* and cotton crops under saline stress.

Number of leaves (NL)
Salinity not only a source of limiting the leaf growth but also a big reason of decreasing number of leaves per plant. The results indicated that SB-9617 (5.60) had the higher value of NL. However, the least number of leaves per plant were recorded in composite (4.24). Irrespective of the maize genotypes, addition of melatonin at1000 µM (5.19) produced the maximum number of leaves compared with control treatment (4.29).

Dikobe *et al.* (2021); Hannachi *et al.* (2022) and Khan *et al.* (2023) reported the declination of leaves number when plants are exposed to salt stress. The declined number of leaves associated with saline stress might be due to the accelerated leaf senescence. However, the addition of melatonin augments the tolerance potential of a plant to withstand salinity stress by delaying senescence process (Jiang *et al*., 2021; Hussain *et al*., 2023).

Leaf area (LA)
When comparing the maize genotypes, SB-9617 (283.12 cm^2 plant^{-1}) attained the highest LA among the other genotypes. The lowest LA was computed in composite (121.30 cm^2 plant^{-1}). By taking priming treatments, the significantly higher LA was recorded with melatonin at 1500µM (232.80 cm^2 plant^{-1}). While the same was non-significant with the treatment when melatonin was applied at 1000 µM (230.20 cm^2 plant^{-1}). Despite this, the lowest LA was recorded in the control treatment (172.90 cm^2 plant^{-1}).

Álvarez-Méndez *et al.* (2022); Hussain *et al.* (2023) and Khan *et al.* (2023) also reported the reduction of leaf area in *ranunculus* during saline stress. Similar reductions were also reported by Álvarez-Méndez *et al.* (2022). Interestingly, Jiang *et al.* (2020) reported the similar findings that the application of melatonin alleviates salinity-induced inhibition of leaf growth in cotton and maize crops.

Pearson's correlation and principal component analysis
Highly significant (P<0.01) correlations were noticed between the recorded parameters (Table 3). Moreover, the positive correlation values of r= 0.978, 0.950, 0.906 and 0.901 were observed between PI and GP, DSW and FSW, DRW and DSW and LA and NL, respectively.

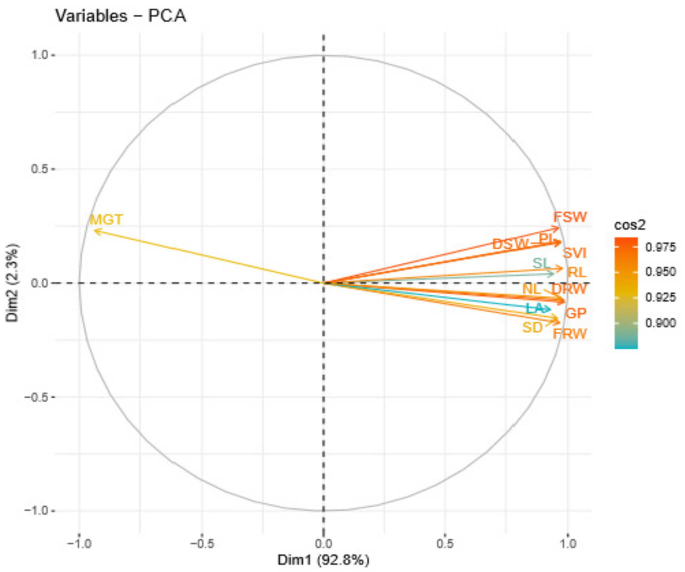

Figure 2: *Variable PCA.*

Figure 2 showed the variable CA which indicates the contribution of each component i.e., Dim1 (92.8%) and Dim2 (2.3 %) towards explaining the total variations. The positively correlated variables i.e., GP, SVI, PI, LS, FSW, DSW- LR, FRW, DRW, SD, NL and LA, were grouped together. However, the negatively correlated variable i.e., MGT was positioned on the opposite side of the plot. Moreover, a high cos2 in thevariable PCA indicated the quality of representation of the variables on the principal component. The variables very close to the center of the circle of the plot were less important for the first component (Dim 1). In our case all the variables were

Table 3: *Pearson's correlations coefficients for different parameters as affected by melatonin priming and maize genotypes under salt stress conditions.*

	GP	MGT	SVI	PI	LS	FSW	DSW	LR	FRW	DRW	SD	NL	LA
GP	1												
MGT	-0.726**	1											
SVI	0.879**	-0.736**	1										
PI	0.978**	-0.842**	0.897**	1									
LS	0.577**	-0.680**	0.645**	0.638**	1								
FSW	0.693**	-0.725**	0.823**	0.748**	0.821**	1							
DSW	0.745**	-0.760**	0.853**	0.791**	0.802**	0.950**	1						
RL	0.697**	-0.751**	0.795**	0.754**	0.777**	0.868**	0.883**	1					
FRW	0.707**	-0.812**	0.750**	0.767**	0.845**	0.857**	0.876**	0.836**	1				
DRW	0.749**	-0.783**	0.812**	0.798**	0.810**	0.872**	0.906**	0.862**	0.892**	1			
SD	0.569**	-0.653**	0.724**	0.636**	0.559**	0.713**	0.710**	0.692**	0.632**	0.731**	1		
NL	0.799**	-0.792**	0.855**	0.832**	0.724**	0.822**	0.870**	0.842**	0.856**	0.865**	0.681**	1	
LA	0.807**	-0.791**	0.892**	0.846**	0.720**	0.848**	0.878**	0.856**	0.857**	0.906**	0.698**	0.901**	1

***Correlation significant at 0.01 level, LS-length of shoot, LR-length of roots, FSW-fresh shoot weight, DSW-dry shoot weight, FRW-fresh root weight, DRW-dry root weight, SD-stem diameter, LA-leaf area and NL-number of leaves.*

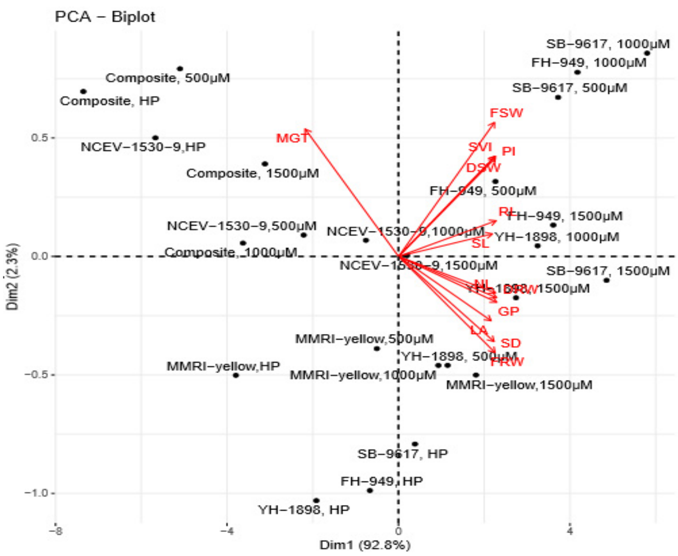

Figure 3: *Bi-plot showing the dispersion of different treatments combinations.*

positioned away from the center of the circle indicated the good representation in first component. Similarly, SB-9617 with melatonin @ 1000µM indicated as the best combination compared with other treatment combinations (Figure 3).

Conclusions and Recommendations

In this study, it was confirmed that salinity stress negatively influences the germination and early stages in maize crop. Moreover, SB-9617 may be used as salt tolerant genotype for improving maize crop yield in saline fields. Furthermore, priming of seed with melatonin at 1000µM proved to be best option for inducing salt tolerance in maize genotypes. Additionally, the present findings can be a supportive tool to explore the exact mechanism underway in achieving salt tolerance in other crops including maize in future.

Acknowledgements

Thank to anonymous reviewers for their valuable suggestions in improving the research article.

Novelty Statement

The impacts of salinity is found to be more pronounced on seed germination and early seedling stage leading to the loss of yield and poor quality of agricultural output. Melatonin improved the ability of the plant defense system to withstand the negative effects of salt, which led to increased tolerance in plants.

Author's Contribution

Syed Saqlain Hussain: Designed the experiment, collected data, and wrote the manuscript.
Muhammad Rasheed, Zammurad Iqbal Ahmed and Ghulam Jilani: Supervised the research work and curate the data.
M. Irfan, M. Kashif Aziz and Fiaz Hussain: Carried out PCA, statistical analysis and graphs.
M. Akhlaq Mudassar and Zuhair Hasnain: Done proof reading. Moreover, all the authors approved the

manuscript final version for publication.

Conflicts of interest

The authors have declared no conflict of interest.

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