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RIBOFLAVIN (VITAMIN B_2) PRIMING MODULATES GROWTH, PHYSIOLOGICAL AND BIOCHEMICAL TRAITS OF MAIZE (*ZEA MAYS* L.) UNDER SALT STRESS

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Abstract

Abiotic stresses are more often to occur because of poor drainage system resulting in agricultural land contaminations. Salt stress is one of the abiotic stresses which highly effects the growth and yield of cereal crops especially maize (*Zea mays* L.). The current experiment following completely randomized design (CRD) along with three replicates of each treatment was performed in the Botanic Garden, Government College University, Faisalabad to evaluate the effect of salt (70 mM NaCl) stress on maize (*Zea mays* L.) plants raised from seeds treated with riboflavin (RF). The temperature range in whole experiment was 30°C - 43°C. This study appraised riboflavin (0, 50 and 75 ppm) role in stress effect mitigation by enhancing growth, enzymatic (SOD, POD, CAT, APX and GPX) and non-enzymatic (flavonoids, phenolics and anthocyanin) antioxidant activities, and by scavenging ROS (MDA, H_2O_2) effect and maintaining osmotic level. Salt (70 mM NaCl) subjected plants showed reduced growth and photosynthetic rate, while increased ROS production (more in Sadaf compared to Pearl). Riboflavin is a novel vitamin which can be used to treat the salinity stress effected plants. Seed priming with RF (vitamin B2) significantly reduced salt stress effects by enhancing growth rate, photosynthesis, increased osmolytes accumulation and improved antioxidant defense system, while decreasing oxidative stress (MDA and hydrogen peroxides). Plants raised from seeds treated with riboflavin showed a significant increase in total leaf area, total free proteins and total soluble sugars than plants without riboflavin application. Gradual increase in RF concentration showed more improved growth under salt stress.

Key words: Antioxidants, Maize, Priming, Riboflavin, Salinity.

Abbreviations: RL- Root length, SL- Shoot length, RFW- Root fresh weight, RDW- Root dry weight, SFW- Shoot fresh weight, SDW- Shoot dry weight, LA- Leaf area, RWC- Relative water contents, Chl.- Chlorophyll, T. Chl.- Total Chlorophyll, MDA- Malondialdehyde, Antho.- Anthocyanin, Flavo.- Flavonoid, Phenol.- Phenolics, Pro.- Proline, T.Pro.- Total proteins, TSS- Total soluble sugars, SOD- Superoxidase dismutase, POD- Peroxidase, Cat.- Catalase, APX- Ascorbate peroxidase, GPX- Guaiacol peroxidase, RF- Riboflavin

Introduction

Soil salinity is the global issue, threating the food production and environmental health by affecting about 954 million hectare of land worlwide (Diaz *et al*., 2021; Wei *et al*., 2021). Increased salinity concentration of either rootzone or surface area decreases the soil fertility rate and ultimately decreases plants production rate (Fu *et al*., 2020). This salinity hinders water intake and results in erosion (Gorji *et al*., 2020) and effects agricultural growth and decreased crop production (Sing, 2022).

Salinity is threating abiotic factor which limits the growth, development, yield and expansion of plants (Zhao *et al*., 2020, Hadia *et al*.,2022). Salinity stress induce several morphophysiological changes that results in decreased shoot and root lengths, fresh and dry weight of plant (Dikobe *et al*.,2021). It is the main growth reducing factor which delays the germination time and causes disruption in nutrients uptake resulting in plant nutrient deficiency (Shiade & Boelt, 2020). Soil salinity subjected plants showed reduced Chl. *a, b* and total chlorophyll contents. Increased soluble sugar accumulation is followed by decreased starch level (Hassanein *et al*., 2002; Waheed AL-Mayahi, 2016). Salt stress causes accumulation of hydroxyl radical and hydrogen peroxide which is signal of oxidative stress in plants that might be the reason of cell damage and if there is no appropriate defence mechanism; cell dies. So plant's antioxidative machinery is activated to mitigate stress effects (Nadarajah, 2020; Abd El-Samad *et al.,* 2017). Both enzymatic and non-enzymatic antioxidants help the plants to detoxify reactive oxygen species (ROS) effect (Hasanuzzaman *et al*., 2020). Soil salinity stimulate activities of superoxide dismutase, peroxidase, ascorbate peroxidase and catalase enzymes to mitigate stress effects (Lee *et al*., 2001).

Maize (*Zea mays* L.), a member of family Poaceae, is the 2nd most demandingly produced cereal crop worldwide (Santpoort, 2020; Vaughan *et al*., 2018). It is known as the king of cereal crops. It is produced in 116 countries all over the world (Ahmad *et al*., 2021; Waqas *et al*., 2021). Environmental changes have a deleterious effect on *Z. mays* growth and yield ultimately decreasing the food availability and leading to corn deficiency (Wichelns & Qadir, 2015). There is a reduced production rate of Z. *mays* and it looks hard to increase its production rate to fulfil the deficiencies (Zahra *et al*., 2020; Kaya *et al*., 2020).

Vitamins have ability to protect plants from singlet oxygen and hygrogen peroxide species which are produced during photosynthetic process. Vitamins are important for the improvement of photosynthetic rate and antioxidant enzymes activities (Chi *et al*., 2021). These are also important for any plant for their growth, yield production and metabolism improvement. These may be fat or water-soluble (Garg *et al*., 2021). Many biological responses are highly affected by vitamins and vitamin B_2 is highly produced in green leaves of vegetables (Yoshii *et al*., 2019). Vitamins are important for competing the environmental biotic and abiotic stresses, especially vitamin B are the base of metabolic cofactors but any harsh stress level may cause decrease in B group vitamins in plants. To mitigate such conditions exogenous vitamin application is practised (Abdulhamed *et al*., 2020). Seed soaking technique is one of the best treatments for seeds to

compete against stress conditions by adapting physiological improvements (Hadia *et al.,* 2022). This is a cost effective technique and give great results. This technique positively increases seed maturation and plant growth rate (Kazemi & Eskandari, 2012; Hafeez *et al.,* 2021).

Riboflavin is known as a water-soluble vitamin B2 (Jiadkong *et al*., 2023). Its importance in every field either food production or medicine is dramatically increased due to its participation in health nutrition (Zhou *et al*., 2021). Riboflavin due to its anti-oxidative characteristic plays an important role in competing the salinity effect by improving osmotic pressure and ultimately enhances abiotic stress resistance (Abdulhamed *et al*., 2020; Singh, 2022). When plants are exposed to salinity stress, exogenous vitamin application activate antioxidant defense system that increase stress tolerance in maize, tomato and rice plants (Alayafi, 2020, Khatun *et al*., 2016).

In order to check the role of riboflavin (vitamins B_2) in decreasing salt stress effects; a pot experiment was performed, where two salt stressed maize cultivars were treated with riboflavin.

Experimental Design: A sand pot (8L) experiment was conducted in the Botanic Garden, Government College University, Faisalabad. Day to night humidity was 61%-70% and day to night temperature was 30°C-43°C. Maize (*Zea mays* L.) seeds of two varieties (Sadaf, Pearl) were collected from Ayub Agricultural Research Institute Faisalabad and Maize and Millet Research Institute, Sahiwal. A completely randomized design (CRD) was followed and two salt stress levels (0 and 70 Mm NaCl) were used to check the effect of salt stress. After gentle wash seeds of both varieties were primed with three riboflavin (vitamin B_2) levels (0, 50 and 75 ppm) for 12 hours and seed sowing was done in form of six experimental sets.

Sampling and data collection: After uniform germination, at four-leaf stage, salt stress was applied along with full strength Hoagland's nutrient solution. Hoagland's solution was applied every 3rd day throughout the experiment. During the fourth week of germination, plants were uprooted, well washed and air dried to wipe out excessive water. Root and shoot fresh weights and lengths were measured. After that shoot and root of each plant sample were placed in an oven for 48 hours at 72^{o}C for dry mass measurement. Total Leaf area per plant was calculated using the method of Carleton & Foote (1965). For the determination of physiological and biochemical parameters, leaves were packed in plastic zipper bags and kept in freezer at -20^{o}C.

Plant analysis

Relative water contents (RWC) (%): Fresh leaves of each plant were taken and weighed. Then, soaked the leaves in de-ionized water for 24 hours. Weight of soaked leaves was measured and these leaves were placed in an oven at 80^{o}C for 48 hours.

And dry weight was measured (Jones & Turner, 1987).

$$RWC\% = [(FW - DW)/ (TW - DW)] \times 100$$

Chlorophyll contents: For the determination of chlorophyll contents the protocol of Arnon (1949) was used. Fresh leaves (0.5 g) were taken, chopped and ground in 10 ml of 80% acetone (20 ml water and 80 ml acetone) and left these at -4^{o}C for one night then centrifuged these samples at 10,000 rpm for 5 minutes. Then the supernatant was used to measure chlorophyll contents at wavelengths of 480 nm, 645 nm and 663 nm through a spectrophotometer (IRMECO U2020).

The following formula was used to measure chl. a, chl. b contents.

$$\text{Chl. } a = [12.7 \text{ (OD 663) } -2.69 \text{ (OD 645)}] \times V/1000 \times W$$

$$\text{Chl. } b = [22.9 \text{ (OD 645) } -4.68 \text{ (OD 663)}] \times V/1000 \times W$$

V = Volume of the extract (mL),

W = Weight of the fresh leaf tissue (g)

Malondialdehyde (MDA) contents: Malondialdehyde (MDA) contents were estimated by following the protocol of Cakmak & Horst (1991). To 0.5 g fresh leaf material added 10 ml of 0.1% w/v TCA (trichloroacetic acid) during grinding. Then centrifuged this solution at 12,000 rpm for 10 minutes and took 1 ml of extract and to it added 4.5 ml of 0.5% TBA (thiobarbituric acid). The mixture was heated in a water bath for 30 minutes at 95^{o}C, cooled in an ice bath and again centrifuged. The readings of above samples were observed at 532 nm and 600 nm wavelengths on a spectrophotometer (IRMECO U2020).

Hydrogen peroxide (H_2O_2): For the estimation of hydrogen peroxide contents method purposed by Velikova *et al*., (2000) was used. Leaf sample of 0.5 gram was ground by adding 5 ml of 0.1% w/v trichloroacetic acid (TCA). Mixture was centrifuged at 12,000 rpm for 15 minutes. Phosphate buffer of neutral pH (7.0) was added with 0.5ml concentration after that 1ml of KI (potassium Iodide) was added and absorbance was noted at 390 nm wavelength on UV visible spectrophotometer (IRMECO U2020).

Anthocyanin contents: For anthocyanin estimation method of Zhang *et al*., (2009) was followed. According to this method fresh leaves (0.1 g) were ground in 5 ml of phosphate buffer and then centrifuged. Values of samples were noted at 600 nm with a spectrophotometer (IRMECO U2020).

Flavonoids: For flavonoids determination protocol of Karadeniz *et al*., (2005) was used, fresh leaves (1.0 g) were taken from each of the plants and then ground with a pestle and mortar by adding 20 ml of 80% ethanol. Samples were filtered through Whatman's filter paper 42. In a test tube 0.5 ml filtrate and 3 ml deionized water along with 3 ml of 0.5% $NaNO_2$ and 0.6 ml of 10% $AlCl_3$ was also added and left the samples for 6 minutes. Added 2ml of 1M NaOH. Deionized water was added to make the volume of each test tube up to 10 ml. The reading of flavonoid at 510 nm was noted with a spectrophotometer (IRMECO U2020).

Phenolic content: Total phenolic contents were determined by the protocol of Julkunen-Titto (1985). Leaf sample (0.05 g) of each replicate was taken and ground with 10 ml of 80% acetone. After homogenizing the leaf sample with acetone, it was centrifuged at 10,000 rpm for ten minutes. Removed supernatant and a little portion of aliquot (100 µ) were treated with 1 ml of Folin-Ciocalteau's phenol reagent and then 2.0 ml of distilled water was added. After that, 5 ml of Na_2CO_3 (20% w/v) was added. Deionized water was added to make the volume up to 10 ml, solution was shaken and the values were observed at 750 nm on UV visible spectrophotometer (IRMECO U2020).

Free proline estimation: Free proline contents were estimated by the method given by Bates *et al*., (1973). A fresh leaf sample (0.5g) was taken and ground in 10mL of sulphosalyslic acid (3%) and was left for 5 minutes. Filtered it, 2mL of filtrate, 2mL acid ninhydrin and 2 ml of glacial acetic acid were poured into the test tube and heated at 100°C for one hour. The sample was cooled in an ice bar. Then 4mL of toluene was added, stirred and passed through the air for 2 minutes. The values were observed at 520 nm absorbance. Toulene was run as blank. Proline concentration was observed through the standard curve.

Total soluble Proteins: Total soluble proteins were determined by the protocol given by Bradford (1976). Fresh leaves (0.5g) were well ground in 10 mL (50 mM) phosphate buffer in a prechilled environment, then centrifuged at 6000 rpm for five minutes at 4°C. 0.1 mL of supernatant and 2mL of Bradford reagent were introduced and this mixture was left for five minutes. Absorbance was noted at 595 nm absorbance using a spectrophotometer.

Total soluble sugars: Total soluble sugars were determined by the method of Yemm & Willis (1954). Dried plant material was ground and passed through sieves of 1mm. The extracted material (0.1 gram) was mixed with 80% acetone (10 mL each), shaken for 6 hours and the extract was used for the determination of soluble sugars, 0.1 mL plant extract and 3mL of anthrone reagent were poured into a 25 mL test tube. Then, heated at boiling temperature for 10 minutes and was cooled it for 10 minutes. Incubated for 20 minutes at room temperature and absorbance was noted at 625 nm using a spectrophotometer (IRMECO U2020).

Determination of antioxidant enzymes activities: Under super chilled conditions, 0.5 gram of fresh leaves were ground in 10 ml (5 mM) phosphate buffer with 7.8 pH, centrifuged at 12000 rpm at 4°C (20 min) and again centrifuged this at 15000 rpm for 10 minutes. Stored this enzyme extract at -20°C for antioxidant enzyme activity analysis.

Superoxide dismutase (SOD): Enzyme inhibition of photochemical reduction of nitroblue tetrazolium (NTB) was determined by the method of Giannopolitis & Ries (1977). For this purpose, a reaction mixture was prepared. This mixture contained 250µL (50 mM) of phosphate buffer, 400µL distilled water, Methionine (100µL), 50 µM NBT, 50 µM riboflavin and 50µL of enzyme extract. Then values were noted at 560 nm absorbance on UV visible spectrophotometer.

Peroxidase (POD) and catalase (CAT): Activities of both POD and CAT were determined by following the method of Chance & Maehly (1955). For CAT determination, a mixture was prepared to consist of 1.9 mL (50 mM) with pH 7, 5.9 mM of hydrogen peroxide (1 mL) and 100 µM of enzyme extract. Readings were noted at 240 nm on a spectrophotometer. Readings were monitored every 20s for 2 minutes. While for POD estimation, a reaction mixture was prepared to contain 250 µL (50 mM) of phosphate buffer, 100 µL (20 mM) guaiacol, 50 µL enzyme extract and distilled water. The enzyme activity change was observed every 20s at 470 nm.

Ascorbate peroxidase (APX): A reaction solution (3mL) which was consisting of 50 mM phosphate buffer with 7.0 pH, 0.5 mM hydrogen peroxide and 0.5 mM ascorbic acid was prepared (Asada, 1992). The reaction was started after adding hydrogen peroxide. The absorbance was recorded at 390 nm for 2 minutes.

Guaiacol peroxidase (GPX): The determination of GPX was done by using (3 mL) guaiacol solution containing 10 mm ($K_2H_2PO_4$) at 7 pH, 20 mm guaiacol, 0.5 mL crude extract and 20 mm guaiacol and values were noted at 436 nm (Chance & Maehly, 1955).

Acid digestion for ion analysis (Allen *et al*., 1985): The dried material (0.1 g) was placed in a digestion flask and 2 ml of sulphuric acid (H_2SO_4) was added. The mixture was left for over night at 25°C. On the next day, 0.5ml of hydrogen peroxide was added and heated at 150°C. then placed the flasks on hot plate at 250°C temperature. The fumes emission and coloration were observed. When the solution turned colorless, volume of the solution was up to 50 ml by adding water. It was filtered and was run on flame photometer for the determination of ions (K, Ca and Na).

Cl^- was determined by AgCl precipitation and titration procedure following the method of Johnson & Ulrich (1959).

Statistical Analysis

The collected data was subjected to analysis of variance (ANOVA) using CoStat software version 6.303 and mean values were compared by least significant difference (LSD) test at 5% level of significance.

Results

Growth traits: The current experiment was conducted to check the effect of seed priming with riboflavin (RF) i.e., 0 ppm, 50 ppm and 75 ppm on two maize (*Zea mays* L.) varieties (Sadaf and Pearl) under two levels of salt (0 and 70 mM NaCl) stress. Root and shoot length of Sadaf showed more reduction than Pearl under salt stress (70 mM). Shoot length showed comparatively more reduction rate than root length ($p \leq 0.001$) and pre-treatment with riboflavin exhibited improved plant ($p \leq 0.01$) response under salt stress. Highly reduced root and shoot fresh weight was observed in the variety Sadaf than that of Pearl. However, this reduction was less in plants raised from the grains treated with RF. The dry mass of Sadaf was less than that of Pearl. Variety Sadaf showed high ($p \leq 0.05$) reduction

rate in dry mass under salt stress and RF treated plants were significantly tolerant to salt stress ($p \leq 0.01$). The Sadaf variety has a greater response under salt stress ($p \leq 0.01$) in terms of leaf area, however, RF treated plants exhibited a less salt stress effect ($p \leq 0.05$). Overall, all growth attributes i.e., root and shoot fresh and dry weights, shoot and root lengths, and total leaf area per plant ($p \leq 0.001$) have decreased under salt stress, while pre-sowing seed treatment with riboflavin improved these growth attributes of both maize varieties under salt stress or non-stress conditions. Pearl was higher in growth parameters than Sadaf variety of maize (Fig. 1, Table 1).

Relative water contents (RWC) and chlorophyll contents: Leaf RWC was decreased ($p \leq 0.001$) under salt stress in both maize varieties (Fig. 1, Table 1). Pre-sowing seed treatment with 50ppm and 75ppm riboflavin *b* significantly ($p \leq 0.001$) increased RWC in both varieties, Pearl variety showed more RWC than Sadaf (Fig.1, Table. 1). Total chlorophyll, chlorophyll *a* and chlorophyll *b* contents significantly ($p \leq 0.001$) decreased under 70 mM NaCl stress in both maize varieties i.e., Pearl and Sadaf (Fig. 2; Table 1). Pre-sowing seed treatment with 50ppm and 75ppm riboflavin *b* significantly ($p \leq 0.001$) increased chlorophyll (Chl. *a,* Chl. *b* and total chlorophyll) contents. Pearl variety of maize accumulated more chlorophyll *b* ($p \leq 0.001$) contents than variety Sadaf (Fig. 2, Table 1).

Hydrogen peroxide (H_2O_2), malondialdehyde (MDA), anthocyanin, flavonoid and total phenolic contents: In the current study, H_2O_2, MDA, flavonoid and total phenolic contents were increased ($p \leq 0.001$) under salt stress in both the varieties (Fig. 2; Table 1). These Riboflavin treated plants showed decreased ($p \leq 0.001$) H_2O_2, MDA contents, while flavonoid and total phenolic contents ($p \leq 0.001$) were increased in both of the varieties under salt stress (Fig. 2; Table 1). Where as more accumulation was observed in Sadaf than of Pearl because this accumulation was the indication of stress exposure. RB application reduced this accumulation rate gradually to minimize stress effect on plant (Fig. 2, Table 1). Anthocyanin contents did not change significantly under salt or riboflavin treatment) in both maize varieties (Fig. 2, Table 1). Total phenolics and flavonoids increased ($p \leq 0.001$) under salt stress in both the varieties. RF treated plants showed increased phenolic and flavonoid contents in both maize varieties. However, Pearl showed higher accumulation of total phenolic and flavonoid contents than variety of Sadaf (Figs. 2 & 6; Table 1).

Free proline, total proteins and total soluble sugars: Proline accumulation was high under salt stress ($p \leq 0.001$) in both varieties. Total proteins and total soluble sugars were decreased ($p \leq 0.001$) under NaCl stress, however, riboflavin treated plants showed increased accumulation of free proline, total proteins and total soluble sugars ($p \leq 0.001$) in both the varieties. However, Pearl variety was higher in free proline, total proteins and total soluble sugars under both control and 70 mM NaCl stress (Table 1; Figs. 3 & 6).

Antioxidant enzymes activities: Activities of antioxidant enzymes i.e., SOD, POD, CAT, APX and GPX significantly increased under salt stress in both maize varieties (Pearl and Sadaf). Thus, Pearl was higher in the activities of antioxidant enzymes compared to Sadaf variety. Pre-sowing seed treatment with riboflavin further enhanced the activities of antioxidant enzymes. Riboflavin played its significant role in the improvement of antioxidants activation rate ($p \leq 0.001$) (Table 1; Fig. 3).

Ion analysis: Salt (NaCl) stress increased Na^+ and Cl^- ions in root and shoot of both varieties, however, its enhancement rate was greater in Sadaf variety than that of Pearl. Salt stress decreased nutrient ions (K^+, Ca^+) in both maize varieties. Their reduction rate was more in root than shoot. Seed priming with riboflavin played vital role to mitigate the adverse effect of salt stress on nutrients (K^+, Ca^+) ions (Table 2; Fig. 4).

Multivariate analysis

Principal component analysis (PCAs): Principal component analysis were conducted for growth, photosynthetic pigments, biochemical of maize under salt stress and Riboflavin (RF) applications. The distance between eigenvectors and values of positive or negative values demonstrated the effect of applied applications. The PCAs for these traits showed a cumulative variability of 87.5%. Both varieties exhibited strong association by overlapping the eclipses. As a result of a higher saline level of 70 mM and RF supplementation (T3-75ppm), the organic osmolytes TSS, TSP, and Pro were significantly enhanced and strongly associated with each other by loading to the PCA1 side and showed higher positive eigenvalues. Both T3 and 70 mM NaCl were strongly interlinked with each other. The GPX and Flavo corresponded with higher positive eigenvalues. The Ribo (T2) exhibited strong relation with MDA contents under influence of 70 mM NaCl treatments. However, the SFW and RDW were associated with the T1 and 0 mM NaCl levels (Fig. 5a).

Pearson correlation matrix: The Pearson correlation matrix showed a significant ($p \leq 0.05$) correlation for growth, photosynthesis and biochemical traits of maize plants under different NaCl and riboflavin treatment (Fig. 5b). The photosynthetic pigments (Chl a, b and T. Chl) were strongly and positively associated with the plant biomass (SFW, RFW, SDW, RDW) and growth traits such as LA, RL and Sl. The organic osmolytes (Prol, TSS, TSP) were strongly correlated with the anthocyanin and weakly associated with the antioxidant enzymes (SOD, POD). Photosynthetic and growth traits were negatively correlated with the MDA and H_2O_2 contents.

Clustered heatmap: A cluster heatmap was constructed to demonstrate the influential response of traits and varieties under salt and riboflavin treatments (Fig. 6). The growth traits (RL, SDW, SL and RDW) were strongly correlated with T3 and S2 of both varieties. The MDA and H_2O_2 contents were negatively associated with photosynthetic and growth traits with higher negative values.

Table 1. Mean square values of riboflavin induced modulation in growth, physiological and biochemical traits of maize (*Zea mays* L.) under salt stress.

SOV	Varieties	NaCl	RF	V×NaCl	V×RF	NaCl×RF	V×S×RF	Error
RL	327.00***	207.8***	497.5***	0.562ns	68.430*	8.763ns	0.541ns	8.60
SL	2523.4***	636.7***	3576.2***	364.81***	74.57ns	184.97**	44.28ns	13.56
RFW	83.11***	32.30***	160.17***	0.0802ns	0.802 ns	0.295ns	1.082ns	0.732
SFW	1242.5 ***	2730.0***	727.86***	5.062ns	21.00***	79.53***	9.301 ns	2.968
RDW	13.08***	137.3***	18.370***	0.667*	0.2205ns	0.2ns	0.004ns	0.136
SDW	11.33***	13.44***	36.015***	0.071 ns	1.160ns	4.6838**	0.037 ns	0.413
LA	347.9***	194.9***	62.80***	10.69**	2.310 ns	1.33147ns	5.210*	305.1
RWC	1591.1***	449.02**	165.301*	56.52 ns	14.58ns	5.8790 ns	6.456 ns	44.51
Chl. a	0.162***	0.484***	0.005***	0.000 ns	0.000ns	0.000ns	0.0006ns	0.000
Chl. b	0.069***	0.018***	0.0033***	0.001***	0.000***	0.000***	0.0005**	0.000
T. Chl	0.444***	0.126***	0.0171***	0.0014*	0.000ns	0.0012*	0.002***	0.0002
MDA	15.24ns	759.7***	202.829*	2.911ns	2.927ns	34.514ns	8.311ns	51.94
H_2O_2	1.7463***	13.09***	21.23***	0.194ns	1.053***	0.2618*	0.543***	0.074
Antho	0.0207ns	1.695ns	0.5691ns	0.000ns	0.137ns	7.5453**	1.256 ns	1.281
Flavo.	0.1514***	1.124***	0.0028***	0.000ns	0.000ns	0.0000ns	0.0002ns	0.000
Phen	10.696***	26.58***	6.7551***	0.052 ns	0.608 ns	0.0142ns	0.0730 ns	0.039
Prol	35.979*	57.49**	92.46***	0.342ns	5.546ns	3.9849ns	1.4137 ns	4.826
TSP	135.6***	9.428***	2.677***	0.5161*	0.354*	0.0487ns	0.3023*	0.0862
TSS	5474.6***	1888.1***	226.51***	77.438***	59.75***	0.440ns	1.0859ns	0.5823
SOD	0.3434ns	4.7930***	1.0687***	0.256 ns	0.001 ns	0.0044 ns	0.0721ns	0.0807
POD	483.01ns	2042.5***	1312.4***	138.01***	0.230ns	72.1630*	12.32ns	120.32
CAT	469.5***	2070.5***	1333.9***	113.10*	0.1236ns	79.801*	7.6705ns	23.26
APX	0.1036*	0.8453***	0.090*	0.000ns	0.013ns	0.0055 ns	0.0027 ns	0.0178
GPX	26.310***	222.4***	12.65***	1.5365 ns	4.157 *	1.734 ns	1.258ns	1.168
Df.	1	2	2	1	2	2	2	24

*, ** and *** = Significant at 0.05, 0.01 and 0.001 levels respectively; ns = Non-significant; df = Degree of freedom.

Abbreviations: RL - Root length, SL - Shoot length, RFW - Root fresh weight, RDW - Root dry weight, SFW - Shoot fresh weight, SDW - Shoot dry weight, LA - Leaf area, RWC- Relative water contents, Chl. - Chlorophyll, T. Chl. - Total Chlorophyll, MDA - Melondialdehyde, Antho - Anyhocyanin, Flavo. - Flavonoid, Phenol. - Phenolics, Pro. - Proline, T.Pro. - Total proteins, TSS - Total soluble sugars, SOD - Superoxidase dismutase, POD - Peroxidase, Cat - Catalase, APX - Ascorbate peroxidase, GPX- Guaicol peroxidase, RF - Riboflavin

Table 2. Mean square values of riboflavin induced modulation in ion contents of maize (*Zea mays* L.) under salt stress.

SOV	Varieties	NaCl (stress)	RF	V × NaCl	V × RF	NaCl × RF	V × S × RF	Error
Shoot Na^+	658.7***	44.44***	100.0***	1.777ns	17.36**	1.361ns	1.361ns	2.055
Root Na^+	744.6***	46.6***	111.4***	2.25ns	23.69***	0.861ns	1.75ns	1.833
Shoot Cl^-	73.25***	3185.25***	119.75***	15.069*	0.583ns	1.083ns	2.027ns	3.277
Root Cl^-	61.36***	2826.7***	186.86***	34.027***	2.1945**	13.36ns	2.027ns	2.334
Shoot Ca^{2+}	9.0**	560.11***	72.86***	0.000***	3.583ns	0.194ns	2.583ns	1.111
Root Ca^{2+}	18.77***	544.44***	72.69***	0.111ns	3.6944*	1.861ns	0.8611ns	1.055
Shoot K^+	1708.45***	53.77***	48.69***	1.778ns	3.027ns	0.361ns	0.3611ns	1.583
Root K^+	1808.4***	54.77***	49.094***	1.787ns	2.927ns	0.359ns	0.3591ns	1.493
df	1	2	2	1	2	2	2	24

*, ** and *** = Significant at 0.05, 0.01 and 0.001 levels respectively; ns = Non-significant; df = Degree of freedom.

Abbreviations: RL - Root length, SL - Shoot length, RFW - Root fresh weight, RDW - Root dry weight, SFW - Shoot fresh weight, SDW - Shoot dry weight, LA - Leaf area, RWC- Relative water contents, Chl. - Chlorophyll, T. Chl. - Total Chlorophyll, MDA - Melondialdehyde, Antho - Anyhocyanin, Flavo. - Flavonoid, Phenol. - Phenolics, Pro. - Proline, T.Pro. - Total proteins, TSS - Total soluble sugars, SOD - Superoxidase dismutase, POD - Peroxidase, Cat - Catalase, APX - Ascorbate peroxidase, GPX- Guaicol peroxidase, RF - Riboflavin

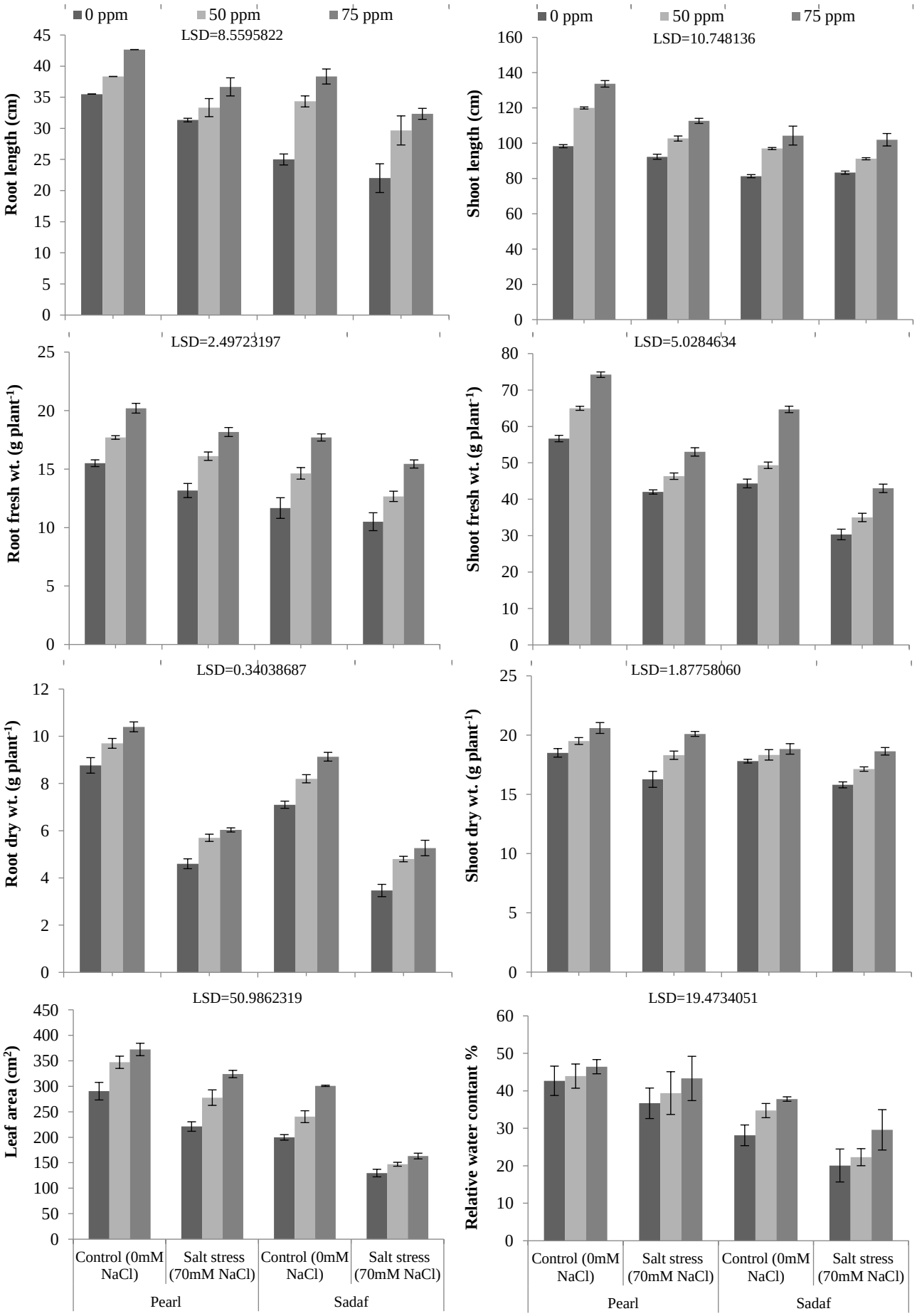

Fig. 1. Morpho-physiological attributes of maize (*Zea mays*) plants seeds of which were pre-treated with different riboflavin levels.

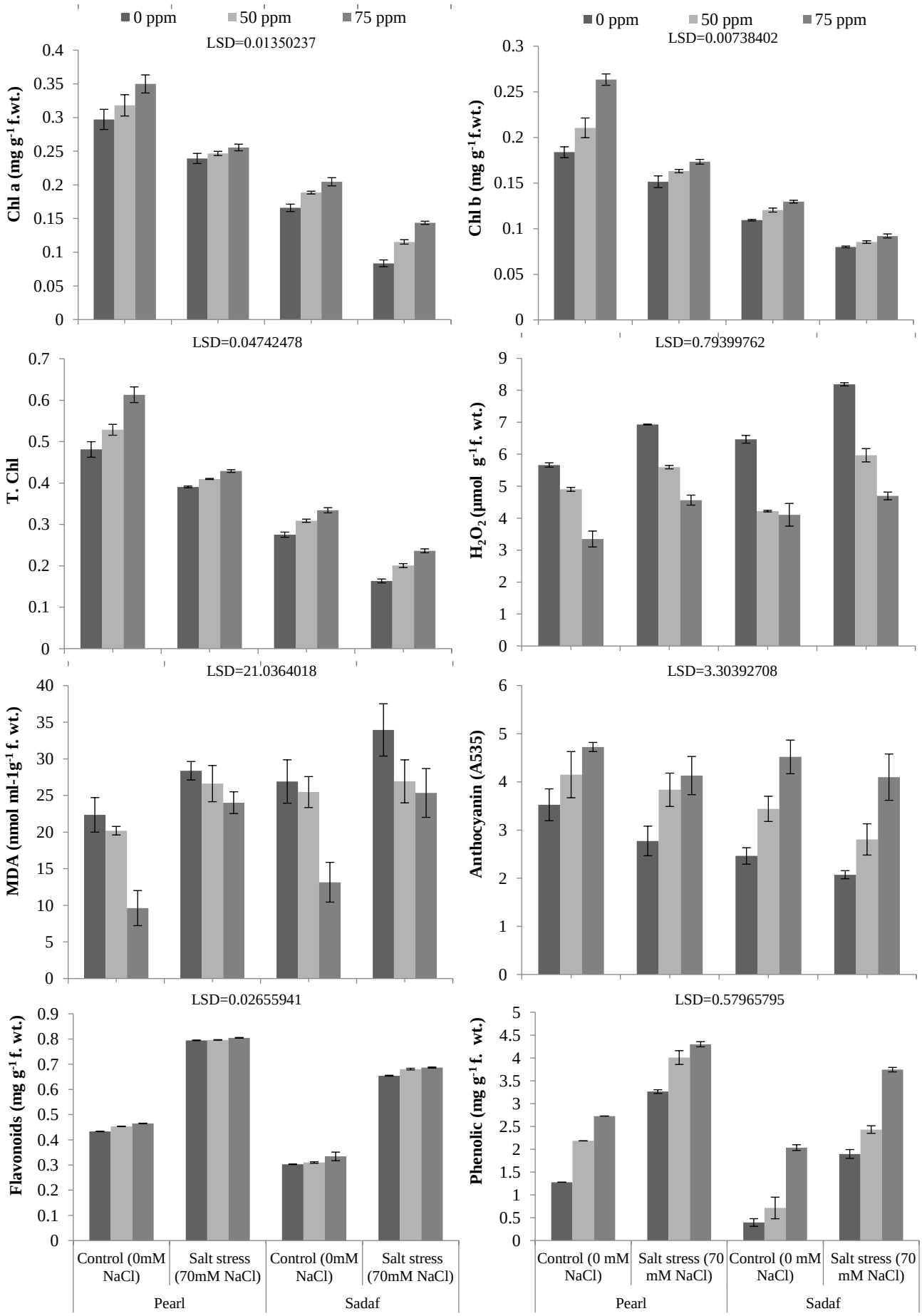

Fig. 2. Biochemical attributes of maize (*Zea mays*) plants seeds of which were pre-treated with different riboflavin levels.

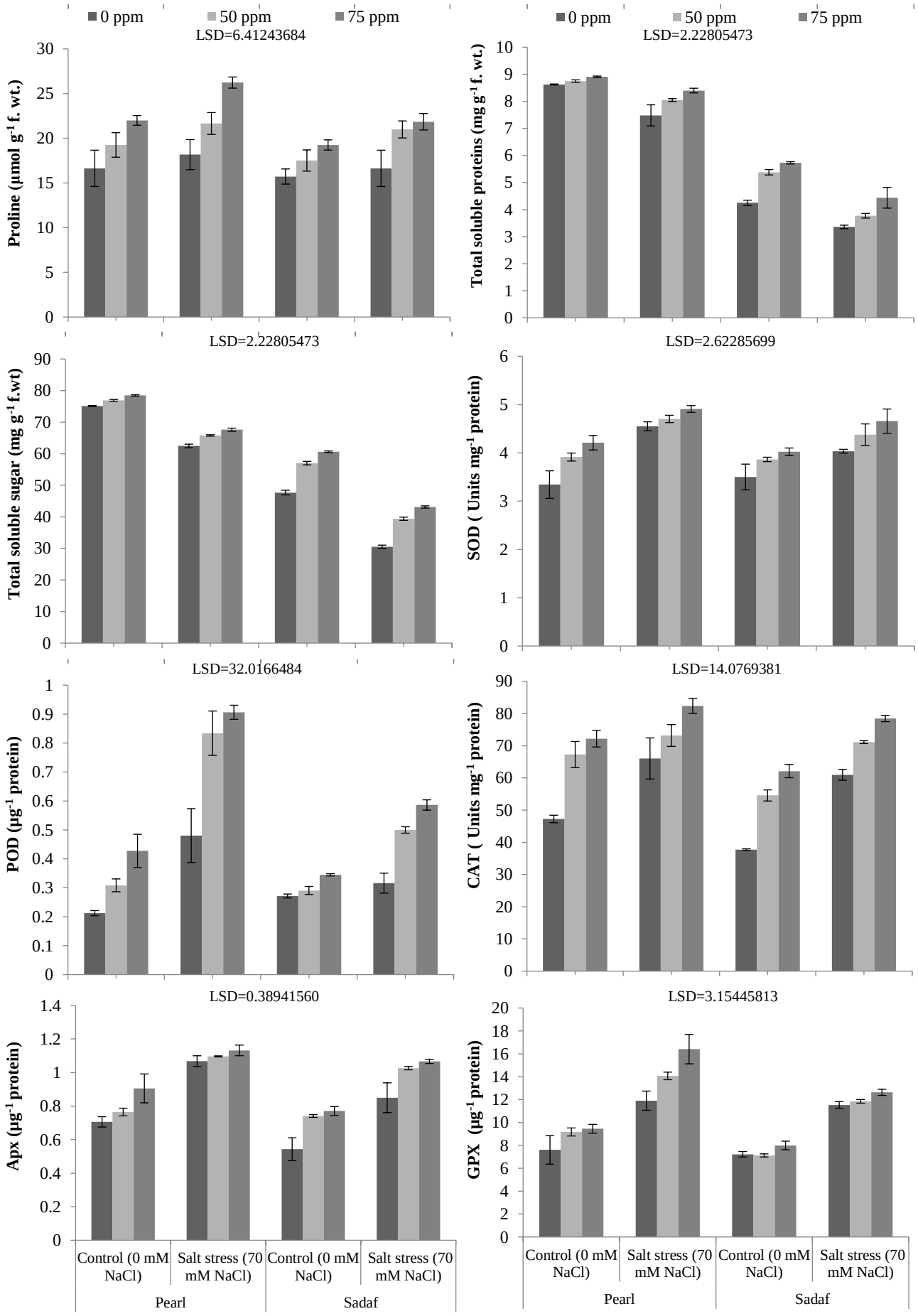

Fig. 3. Organic osmolytes and antioxidant enzyms activities of maize (*Zea mays* L.) plants seeds of which were pre-treated with different riboflavin levels.

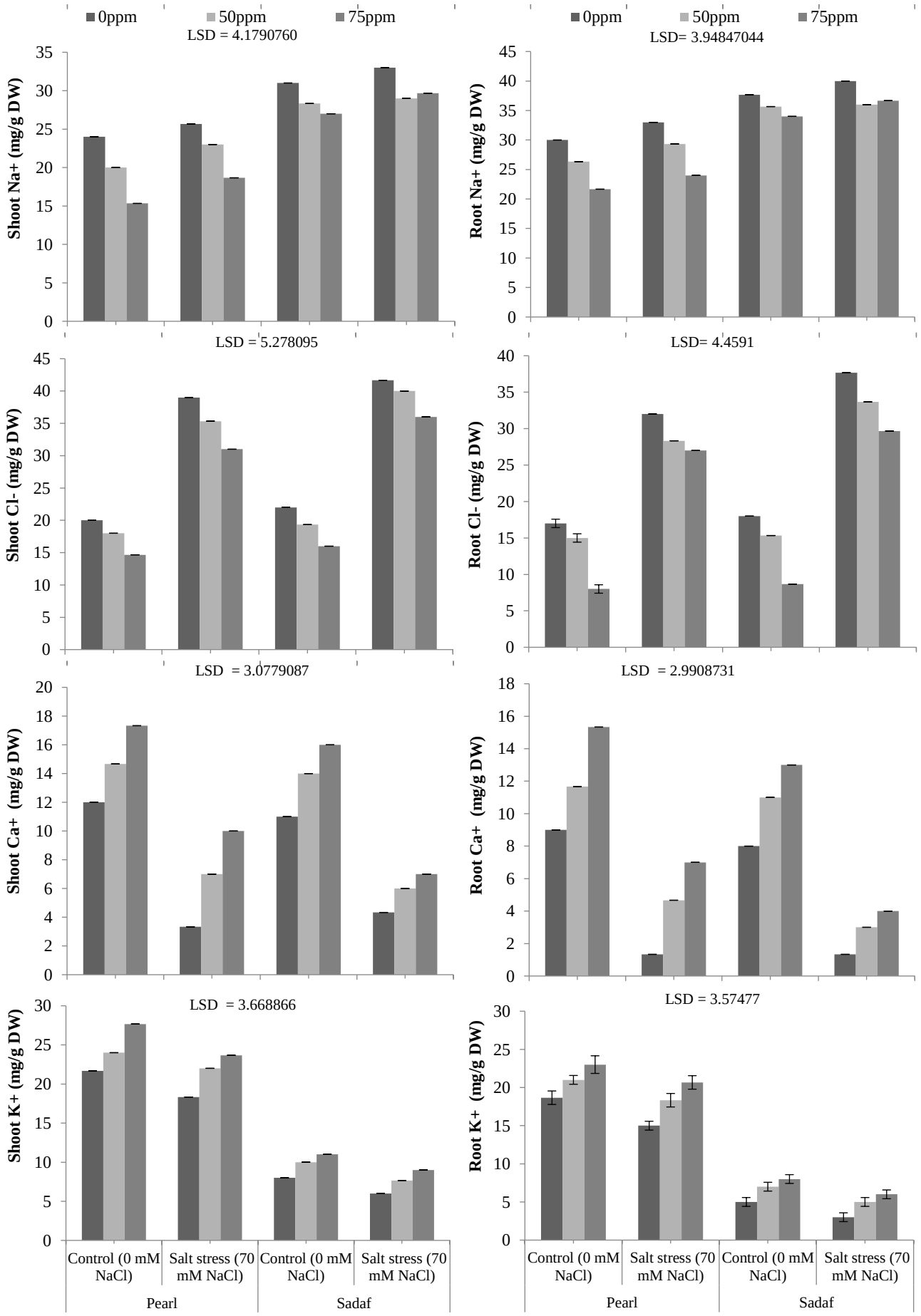

Fig. 4. Mineral ion contents of maize (*Zea mays* L.) plants seeds of which were pre-treated with different riboflavin levels.

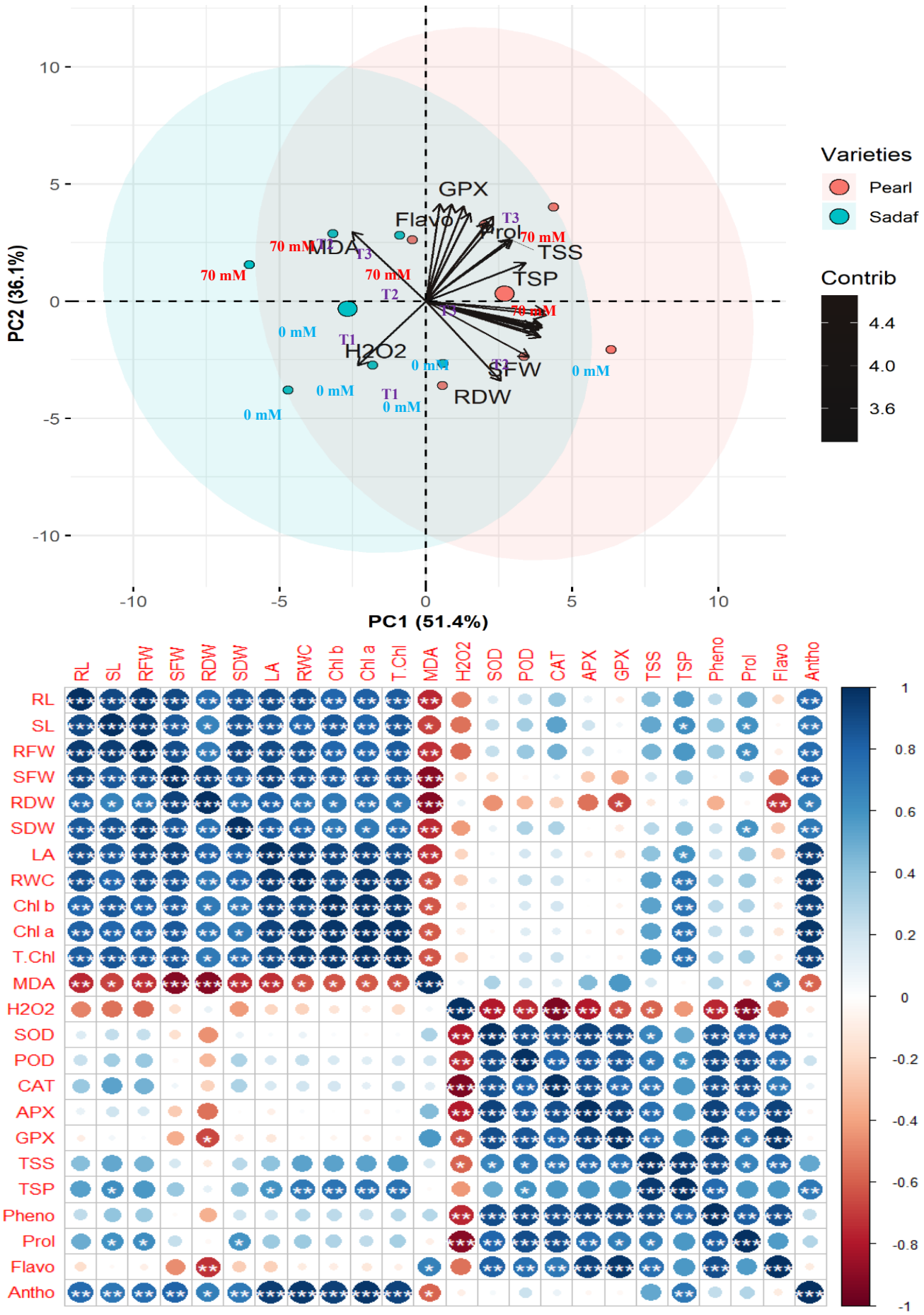

Fig. 5. **a)** PCA biplot, **b)** Pearson correlation 4, **a)** 441(T1- 0 ppm, T2-50 ppm, T3-75 ppm).

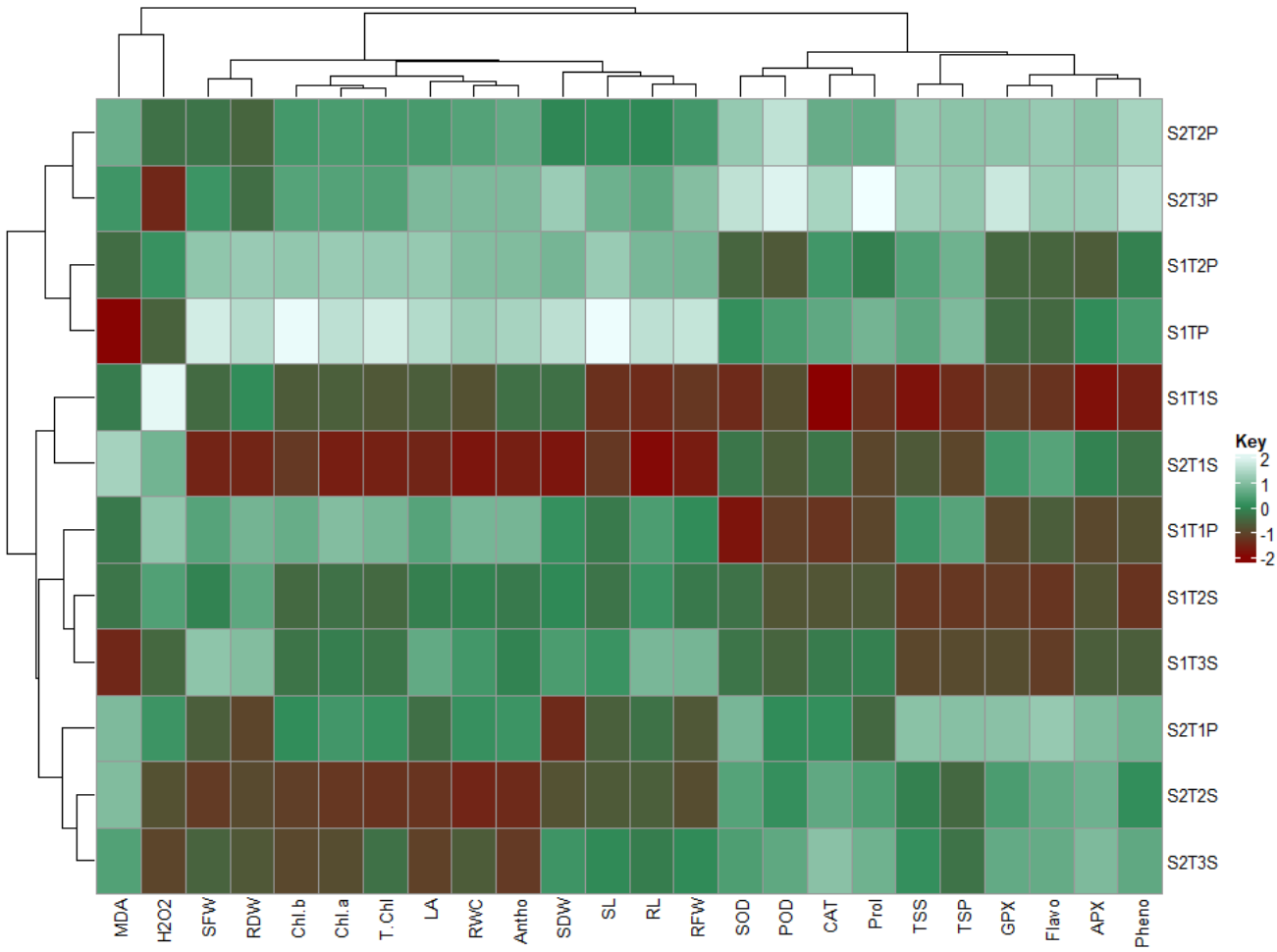

Fig. 6. Cluster heatmap for growth, photosynthetic pigments, biochemical of maize under Salt stress (S1-0 mM & S2-70 mM NaCl), Riboflavin (T1-0 ppm, T2-50 ppm, T3-75 ppm) and varieties (P-pearl, S, Sadaf).

Discussion

Salt stress is ubiquitously threatening factor for crop growth, development and yield. Salt containing soils consist of large amount of soluble salts and exchangeable sodium ions in root area. Sodium chloride stress restricts the growth rate of commercial crops; as it harms more than 800 million ha of land worldwide (Anon., 2000). Salt stress reduces plant growth as it provokes oxidative stress, ion imbalance and many secondary stresses like oxidative stress and lack of nutrients (Deinlein *et al*., 2014). In this experiment, two maize cultivars were examined to measure the morpho-physiological responses against saline soil (salt stress) and mitigating effect of vitamin B_2. A significant reduction in the plant growth and dry weight along with leaf area was noticed with exposure of salt stress to these maize cultivars. But riboflavin (RF) priming showed enhanced plant growth rate and biomass (Fig. 1, Table. 1). The mechanism behind this growth, leaf area and relative water content (RWC) is low amount of water uptaking by plant which causes slow plant growth. Another reason might be entrance of salt in the plant cell and cause cell injury and cell death (Munns, 2005). The limited supply of water, aeration and nutrients due to salt stress leads to decline in plant biomass (Attia *et al*., 2008). Another reason of growth suppression under salt stress is the shrinkage of cell, imbalanced supply of nutrients and damages to membrane integrity (Akhtar *et al*., 2015, Sobhanian *et al*., 2010). Vitamins play important role to enhance plant growth rate and biomass by reducing reactive oxygen species (ROS) production and enabling plants to uptake essential nutrients (Kaya *et al*., 2015). Similar results were defined by Safdar *et al*., (2019).

Photosynthesis is important food producing process in plants. Plants subjected to salinity stress show reduced photosynthetic rate. Present study depicted reduced photosynthetic pigments (chl. *a* chl. *b* and total chlorophyll) after plants subjected to salinity stress. However, riboflavin application played a positive role to enhance these photosynthetic pigments (Fig. 2, Table. 1). Chlorophyll reduction may be due to increased cholorophylase, a chlorophyll degradation enzyme produced as a result of elevated salinity level (Noreen & Ashraf, 2009). Another reason might be stomatal closure due to water deficiency by increased nutrient uptake in presence of high salt level (Chatrath *et al*., 2000). One more reason for reduced photosynthetic rate might be decreased efficiency of PS-II and reduced photon yield under salinity stress (Yang & Lu, 2005; Chu-Um & Kirdmanee, 2009). Vitamins showed positive response in increasing chlorophyll contents by increasing stomatal opening time and supressing production of chlorophyll

degradation enzyme in salt subjected plants (Wahid & Jamil, 2009). Same findings were observed in pumpkin (Sevengor *et al*., 2011).

Malondialdehyde (MDA) is the indicator of stress exposure, which results in membrane impairment when plant is exposed to salt stress (Katsuhara *et al*., 2005). In this experiment, MDA and hydrogen peroxide were accumulated under salinity stress and RF application decreased this accumulation rate (Fig. 2; Table 1). Membrane breakdown, ion leaking, lipid peroxidation and difficulty in nutrient uptake may be the reason of accumulated MDA (as membrane damage is associated with MDA accumulation) and hydrogen peroxides in plants to compete with increased production of oxidative stress indicators (Sacała, 2017). Vitamins decrease ion leakage and membrane breakdown because they act as growth regulator. Exogenously applied vitamin to seeds showed reduced accumulation of MDA and hydrogen peroxide under salinity stress (Tunc-Ozdemir *et al*., 2009). Khan *et al*., (2002) also concluded that MDA and H_2O_2 are accumulated in rice on salinity stress exposure. Same findings were observed in sorghum (Huang, 2018).

Anthocyanin and flavonoids are water soluble pigments the accumulation rate of which varied in plants exposed to abiotic stresses. Anthocyanin accumulation decreased, while flavonoids accumulation was increased in vegetative tissues of plants that were subjected to salinity stress. The current study showed that anthocyanin were accumulated under non stress conditions, while flavonoids were accumulated under salt stress conditions. RF application increased their accumulation rate (Fig. 2, Table. 1). Both anthocyanin and flavonoids play crucial role towards oxidative stress (Pervaiz *et al*., 2017) under salinity stress. When vitamins are applied exogenously, they show positive response to compete stress in order to produce increased anthocyanin and flavonoid concentration (Bahmani *et al*., 2015). When a plant is exposed to abiotic stress, reactive oxygen species are generated as a stress signal and ultimately activation of flavonoid and anthocyanin is regulated. This differential accumulation is due to increased oxidative stress and enhanced ROS which result in reduced osmotic damage, photo-protection and quenching of ROS.

Plants are adaptive to face many abiotic stresses i.e., salinity stress (Zhoa *et al*., 2020). This ability is increased due to metabolites (phenolics) in plants (Ali *et al*., 2006). Phenolics are important non enzymatic antioxidants having ability to donate hydrogen ions and ultimately accumulated under salinity stress (Posmyk *et al*., 2009). In this study, increased total phenolic content was observed in both *Z. mays* cultivars. Under salt stress Vitamin application showed positive accumulation of phenolic concentration under stress conditions (Fig. 3, Table 1). Phenolics have ability of ROS scavenging and hinders the conversion of H_2O_2 to free radicals under salinity stress (Pearse *et al*., 2005). Phenolics protect plasma membrane by reducing the oxidative stress effect of ROS and increasing the production rate of antioxidants. Navarro *et al*., (2006) conducted similar studies on pepper plant.

Prolines are water soluble compatible solutes. These are member of amino acid group the accumulation of which is elevated under salinity stress resulting in osmotic potential regulation. This is key amino acid in scavenging free radicals (Ashraf & Harris, 2004). Present experiment showed that free proline accumulation under salt stress and riboflavin (vitamin) application improved its accumulation rate (Fig. 3, Table 1). Plants facing high salinity stress accumulate more proline contents in order to resist abiotic stresses and to produce plant tolerance against these environmental stresses. Proline accumulation might be due to osmotic adjustment, and maintaining plant cell structure under salinity stress (Turan *et al*., 2009). Another possible reason of increased free proline accumulation might be the upregulation of pyroline-5-carboxylate and down regulation of PDH (proline dehydrogenase enzyme). Proline plays an important role in radicle detoxification and enzyme protection (Ashraf & Foolad, 2007). Increaesd proline accumulation under salinity stress was also observed in tomato (Amini & Ehsanpour, 2005) and in wheat (Turan *et al*., 2007). Application of vitamin (vit. B_2) showed positive improvement in proline content. Tuna *et al*., (2013) studied the effects of vitamins (vit. B_2) on proline accumulation in maize under salinity stress. Similar findings were observed in sunflower (Sayed & Gadallah, 2002).

Soluble proteins are the stress indicators in plants. In the present study, total soluble proteins showed an increase under salt stress in both maize cultivars, more accumulation was observed in salt tolerant variety than sensitive one. It has been described that after vitamin application plants species accumulate greater contents of protein under salinity stress (Fig. 3, Table 1). This protein accumulation might be due to synthesis of osmotin like protein which are involved in cell wall modification under abiotic stress condition to enhance osmotic adjustment and plant survival rate (Abdel Latef, 2010). Same results were concluded under salinity stress in wheat and in maize (Ali *et al*., 2022).

Data of current study showed increased soluble sugars accumulation under salt stress, however, maize plants raised from seeds treated with RF showed further accumulation in order to combat with salt stress conditions (Fig. 3; Table 1). This increase in total soluble sugars might be due to increased osmotic potential and increased water absorption after salinity induction (Abdelgawad *et al*., 2016, Nemati *et al*., 2011). It is an important feature of any plant to accumulate sugars under salinity exposure (Ashraf & Harris, 2004). Vitamins play antioxidant role in plants to mitigate deleterious effect of salinity stress. Soluble sugars are increased due to vitamin priming thus strengthening osmotic potential (Sayed & Gadallah, 2002). Similiar findings were reported in tomato (Shibli *et al*., 2007; Turan *et al.,* 2007) and in *Brassica napus* (Ahmadi *et al*., 2018).

Abiotic stresses produce reactive oxygen species in plants resulting in membrane damaging, deoxyribose nucleic acid damage, loss of carbohydrates and lipids ultimately result in oxidative stress. Present study showed increased antioxidant enzymes (POD, SOD, CAT, APX and GPX) activities under sodium chloride stress and riboflavin pre-treatment showed more

increase in the activities of these antioxidant enzymes (Fig. 3; Table 1). Antioxidant enzymes (SOD, POD, CAT, APX and GPX) are able to scavenge deleterious effect of ROS, where POD has hydrogen peroxide scavenging ability and SOD has singlet oxygen scavenging ability. Antioxidant enzymes i.e., CAT and APX with their high activity rate decrease the hydrogen peroxide level in plant cells resulting in improved stability of membrane and carbon dioxide fixation, because many chloroplast consisting of Calvin cycle enzymes are sensitive to H_2O_2 (restrict carbon dioxide fixation) (Yamazaki *et al*., 2003; Esfandiari *et al.,*2007). Increased antoxidative activities of enzymes are closely related to decreased oxidative stress (Candan & Tarhan, 2003). A high level of H_2O_2 directly inhibits CO_2 fixation. Antioxidant enzymes showed increased activities after vitamin (vitamin B2) treatment to seeds (Chi *et al*., 2021). Because vitamins act as coenzymes in metabolic pathways (Goyer *et al*., 2010) to protect the plant from abiotic stress by increasing tolerance level of plants against oxidative stress (Ahn *et al*., 2005). Similar results were also recorded in potato (Sattar *et al*., 2021) and tobacco (Wang *et al*., 2010).

This excessive increase in Na^+ and Cl^- ions results in nutrients imbalance, effects osmotic regulation which ultimately results in ion toxicity (Katerji *et al*., 2004; Arzani, 2008). Uptaking mechanism of ions is disturbed in membranes which results in increased Cl^- translocation in shoots (Yousif *et al*., 1972; Yong *et al.,* 2020). In this study, salt stress increased the sodium and chloride ion concentration in roots and shoots which ultimately disturbed growth rate and proper functioning of plant. Same results were observed by Chavan & Karadge (1986) and Turan *et al*., (2007). With the increase in salinity level, potassium is decreased in root and shoot because plasma membrane is depolarized by sodium ions and results in potassium ion leakage (Cramer *et al*., 1985). In current study, potassium ions decreased under salt stress. Similar, results were observed by Karmoker *et al*., (2008).

Conclusion

Present study concluded that salt stress exerted drastic effect on growth of maize plants by increasing reactive oxygen species concentration. Pre-sowing seed treatment with riboflavin showed positive response in improving salt stress tolerance in maize cultivars (Pearl and Sadaf). Of all the studied attributes including shoot and root fresh and dry weight and shoot and root length, chlorophyll contents, flavonoids, total phenolics, total soluble sugars, total soluble proteins, free proline and activities of catalase, ascorbate peroxidase and guaiacol peroxidase and mineral ions (K^+ and Ca^{2+}) Pearl variety of maize than that of Sadaf. The results obtained showed were higher in variety Pearl was more tolerant to salt stress compared to Sadaf.

References

Abd El-Samad, H.M., D. Mostafa and K.N. Abd El-Hakeem. 2017. The combined action strategy of two stressses, salinity and Cu++ on growth, metabolites and protein pattern of wheat plant. *Amer. J. Plant Sci*., 8(3): 625-643.

Abdel Latef, A. 2010. Changes of antioxidative enzymes in salinity tolerance among different wheat cultivars. *Cereal Res. Comm.*, 38(1): 43-55.

Abdelgawad, H., G, Zinta, M.M. Hegab, R. Pandey, H. Asard and W. Abuelsoud. 2016. High salinity induces different oxidative stress and antioxidant responses in maize seedlings organs. *Front. Plant Sci.*, 7: 276.

Abdulhamed, Z.A., A.O. Alfalahi and N.M. Abood. 2020. December. Riboflavin and Cultivars affecting genetic parameters in maize (*Zea mays* L.). In: *AIP Conference proceedings* (Vol. 2290, No. 1, p. 020020). AIP Publishing LLC.

Ahmad, M., Q. Ali, M. M. Hafeez and A. Malik. 2021. Improvement for biotic and abiotic stress tolerance in crop plants. *J. Biol. Res.*, 2021: 1 https://doi.org/10.54112/bcsrj.v2021i1.50

Ahmadi, F.I., K. Karimi and P.C. Struik. 2018. Effect of exogenous application of methyl jasmonate on physiological and biochemical characteristics of *Brassica napus* L. cv. Talaye under salinity stress. *S. Afr. J. Bot.*, 115: 5-11.

Ahn, I.P., S. Kim and Y.H. Lee. 2005. Vitamin B1 functions as an activator of plant disease resistance. *Plant Physiol*., 138(3): 1505-1515.

Akhtar, S.S., M.N. Andersen, M. Naveed, Z.A. Zahir and F. Liu. 2015. Interactive effect of biochar and plant growth-promoting bacterial endophytes on ameliorating salinity stress in maize. *Funct. Plant Biol.*, 42(8): 770-781.

Alayafi, A.A.M. 2020. Exogenous ascorbic acid induces systemic heat stress tolerance in tomato seedlings: Transcriptional regulation mechanism. *Environ. Sci. Pollut. Res*., 27(16): 19186-19199.

Ali, B., X. Wang, M.H. Saleem, M.A. Azeem, M.S. Afridi, M. Nadeem, M. Ghazal, T. Batool, A. Qayuum, A. Alatawi and S. Ali. 2022. *Bacillus mycoides* PM35 reinforces photosynthetic efficiency, antioxidant defense, expression of stress-responsive genes, and ameliorates the effects of salinity stress in maize. *Life*, 12(2): 219.

Ali, M.B., S. Khatun, E.J. Hahn and K.Y. Paek. 2006. Enhancement of phenylpropanoid enzymes and lignin in Phalaenopsis orchid and their influence on plant acclimatisation at different levels of photosynthetic photon flux. *Plant Growth Regul*., 49(2): 137-146.

Amini, F. and A.A. Ehsanpour. 2005. Soluble proteins, proline, carbohydrates and Na+/K+ changes in two tomato (*Lycopersicon esculentum* Mill.) cultivars under in vitro salt stress. *Amer. J. Biochem. Biotechnol.*, 1(4): 204-208.

Anonymous. 2000. Global network on integrated soil management for sustainable use of salt-affected soils. Available in: http://www.fao.org/ag/AGL/agll/spush/intro.htm (28 Jan.2015).

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant Physiol.*, 24(1): 1.

Arzani, A. 2008. Improving salinity tolerance in crop plants: a biotechnological view. *Vitro Cell. Dev. Biol. Plant*, 44: 373-383.

Asada, K. 1992. Ascorbate peroxidase–a hydrogen peroxide-scavenging enzyme in plants. *Physiol. Plant.*, 85(2): 235-241.

Ashraf, M. and M.R. Foolad. 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.*, 59(2): 206-216.

Ashraf, M. and P.J.C. Harris. 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Sci.*, 66(1): 3-16.

Attia, H., N. Arnaud, N. Karray and M. Lachaâl. 2008. Long-term effects of mild salt stress on growth, ion accumulation and superoxide dismutase expression of Arabidopsis rosette leaves. *Physiol. Plant*, 132(3): 293-305.

Bahmani, K., S.A.S. Noori, A.I. Darbandi and A. Akbari. 2015. Molecular mechanisms of plant salinity tolerance: A review. *Aust. J. Crop Sci*., 9(4): 321-336.

Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil*, 39(1): 205-207.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72(1-2): 248-254.

Cakmak, I. and W.J.T. Hors. 1991. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiol. Plant*, 83(3): 463-468.

Candan, N. and L. Tarhan. 2003. The correlation between antioxidant enzyme activities and lipid peroxidation levels in Mentha pulegium organs grown in Ca2+, Mg2+, Cu2+, Zn2+ and Mn2+ stress conditions. *Plant Sci.*, 165(4): 769-776.

Carleton, A.E. and W.H. Foote. 1965. A comparison of methods for estimating total leaf area of barley plants 1. *Crop Sci.*, 5(6): 602-603.

Chance, B. and A.C. Maehly. 1955. Assay of catalases and peroxidases. 136: 764-775.

Chatrath, A., P.K. Mandal and M. Anuradha. 2000. Effect of secondary salinization on photosynthesis in fodder oat (*Avena sativa* L.) genotypes. *J. Agron. Crop Sci.*, 184(1): 13-16.

Cha-Um, S. and C. Kirdmanee. 2009. Effect of salt stress on proline accumulation, photosynthetic ability and growth characters in two maize cultivars. *Pak. J. Bot.*, 41(1): 87-98.

Chavan, P.D. and B.A. Karadge. 1986. Growth, mineral nutrition, organic constituents and rate of photosynthesis in *Sesbania grandiflora* L. grown under saline conditions. *Plant Soil*, 93: 395-404.

Chi, Y.X., L. Yang, C.J. Zhao, I. Muhammad, X.B. Zhou and H. De Zhu. 2021. Effects of soaking seeds in exogenous vitamins on active oxygen metabolism and seedling growth under low-temperature stress. *Saudi J. Biol. Sci*., 28(6): 3254-3261.

Cramer, G.R., A. Lauchli and V.S. Polito. 1985. Displacement of Ca2+ by Na+ from the plasmalemma of root cell. A primary response to salt stress? *Plant Physiol.*, 79: 207-211.

Deinlein, U., A.B. Stephan, T. Horie, W. Lu, G. Xu and J.I. Schroeder. 2014. Plant salt-tolerance mechanisms. *Trends Plant Sci.*, 19(6): 371-379.

Díaz, F.J., J.C. Sanchez-Hernandez and J.S. Notario. 2021. Effects of irrigation management on arid soils enzyme activities. *J. Arid Environ.*, 185: 104330.

Dikobe, T.B., B. Mashile, R.P. Sinthumule and O. Ruzvidzo. 2021. Distinct morpho-physiological responses of maize to salinity stress. *Amer. J. Plant Sci.*, 12(6): 946-959.

Esfandiari, E., M.R. Shakiba, S.A. Mahboob, H. Alyari and M. Toorchi. 2007. Water stress, antioxidant enzyme activity and lipid peroxidation in wheat seedling. *J. Food Agric. Environ.*, 5(1): 149.

Fu, Z., P. Wang, J. Sun, Z. Lu, H. Yang, J. Liu and T. Li. 2020. Composition, seasonal variation, and salinization characteristics of soil salinity in the Chenier Island of the Yellow River Delta. *Glob. Ecol. Conserv.*, 24: e01318.

Garg, M., A. Sharma, S. Vats, V. Tiwari, A. Kumari, V. Mishra and M. Krishania. 2021. Vitamins in cereals: a critical review of content, health effects, processing losses, bioaccessibility, fortification, and biofortification strategies for their improvement. *Front. Nutr*., 8: 586815.

Giannopolitis, C.N. and S.K. Ries. 1977. Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiol*., 59(2): 309-314.

Gorji, T., A. Yildiri, N. Hamzehpou, A. Tanik and E. Sertel. 2020. Soil salinity analysis of Urmia Lake Basin using Landsat-8 OLI and Sentinel-2A based spectral indices and electrical conductivity measurements. *Ecol. Indic.*, 112: 106173.

Goyer, A. 2010. Thiamine in plants: aspects of its metabolism and functions. *Phytochemistry*, 71(14-15): 615-1624.

Hadia, E., A. Slama, L. Romdhane, M. Cheikh, M.A.S. Fahej and L. Radhouane. 2022. Seed priming of bread wheat varieties with growth regulators and nutrients improves salt stress tolerance particularly for the local genotype. *J. Plant Growth Regul.*, 1-15.

Hafeez, M.B., N. Zahra, K. Zahra, A. Raza, A. Khan, K. Shaukat and S. Khan. 2021. Brassinosteroids: Molecular and physiological responses in plant growth and abiotic stresses. *Plant Stress*, 2: 100029.

Hasanuzzaman, M., M.H.M. Bhuyan, F. Zulfiqar, A. Raza, S.M. Mohsin and J.A. Mahmud. 2020. Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. *Antioxidants*, 9(8): 681.

Hassanein, A.M., H.M. Azooz and F.A. Faheed. 2002. Riboflavin, vitamin B2 treatments counteract the adverse effect of salinity on growth and some relevant Physiologyical responses of *Hibiscus sabdariffa* L. seedlings. *Bull. Fac. Sci., Assiut. Univ. (Egypt)*, 31: 295-303.

Huang, R.D. 2018. Research progress on plant tolerance to soil salinity and alkalinity in sorghum. *J. Integr. Agric*., 17(4): 739-746.

Jiadkong, K., M. Nampei, S. Wangsawang and A. Ueda. 2023. Riboflavin seed priming activates OsNHXs expression to alleviate salinity stress in rice seedlings. *J. Plant Growth Regul.*, 42(5): 3032-3042.

Johnson, C.M. and A. Ulrich. 1959. Analytical methods for use in plant analysis. *California Agric*. *Exp. Station Bull.*, 766: 44-45.

Jones, M.M. and N.C. Turner. 1978. Osmotic adjustment in leaves of sorghum in response to water deficits. *Plant Physiol.*, 61(1): 122-126.

Julkunen-Titto, R. 1985. Phenolic constituents in the levels of northern willows: methods for precursors of clarified apple juice sediment. *J. Food Sci.*, 33: 254-257.

Karadeniz, F., H.S. Burdurlu, N. Koca and Y. Soyer. 2005. Antioxidant activity of selected fruits and vegetables grown in Turkey. *Turk. J. Agric. For.*, 29(4): 297-303.

Karmoker, J.L., S. Farhana and P. Rashid. 2008. Effects of salınıty on ıon accumulatıon ın maıze (*Zea mays* L. CV. BARI-7). *Bangladesh J. Bot.*, 37: 203-205.

Katerji, N., J. W. van Hoorn, A. Hamdy and M. Mastrorilli. 2004. Comparison of corn yield response to plant water stress caused by salinity and by drought. *Agric. Water Manag*., 65: 95-101.

Katsuhara, M., T. Otsuka and B. Ezaki. 2005. Salt stress-induced lipid peroxidation is reduced by glutathione S-transferase, but this reduction of lipid peroxides is not enough for a recovery of root growth in Arabidopsis. *Plant Sci*., 169(2): 369-373.

Kaya, C., M. Ashraf, M.N. Alyemeni, F.J. Corpas and P. Ahmad. 2020. Salicylic acid-induced nitric oxide enhances arsenic toxicity tolerance in maize plants by upregulating the ascorbate-glutathione cycle and glyoxalase system. *J Hazard. Mater.*, 399: 123020.

Kaya, C., M. Ashraf, O. Sonmez, A.L. Tuna, T. Polat and S. Aydemir. 2015. Exogenous application of thiamin promotes growth and antioxidative defense system at initial phases of development in salt-stressed plants of two maize cultivars differing in salinity tolerance. *Acta. Physiol. Plant*, 37(1): 1-12.

Kazemi, K. and H. Eskandari. 2012. Does priming improve seed performance under salt and drought stress. *J. Basic Appl. Sci. Res.*, 2(4): 3503-3507.

Khan, M.H., K.L. Singha and S.K. Panda. 2002. Changes in antioxidant levels in *Oryza sativa* L. roots subjected to NaCl-salinity stress. *Acta. Physiol. Plant*, 24(2): 145-148.

Khatun, S., T.S. Roy, M.N. Haque and B. Alamgir. 2016. Effect of plant growth regulators and their time of application on yield attributes and quality of soybean. *Int. J. Plant Soil Sci.*, 11(1): 1-9.

Lee, D.H., Y.S. Kim and C.B. Lee. 2001. The inductive responses of the antioxidant enzymes by salt stress in the rice (*Oryza sativa* L.). *J. Plant Physiol.*, 158(6): 737-745.

Munns, R., S. Goyal and J. Passioura. 2005. Salinity stress and its mitigation. University of California, Davis.

Nadarajah, K.K. 2020. ROS homeostasis in abiotic stress tolerance in plants. *Int. J. Mol. Sci.*, 21(15): 5208.

Navarro, J.M., P. Flores, C. Garrido and V. Martinez. 2006. Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. *Food Chem*., 96(1): 66-73.

Nemati, I., F. Moradi, S. Gholizadeh, M.A. Esmaeili and M.R. Bihamta. 2011. The effect of salinity stress on ions and soluble sugars distribution in leaves, leaf sheaths and roots of rice (*Oryza sativa* L.) seedlings. *Plant Soil & Environ.*, 57(1): 26-33.

Noreen, Z. and M. Ashraf. 2009. Changes in antioxidant enzymes and some key metabolites in some genetically diverse cultivars of radish (*Raphanus sativus* L.). *Environ. Exp. Bot.*, 67(2): 395-402.

Pearse, I., K.D. Heath and J.M. Cheeeseman. 2005. A partial characterization of peroxidase in Rhizophora mangle. *Plant Cell Environ*., 28: 612-622.

Pervaiz, T., J. Songtao, F. Faghihi, M.S. Haider and J. Fang. 2017. Naturally occurring anthocyanin, structure, functions and biosynthetic pathway in fruit plants. *J. Plant Biochem. Physiol*., 5(2): 1-9.

Posmyk, M.M., R. Kontek and K.M. Janas. 2009. Antioxidant enzymes activity and phenolic compounds content in red cabbage seedlings exposed to copper stress. *Ecotoxicol. Environ. Saf*., 72(2): 596-602.

Sacała, E. 2017. The influence of increasing doses of silicon on maize seedlings grown under salt stress. *J. Plant Nutr*., 40(6): 819-827.

Safdar, H., A. Amin, Y. Shafiq, A. Ali, R. Yasin, A. Shoukat, M.U. Hassan and M.I. Sarwar. 2019. A review: Impact of salinity on plant growth. *Nat. Sci*., 17(1): 34-40.

Santpoort, R. 2020. The drivers of maize area expansion in Sub-Saharan Africa. How policies to boost maize production overlook the interests of smallholder farmers. *Land*., 9(3): 68.

Sattar, F.A., B.T. Hamooh, G. Wellman, M. Ali, S.H. Shah, Y. Anwar and M.A.A. Mousa. 2021. Growth and biochemical responses of potato cultivars under *In vitro* lithium chloride and mannitol simulated salinity and drought stress. *Plants*, 10(5): 924.

Sayed, S.A. and M.A.A. Gadallah. 2002. Effects of shoot and root application of thiamin on salt-stressed sunflower plants. *Plant Growth Regul*., 36(1): 71-80.

Sevengor, S., F. Yasar, S. Kusvuran and S. Ellialtioglu. 2011. The effect of the effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidative enzymes of pumpkin seedling. *Afr. J. Agric. Res*., 6(21): 4920-4924.

Shiade, S.R.G. and B. Boelt. 2020. Seed germination and seedling growth parameters in nine tall fescue varieties under salinity stress. *Acta Agri. Scand*., 70(6): 485-494.

Shibli, R.A.M., G.G. Kushad, Yousef and M.A. Lila. 2007. Physiological and biochemical responses of tomato microshoots to induce salinity stress with associated ethylene accumulation. *J. Plant Growth Regul*., 51: 159-169.

Singh, A. 2022. Soil salinity: A global threat to sustainable development. *Soil Use Manag*., 38(1): 39-67.

Sobhanian, H., N. Motamed, F.R. Jazii, T. Nakamura and N. Komatsu. 2010. Salt stress induced differential proteome and metabolome response in the shoots of *Aeluropus lagopoides* (Poaceae), a halophyte C4 plant. *J. Proteome. Res*., 9(6): 2882-2897.

Tuna, A.L., C. Kaya, H. Altunlu and M. Ashraf. 2013. Mitigation effects of non-enzymatic antioxidants in maize (‘*Zea mays’* L.) Plants under salinity stress. *Aust. J. Crop. Sci.*, 7: 1181-1188.

Tunc-Ozdemir, M., G. Miller, L. Song, J. Kim, A. Sodek, S. Koussevitzky, A.N. Misra, R. Mittler and D. Shintani. 2009. Thiamin confers enhanced tolerance to oxidative stress in Arabidopsis. *Plant Physiol.*, 151(1): 421-432.

Turan, M. A., V. Katkat and S. Taban. 2007. Variations in proline, chlorophyll and mineral elements contents of wheat plants grown under salinity stress. *J. Agron*., 6(1):

Turan, M.A., A.H.A. Elkarim, N. Taban and S. Taban. 2009. Effect of salt stress on growth, stomatal resistance, proline and chlorophyll concentrations on maize plant. *Afr. J. Agric. Res.*, 4(9): 893-897.

Turan, M.A., N. Türkmen and N. Taban. 2007. Effect of NaCl on stomatal resistance and proline, chlorophyll, Na, Cl and K concentrations of lentil plants *J. Agron*., 6: 378-381.

Vaughan, M.M., A. Block, S.A. Christensen, L.H. Allen and E.A. Schmelz. 2018. The effects of climate change associated abiotic stresses on maize phytochemical defenses. *Phytochem. Rev*., 17(1): 37-49.

Velikova, V., I. Yordanov and A. Edreva. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. *Plant Sci.*, 151(1): 59-66.

Waheed AL-Mayahi, A.M. 2016. Influence of salicylic acid (SA) and ascorbic acid (ASA) on '*In vitro*' propagation and salt tolerance of date palm (*'Phoenix dactylifera'*L.) cv.'Nersy'. *Aust. J. Crop Sci.*, 10(7): 969-976.

Wahid, A. and A. Jamil. 2009. Inducing salt tolerance in canola (*Brassica napus* L.) by exogenous application of glycinebetaine and proline: response at the initial growth stages. *Pak. J. Bot*., 41(3): 1311-1319.

Wang, Y., C. Gao, Y. Liang, C. Wang, C. Yang and G. Liu. 2010. A novel bZIP gene from Tamarix hispida mediates physiological responses to salt stress in tobacco plants. *J. Plant Physiol.*, 167(3): 222-230.

Waqas, M.A., X. Wang, S.A. Zafar, M.A. Noor, H.A. Hussain, M.A. Nawaz and M. Farooq. 2021. Thermal stresses in maize: Effects and management strategies. *Plants*, 10(2): 293.

Wei, Y., J. Ding, S. Yang, F. Wang and C. Wang. 2021. Soil salinity prediction based on scale-dependent relationships with environmental variables by discrete wavelet transform in the Tarim Basin. *Catena*, 196: 104939.

Wichelns, D. and M. Qadir. 2015. Achieving sustainable irrigation requires effective management of salts, soil salinity, and shallow groundwater. *Agric. Water Manag*., 157: 31-38.

Yamazaki, J.Y., A. Ohashi, Y. Hashimoto, E. Negish, S. Kumagai, T. Kubo, T. Oikawa, E. Maruta and Y. Kamimura. 2003. Effects of high light and low temperature during harsh winter on needle photodamage of Abies mariesii growing at the forest limit on Mt. Norikura in Central Japan. *Plant Sci*., 165(1): 257-264.

Yang, X. and C. Lu. 2005. Photosynthesis is improved by exogenous glycinebetaine in salt-stressed maize plants. *Physiol. Plant*, 124(3): 343-352.

Yemm, E.W. and A. Willis. 1954. The estimation of carbohydrates in plant extracts by anthrone. *Biochem. J.*, 57(3): 508.

Yong, M.T., C.A. Solis, B. Rabbi, S. Huda, R. Liu, M. Zhou, L. Shabala, G. Venkatraman, S. Shabala and Z. Chen 2020. Leaf mesophyll K+ and Cl− fluxes and reactive oxygen species production predict rice salt tolerance at reproductive stage in greenhouse and field conditions. *Plant Growth Regul*., 92(1): 53-64.

Yoshii, K., K. Hosomi, K. Sawane and J. Kunisawa. 2019. Metabolism of dietary and microbial vitamin B family in the regulation of host immunity. *Front. Nutr.*, 6: 48.

Yousif, H.Y., F.T. Bingham and D.M. Yermason. 1972. Growth, mineral composition, and seed oil of sesame (*Sesamum indicum* L.) as affected by NaCl. *Soil Sci. Soc. Amer. Proc*., 36: 450-453.

Zahra, N., Z. Raza and S. Mahmood. 2020. Effect of salinity stress on various growth and physiological attributes of two contrasting maize genotypes. *Braz. Arch. Biol. Tech.*, 63.

Zhang, C., Y. Ma, X. Zhao and J. Mu. 2009. Influence of copigmentation on stability of anthocyanins from purple potato peel in both liquid state and solid state. *J. Agric. Food Chem*., 57: 9503-9508.

Zhao, C., H. Zhang, C. Song, J.K. Zhu and S. Shabala. 2020. Mechanisms of plant responses and adaptation to soil salinity. *The Innovation*, 1(1): 100017.

Zhou, T., H. Li, M. Shang, D. Sun, C. Liu and G. Che. 2021 Recent analytical methodologies and analytical trends for riboflavin (vitamin B2) analysis in food, biological and pharmaceutical samples. *TrAC Trends Anal. Chem*., 143: 116412.

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