Article

Deciphering *Plantago ovata* Forsk Leaf Extract Mediated Distinct Germination, Growth and Physio-Biochemical Improvements under Water Stress in Maize (*Zea mays* L.) at Early Growth Stage

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Abstract: Use of *Plantago ovata* Forsk leaf (also known as blond plantain or isabgol) extract is a novel approach for ameliorating water stress in various agronomic crops such as maize (*Zea mays* L.). To examine the potential roles of *P. ovata* extract (0, 20 and 40%) in increasing seed germination, plant growth, photosynthetic measurements, stomatal properties, oxidative stress and antioxidant response, ions uptake and the relationship between studied parameters, we investigated the impacts of its short-term seed priming on *Z. mays* L. elite cultivar "Cimmyt-Pak" under a control environment and a water deficit stress environment (induced by PEG). It was evident that water deficit stress conditions induced a negative impact on plant growth, stomatal properties and ion uptake in different organs of *Z. mays*. The decrease in growth-related attributes might be due to overproduction of oxidative stress biomarkers, i.e., malondialdehyde (MDA) contents, hydrogen peroxide (H_2O_2) initiation, and electrolyte leakage (%), which was also overcome by the enzymatic antioxidants, i.e., superoxidase dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) and non-enzymatic antioxidants, which increased under the water stress environment. However, seed priming with *P. ovata* extract positively increased germination rate and growth profile, and protected photosynthetic apparatus and stomatal properties by decreasing oxidative stress indicators and increasing activities of antioxidant compounds. Our results also depicted that the optimum concentration of *P. ovata* extract for *Z. mays* seedlings under water stress conditions was 20%, while a

further increase in *P. ovata* extract (40%) induced a non-significant negative impact on growth and biomass of *Z. mays* seedling. In addition, the effect was more promising on *Z. mays* seedlings when grown under controlled conditions. Here, we concluded that the understanding of the role of seed priming with *P. ovata* extract in the increment of growth-related attributes, photosynthetic apparatus (*Pn*, *Gs*, *Ts* and *Ci*) and nutrient uptake (Ca^{2+}, Fe^{2+}, P and Mg^{2+}) introduces new possibilities for their effective use in water deficit stress environments and shows a promising foundation for *Z. mays* tolerance against water deficit stress conditions.

Keywords: agronomic crop; antioxidant compounds; isabgol extract; nutrient uptake; oxidative stress; drought stress

1. Introduction

Environmental variations due to abiotic stresses, such as drought, heat, cold and salinity, adversely affect and limit agricultural productivity in developing countries including Pakistan [1,2]. About 33% of the world's agricultural land is facing water imbalance and promoting drought vulnerability, which may drastically decrease the growth and yield of cereal crops [3]. Abiotic stresses, such as drought, can lead to a number of alterations in plant growth and composition, such as a decrease in growth-related attributes, affecting photosynthetic machinery, which ultimately causes a reduction in the dry biomass of the plant and the plant is unable to uptake to accumulate essential nutrients from the soil [4–6]. The decline in photosynthetic pigments in the plant leaves and induced oxidative stress indicators and disturbance in protein biosynthesis are factors that have negatively affected plants with generations of reactive oxygen species (ROS) [7,8]. In addition, water deficient conditions also depend upon the length of harvest of the plant, total water contents in the soil, ecological growth factors and the specific type of plant [4,9]. By nature, plants have enzymatic antioxidants, i.e., superoxidase dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX), non-enzymatic antioxidants (flavonoids, phenolics, ascorbic acid and anthocyanin) and glyoxalase detoxification active mechanisms to overcome the destructive damage [10–12]. A slow photosynthesis rate, less leaf expansion, narrowing of stomata followed by blocking, ROS production and less transportation are the significant drought driving factors that ultimately reduce crop production and yield [5,13]. From preventing membrane degeneration, enzymes and macromolecule's lysis activation of the plant's stress defensive mechanism is highly important for the plant's survival.

Zea mays (maize), *Triticum aestivum* (wheat) and *Oryza sativa* (rice) are the major staple crops; among them, maize holds prime importance due to its different uses in the food and feed industry [14]. As *Zea mays* is an important food crop, it is known to be a social security for farmers [15]. Maize is an important feed and industrial source. Drought conditions limit its sowing and production across the globe [16] and its yield has been compromised by up to 25–30% in some fragile zones [17]. From 1980–2015, drought has reduced wheat and maize yields by up to 21 and 40%, respectively [18]. According to previous studies, loss in maize yield depends on drought intensity and the growth factors affected due to it [19–21]. Drought induced at the seedling stage reduces plant biomass dramatically in maize seedlings.

Accumulation of salts in the plant cell triggers water influx that is helpful to maintain the osmotic balance. Exogenously applied solutes may help out in these particular conditions by promoting the endosmosis of water in the plant cell [22,23]. Various organic mixtures (consisting of a heterocyclic setup) actively play a crucial role in existing conditions. Derivatives of heterocyclic thiazine are biologically present in biomolecules, which exhibit antimycotic and antiviral properties as well as a growth regulators [24]. Changes in the protein content and late embryogenic abundant protein formation due to priming treatment makes seeds more efficient and able to tolerate the drought [25].

Activation of a number of cellular and molecular processes effectively reduces the effects of various abiotic stresses after priming [25]. In the current scenario, the use of *P. ovata* leaf extract is becoming more efficient for the priming purpose in tackling the effects of the elevation of stresses in many agricultural crops [26,27]. *Plantago ovata*, also known as ispaghula or desert Indianwheat, is a medicinal plant. It is used for the treatment of intestinal disease and bowel habits [28]. Phytochemical studies of *Plantago ovata* have determined the presence of various metabolites, such as alkaloids, caffeic acid derivatives, coumarins, fats and oils, mucilage, polysaccharides, sterols and salicylic acid. A hypothesis made is that *P. ovata* extract, as an agent against abiotic stress, may be studied in *Zea mays* as a drought tolerant agent. The main objectives of this study were: (i) measurement of the oxidative stress levels, activation of antioxidant mechanisms and ion uptake in maize in water deficit conditions; (ii) investigation of the drought elevating impact of *P. ovata* extract through seed priming in maize.

2. Materials and Methods

2.1. Site and Conditions

In this experiment, seeds of the elite maize variety "Cimmyt-Pak" were obtained from the Ayyub Agricultural Research Institute, Faisalabad, Pakistan (AARI). The experiment was conducted in Department of Botany, Government College University, Faisalabad, Pakistan (Coordinates: 31.4162° N, 73.0699° E; Elevation m a.s.l.: 186). The collected seeds were sterilized with bleaching powder and washed with distilled water. *Plantago ovata* leaf extract solutions (0, 20 and 40%) were prepared for seed priming. The *P. ovata* leaves were collected from the botanical garden, department of Botany, Government College University, Faisalabad, Pakistan, where *P. ovata* has grown annually for research purposes for many years. The leaves of P. ovata were washed carefully with distilled water and then dried and crushed with the help of grinding machine. Then the filtrate (as it is water soluble) was used for seed priming at various concentrations. For seed priming, seeds were soaked in *P. ovata* extract for one night, then they were air dried to attain the original moisture. Fifteen seeds were placed per plate covered with double layered filter paper with three replicates making CRD (completely randomized design). Half of the petri plates were provided in PEG-8000 solution to create water deficit experimental group and half were used as control group free from PEG-8000. The standard full strength Hoagland's solution with following composition in μmol L^{-1} ($Ca(NO_3)_2$, 2000; KH_2PO_4, 100; KNO_3, 3000; $MgSO_4$, 1000; H_3BO_3, 50; $MnCl_2 \cdot 4H_2O$, 0.05; $ZnSO_4 \cdot 7H_2O$, 0.8; $CuSO_4 \cdot 5H_2O$, 0.3; $H_2MO_4 \cdot H_2O$, 0.10; FeNa-CA, 12.5) was used as nutrient solution and 10 mL of it was poured in every plate. After planting the experiment by providing PEG-8000 and Hoagland's solution, the water level was maintained on daily basis. Upon germination, thinning was carried out to keep eight healthy plants of same size and vigor in every plate. The germination data was recorded for 10 days after sowing and plants were harvested for analysis three weeks after germination.

2.2. Morphological Traits and Data Collection

All seedlings were rooted-up in July 2019 to study different growth, germination and other morphological and physiological parameters. Analysis of different biological parameters were performed in Government College University, Pakistan. The leaf in each treatment was picked at a rapid growth stage during 09:00–10:30 a.m. The sampled leaves were washed with distilled water, immediately placed in liquid nitrogen and stored in a freezer at low temperature (−80 °C) for further analysis. Germination index, time to 50% germination, coefficient of uniformity of emergence, mean germination time and germination energy (E) was measured by following the method presented by Wiesner [29], Coolbear et al. [30], Bewley and Black [31] and Ruan et al. [32]. Germination percentage (%) was calculated by the following formula

$$G\% = \text{No. of germinated seeds}/\text{Total number of seeds} \times 100 \quad (1)$$

Stomata were counted at random in 30 visual sections on the abaxial epidermis, and final tallies were used to calculate stomatal density. We used Image J software for measuring stomatal lengths, widths, and apertures.

Plants in each treatment were harvested and separated into roots and shoots for growth and morphology traits. Shoot length was defined as the length of the plant from the surface growth medium line of the Petri dish to the tip of the uppermost shoot and root length was also measured. Shoot fresh weight was measured with the help of a digital weighing balance and root fresh weight was also measured. After that, plant samples were oven dried for 1 h at 105 °C, then 65 °C for 72 h until the weight become uniform and dry biomass was recorded. Roots were washed with distilled water and dipped in 20 mM Na_2EDTA for 15–20 min, washed thrice with distilled water and finally with deionized water, and then oven dried for further analysis.

2.3. Determination of Photosynthetic Pigments and Gas Exchange Parameters

Leaves were collected for determination of their chlorophyll and carotenoid contents. For chlorophylls, 0.1 g of fresh leaf sample was extracted with 8 mL of 95% acetone for 24 h at 4 °C in the dark. The absorbance was measured by a spectrophotometer (UV-2550; Shimadzu, Kyoto, Japan) at 646.6, 663.6 and 450 nm. Chlorophyll content was calculated by the standard method of Arnon [33].

Gas exchange parameters were also measured during the same days. Net photosynthesis (*Pn*), leaf stomatal conductance *(Gs)*, transpiration rate (*Ts*), and intercellular carbon dioxide concentration (*Ci*) were measured from three different plants in each treatment group. Measurements were conducted between 11:30 and 13:30 on days with clear sky. Rates of leaf *Pn*, *Gs*, *Ts* and *Ci* were measured with a LI-COR gas exchange system (LI-6400; LI-COR Biosciences, Lincoln, NE, USA) with a red-blue LED light source on the leaf chamber. In the LI-COR cuvette, CO_2 concentration was set as 380 mmol mol^{-1} and LED light intensity was set at 1000 mmol m^{-2} s^{-1}, which is the average saturation intensity for photosynthesis in *O. sativa* [34].

2.4. Determination of Oxidative Stress Indicators

The degree of lipid peroxidation was evaluated as malondialdehyde (MDA) contents. Briefly, 0.1 g of frozen leaves were ground at 4 °C in a mortar with 25 mL of 50 mM phosphate buffer solution (pH 7.8) containing 1% polyethene pyrrole. The homogenate was centrifuged at 10,000× *g* at 4 °C for 15 min. The mixtures were heated at 100 °C for 15–30 min and then quickly cooled in an ice bath. The absorbance of the supernatant was recorded by using a spectrophotometer (xMark™ Microplate Absorbance Spectrophotometer; Bio-Rad, Hercules, CA, USA) at wavelengths of 532, 600 and 450 nm. Lipid peroxidation was expressed as l mol g^{-1} by using the formula: 6.45 (A532–A600)–0.56 A450. Lipid peroxidation was measured by using a method previously published by Heath and Packer [35].

To estimate H_2O_2 content of plant tissues (root and leaf), 3 mL of sample extract was mixed with 1 mL of 0.1% titanium sulfate in 20% (*v/v*) H_2SO_4 and centrifuged at 6000× *g* for 15 min. The yellow color intensity was evaluated at 410 nm. The H_2O_2 level was computed by extinction coefficient of 0.28 $mmol^{-1}$ cm^{-1}. The contents of H_2O_2 were measured using the method presented by Jana and Choudhuri [36].

Stress-induced electrolyte leakage (EL) of uppermost stretched leaves was determined by using methodology of Dionisio-Sese and Tobita [37]. The leaves were cut into minor slices (5 mm length) and placed in test tubes having 8 mL distilled water. These tubes were incubated and transferred into water bath for 2 h prior to measuring the initial electrical conductivity (EC_1). The samples were autoclaved at 121 °C for 20 min, and then cooled down to 25 °C before measuring the final electrical conductivity (EC_2). Electrolyte leakage was calculated by the following formula

$$EL = (EC_1/EC_2) \times 100 \quad (2)$$

2.5. Determination of Antioxidant Enzyme Activities

To evaluate enzyme activities, fresh leaves (0.5 g) were homogenised in liquid nitrogen and 5 mL of 50 mmol sodium phosphate buffer (pH 7.0) including 0.5 mmol Ethylenediaminetetraacetic Acid (EDTA) and 0.15 mol NaCl. The homogenate was centrifuged at 12,000× *g* for 10 min at 4 °C, and the supernatant was used for measurement of superoxidase dismutase (SOD) and peroxidase (POD) activities. SOD activity was assayed in 3 mL reaction mixture containing 50 mM sodium phosphate buffer (pH 7), 56 mM nitro blue tetrazolium, 1.17 mM riboflavin, 10 mM methionine and 100 µL enzyme extract. Finally, the sample was measured by using a spectrophotometer (xMark™ Microplate Absorbance Spectrophotometer; Bio-Rad, Hercules, CA, USA). Enzyme activity was measured using a method by Chen and Pan [38] and expressed as U g^{-1} FW.

POD activity in the leaves was estimated using the method of Sakharov and Ardila [39] by using guaiacol as the substrate. A reaction mixture (3 mL) containing 0.05 mL of enzyme extract, 2.75 mL of 50 mM phosphate buffer (pH 7.0), 0.1 mL of 1% H_2O_2 and 0.1 mL of 4% guaiacol solution was prepared. Increases in the absorbance at 470 nm because of guaiacol oxidation were recorded for 2 min. One unit of enzyme activity was defined as the amount of the enzyme.

Catalase (CAT) activity was analyzed according to Aebi [40]. The assay mixture (3.0 mL) was comprised of 100 µL enzyme extract, 100 µL H_2O_2 (300 mM) and 2.8 mL 50 mM phosphate buffer with 2 mM ETDA (pH 7.0). The CAT activity was measured from the decline in absorbance at 240 nm as a result of H_2O_2 loss (ε = 39.4 mM^{-1} cm^{-1}).

Ascorbate peroxidase (APX) activity was measured according to Nakano and Asada [41]. The mixture containing 100 µL enzyme extract, 100 µL ascorbate (7.5 mM), 100 µL H_2O_2 (300 mM) and 2.7 mL 25 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0) was used for measuring APX activity. The oxidation pattern of ascorbate was estimated from the variations in wavelength at 290 nm (ε = 2.8 mM^{-1} cm^{-1}).

2.6. Determination of Non-Enzymatic Antioxidants, Sugars and Proline Contents

Plant ethanol extracts were prepared for the determination of non-enzymatic antioxidants and some key osmolytes. For this purpose, 50 mg of plant dry material was homogenized with 10 mL ethanol (80%) and filtered through Whatman No. 41 filter paper. The residue was re-extracted with ethanol and the two extracts were pooled together to a final volume of 20 mL. The determination of flavonoids [42], phenolics [43], ascorbic acid [44], anthocyanin [45] and total sugars [46] was performed from the extracts.

Fresh leaf material (0.1 g) was mixed thoroughly in 5 mL aqueous sulphosalicylic acid (3%). The mixture was centrifuged at 10,000× *g* for 15 min and aliquot (1 mL) was poured into a test tube having 1 mL acidic ninhydrin and 1 mL glacial acetic acid. The reaction mixture was first heated at 100 °C for 10 min and then cooled in an ice bath. The reaction mixture was extracted with 4 mL toluene and test tubes were vortexed for 20 s and cooled. Thereafter, the light absorbance at 520 nm was measured by using UV–VIS spectrophotometer (Hitachi U-2910, Tokyo, Japan). The free proline content was determined on the basis of standard curve at 520 nm absorbance and expressed as µmol $(g\ FW)^{-1}$ [47].

2.7. Determination of Nutrient Contents

For nutrient analysis, plant roots and shoots were washed twice in redistilled water, dipped in 20 mM EDTA for 3 s and then, again washed with deionized water twice for the removal of adsorbed metal on plant surface. The washed samples were then oven dried for 24 h at 105 °C. The dried roots and shoots were digested by using wet digestion method in HNO_3: $HClO_4$ (7:3 *v/v*) until clear samples were obtained. Each sample was filtered and diluted with redistilled water up to 50 mL. The root and shoot contents of Fe^{2+}, Mg^{2+}, Ca^{2+} and P were analyzed by using Atomic Absorption Spectrophotometer (AAS) model Agilent 240FS-AA.

2.8. Statistical Analysis

Statistical analysis of data was performed with analysis of variance (ANOVA) by using a statistical program Co-Stat version 6.2, Cohorts Software, 2003, Monterey, CA, USA. All the data obtained was tested by one-way analysis of variance (ANOVA). Thus, the differences between treatments were determined by using ANOVA, and the least significant difference test ($p < 0.05$) was used for multiple comparisons between treatment means. Logarithmic or inverse transformations were performed for data normalization, where necessary, prior to analysis. Pearson's correlation analysis was performed to quantify relationships between various analyzed variables. The graphical presentation was carried out by using Origin-Pro 2017 (Systat Software Inc., San Jose, CA, USA). The RStudio was used to calculate Pearson's correlation. Furthermore, the plots of principal component analysis and heatmap on *T. aestivum* parameters were carried out by using the RStudio.

3. Results

3.1. Germination and Post-Germinating Growth Characters

In the present study, we observed the effects of different regimes of *P. ovata* extract through seed priming of a maize cultivar (Cimmyt-Pak), under control and PEG-induced water deficit conditions. Graphical data in Figures 1 and 2 clearly represents germination and other growth attributes under the control and stress conditions. All of the germination and growth parameters significantly ($p > 0.05$) increased in seeds that were provided with *P. ovata* extract treatment for one night, when contrasted with the seeds that were not provided with the priming solution in the control condition. Figures 1 and 2 also indicate that growth attributes decreased in drought conditions with respect to the control sample. Priming of seeds accelerated growth parameters even in drought. A decreasing trend in germination and growth parameters was noted with increases of the extract concentration to 40% and a maximum increase in these parameters was noted at the 20% treatment level (Figures 1 and 2).

3.2. Photosynthetic Measurements and Stomatal Properties

Drought significantly ($p > 0.05$) reduced the photosynthetic running machinery content (Chl a, b and carotenoids) and stomatal properties in the elite maize cultivar under study, as presented in Figures 3 and 4. Graphics of data related to photosynthetic pigment content and stomatal attributes indicate a significant increase in seeds that were given priming treatment in both control and drought conditions. However, different increasing and decreasing trends in stomatal conductivity were observed according to Figures 3 and 4. Stomatal conductivity level accelerated with drought; a reduction was noted for maize seeds that were provided with enough water. Treatment with 20% extract efficiently increased the photosynthetic pigments and stomatal characters, both in drought and stress conditions. While, higher regime concentrations of *P. ovata* extract (40%) did not increase described properties any further.

3.3. Oxidative Stress, Antioxidant Response and Sugars

Oxidative stress indicators in maize seedlings primed with *P. ovata* extract under control and drought conditions are represented in Figure 5. An increasing trend in oxidative stress factors existed in maize seedlings facing minimal water availability, in contrast to those seedlings primed with *P. ovata* leaf extract. Oxidative stress level declined in maize seedlings treated with 20% *P. ovata* extract in both control and experimental groups, while oxidative stress biomarkers were enhanced more with further increases in priming agent concentration (40%) in the maize cultivar seedlings under drought and enough water available conditions, when compared with seeds with no treatment.

The antioxidant (enzymatic and non-enzymatic) activities increased in the maize cultivar (Cimmyt-Pak) seedlings that were given PEG-induced water stress. Figure 6 represents data related to enzymatic antioxidants and Figure 7 is related to non-enzymatic antioxidants. Antioxidant activity was elevated in all maize (Cimmyt-Pak) seeds treated

with 20% solution of *P. ovata* extract, while an increase in the solution concentration (40%) caused a decline in the antioxidant activity in both (control and drought) conditions, as represented in Figures 6 and 7.

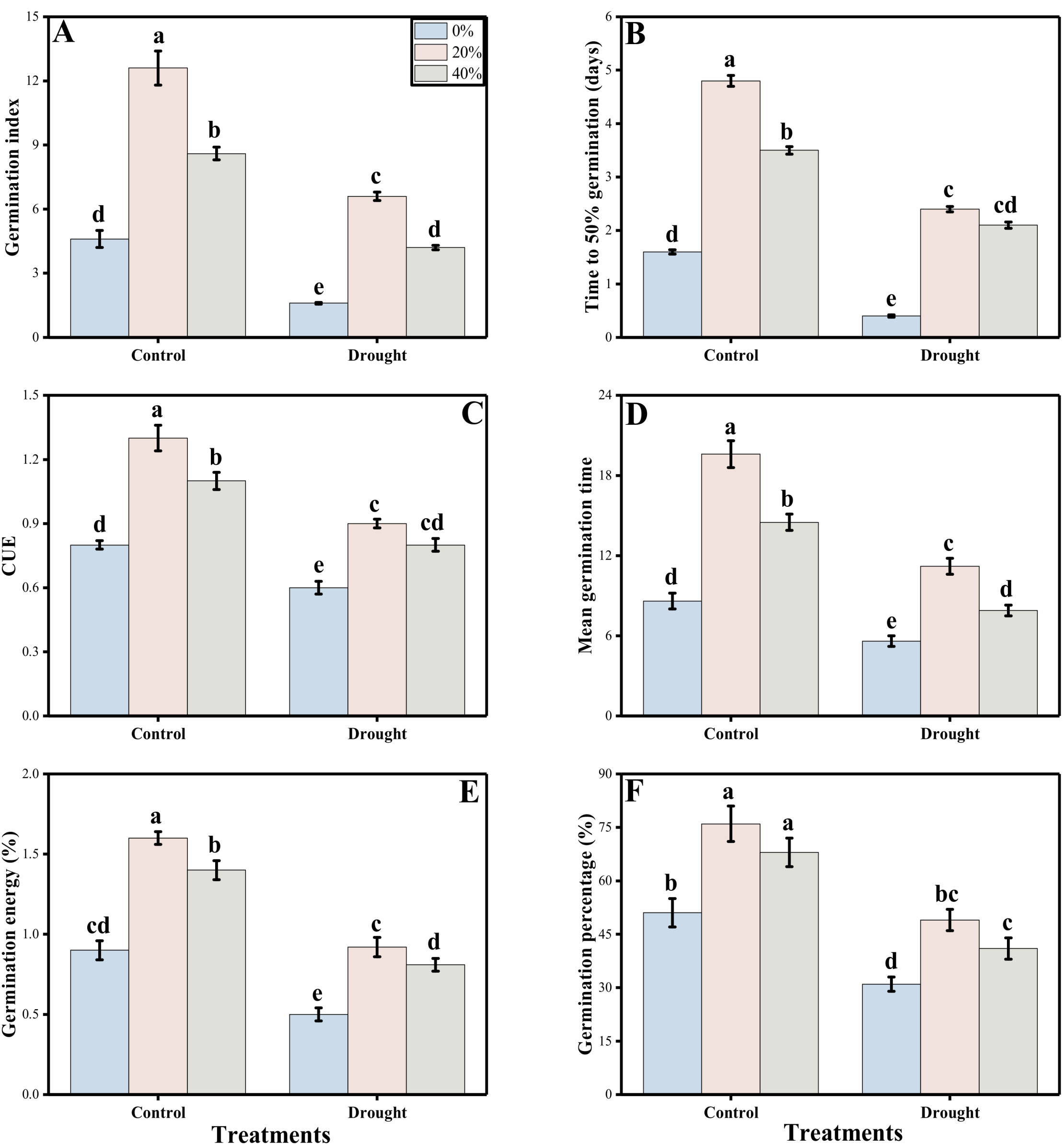

Figure 1. Effect of seed priming with *Plantago ovata* leaf extract on germination index (**A**), time to total 50% germination (**B**), coefficient of uniformity of emergence (**C**), mean germination time (**D**), germination energy (**E**) and germination percentage (**F**) under water stressed and non-stressed environments in maize cultivar Cimmyt-Pak. Means sharing similar letter(s) within a column for each parameter do not differ significantly at $p < 0.05$. Data in the figures are means of three repeats ($n = 3$) of just one harvest of *Zea mays* plants ± standard deviation (SD). Different lowercase letters on the error bars indicate significant differences between the treatments. Different treatments (*P. ovata* extract) used in this study are as follow: 0% (0% *P. ovata* extract), 20% (20% *P. ovata* extract) and 40% (40% *P. ovata* extract).

PEG-induced water stress significantly decreased the sugars content in maize (Cimmyt-Pak). Pre-night soaking of maize seeds in *P. ovata* leaf extract solutions (0, 20 and 40%) resulted in significant ($p > 0.05$) increases in sugars content under water stress. There was an ideal increase in the sugar contents of maize (Cimmyt-Pak) seedlings that were provided with 20% extract solution before placing. Figure 7 represents data related to sugars and proline content in maize seedlings.

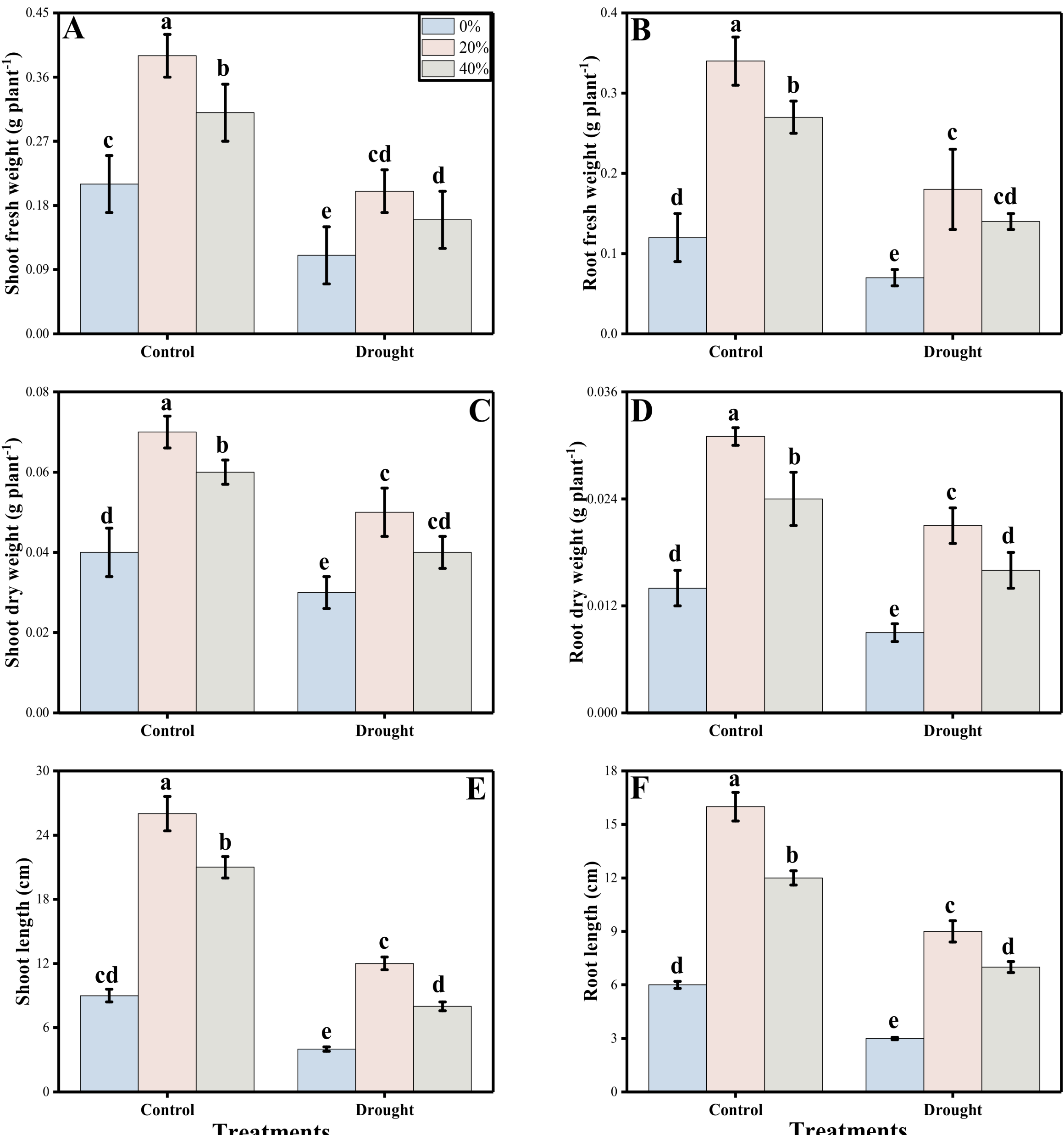

Figure 2. Effect of seed priming with *Plantago ovata* leaf extract on shoot fresh weight (**A**), root fresh weight (**B**), shoot dry weight (**C**), root dry weight (**D**), shoot length (**E**) and root length (**F**) under water stressed and non-stressed environments in maize cultivar Cimmyt−Pak. Means sharing similar letter(s) within a column for each parameter do not differ significantly at $p < 0.05$. Data in the figures are means of three repeats ($n = 3$) of just one harvest of *Zea mays* plants ± standard deviation (SD). Different lowercase letters on the error bars indicate significant difference between the treatments. Different treatments (*P. ovata* extract) used in this study are as follows: 0% (0% *P. ovata* extract), 20% (20% *P. ovata* extract) and 40% (40% *P. ovata* extract).

Proline content significantly ($p > 0.05$) increased under the PEG-induced water deficit condition, in contrast to the normal condition. Seed priming with *P. ovata* extract reduced the proline content under the water stressed condition. With an increase in the extract concentration (20%), proline content decreased in maize seedlings under drought but this decrease was not continuous, further increases in the concentration (40%) enhanced the proline content in primed seeds further, as shown in Figure 7.

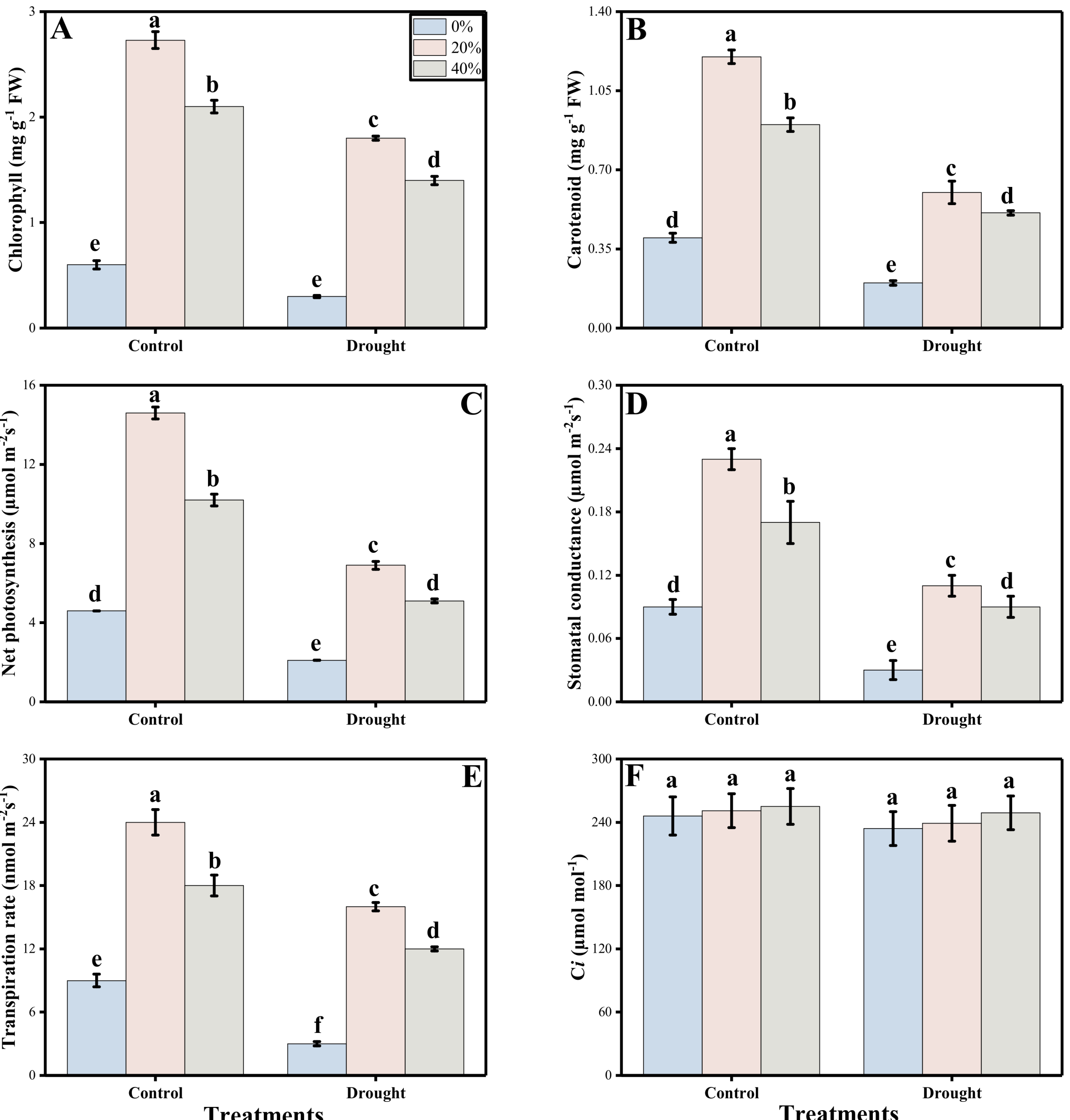

Figure 3. Effect of seed priming with *Plantago ovata* leaf extract on total chlorophyll contents (**A**), carotenoid contents (**B**), net photosynthesis (**C**) stomatal conductance (**D**), transpiration rate (**E**) and intercellular CO_2 (**F**) under water stressed and non-stressed environments in maize cultivar Cimmyt−Pak. Means sharing similar letter(s) within a column for each parameter do not differ significantly at $p < 0.05$. Data in the figures are means of three repeats ($n = 3$) of just one harvest of *Zea mays* plants ± standard deviation (SD). Different lowercase letters on the error bars indicate significant difference between the treatments. Different treatments (*P. ovata* extract) used in this study are as follows: 0% (0% *P. ovata* extract), 20% (20% *P. ovata* extract) and 40% (40% *P. ovata* extract).

3.4. Ion Uptake

The essential ions uptake ability of maize seedlings decreased with PEG-induced water stress, as indicated in Figure 8. The graphical data elucidates a non-significant increase in the essential ion uptake ability of plants, even in primed seeds under the water deficit condition. Among the varying concentrations of extract solutions (0, 20 and 40%), the 20% extract level accelerated essential ion uptake ability in maize seedlings.

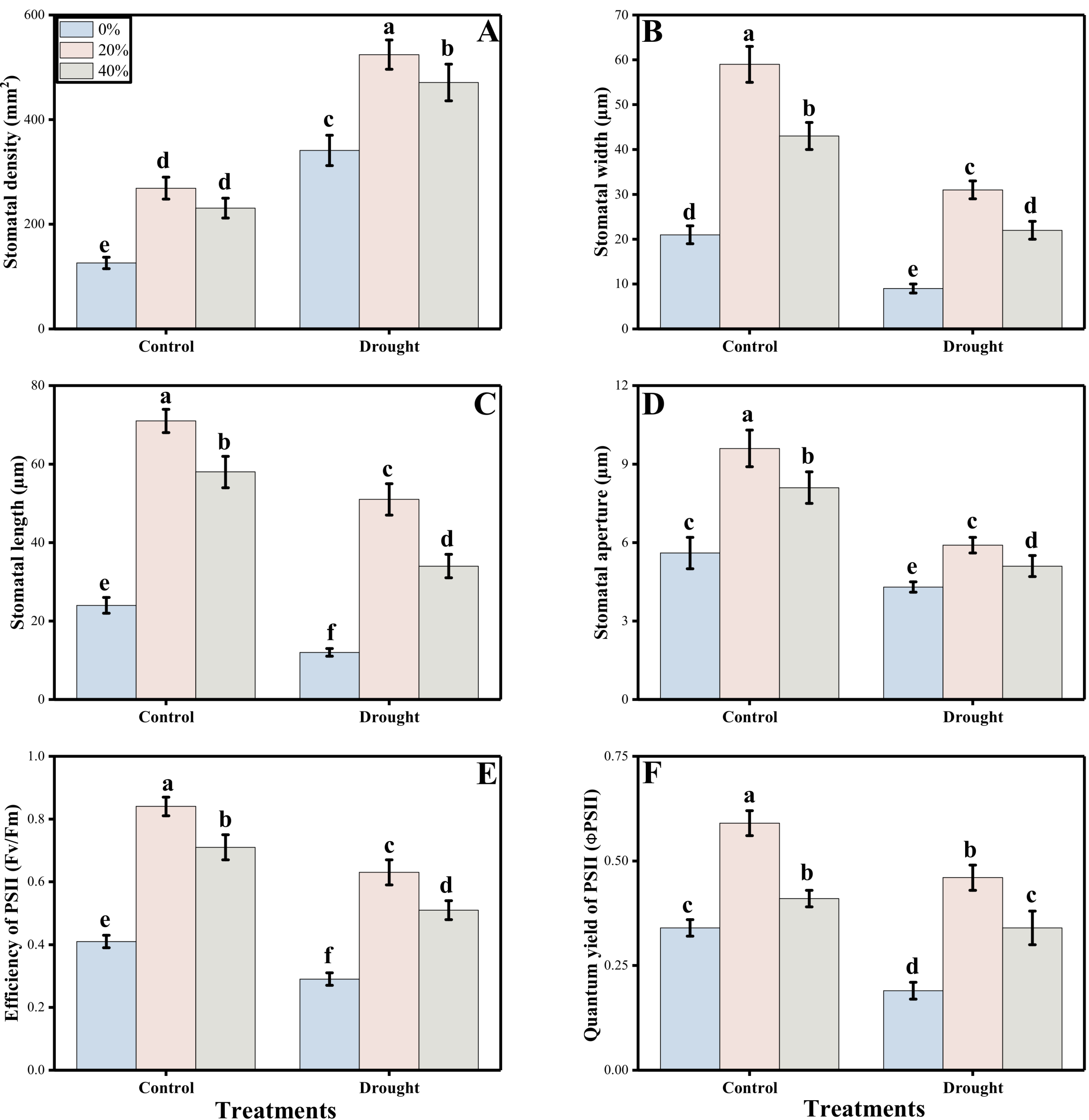

Figure 4. Effect of seed priming with *Plantago ovata* leaf extract on stomatal density (**A**), stomatal width (**B**), stomatal length (**C**) stomatal aperture (**D**), efficiency of PSII (**E**) and quantum yield of PSII (**F**) under water stressed and non-stressed environments in maize cultivar Cimmyt-Pak. Means sharing similar letter(s) within a column for each parameter do not differ significantly at $p < 0.05$. Data in the figures are means of three repeats ($n = 3$) of just one harvest of *Zea mays* plants ± standard deviation (SD). Different lowercase letters on the error bars indicate significant difference between the treatments. Different treatments (*P. ovata* extract) used in this study are as follows: 0% (0% *P. ovata* extract), 20% (20% *P. ovata* extract) and 40% (40% *P. ovata* extract).

3.5. Correlation Analysis

A Pearson's connection indicated the relationship between different parameters concentrated on in this trial; however, we developed a connection between the dry season conditions focused on plants (as the two conditions (control and dry spell) demonstrated the same trend). As per Pearson's correlation, the particle take-up in different organs of the maize seedlings was emphatically associated with each other's development and photosynthetic estimations, yet they were adversely connected with the oxidative pressure biomarkers and proline substance (Figure 9). Comparative relationships we saw in the heatmap histogram were that 0% seed preparation with *P. ovata* leaf removed demonstrated a critical relationship with oxidative pressure biomarkers and proline substance; however,

the rest of the heatmap indicated non-huge outcomes in any remaining parameters concentrated on in this analysis (Figure 10). Essentially, PCA indicated a reasonable connection between different examined parameters and showed that the majority of development parameters, e.g., particles take-up, cell reinforcements and photosynthetic estimations were decidedly associated, while a negative relationship was seen in oxidative pressure biomarkers and proline substance (Figure 11). These connections portray a nearby association between different contemplated credits in *T. aestivum* seedlings in the dry season focused on climate.

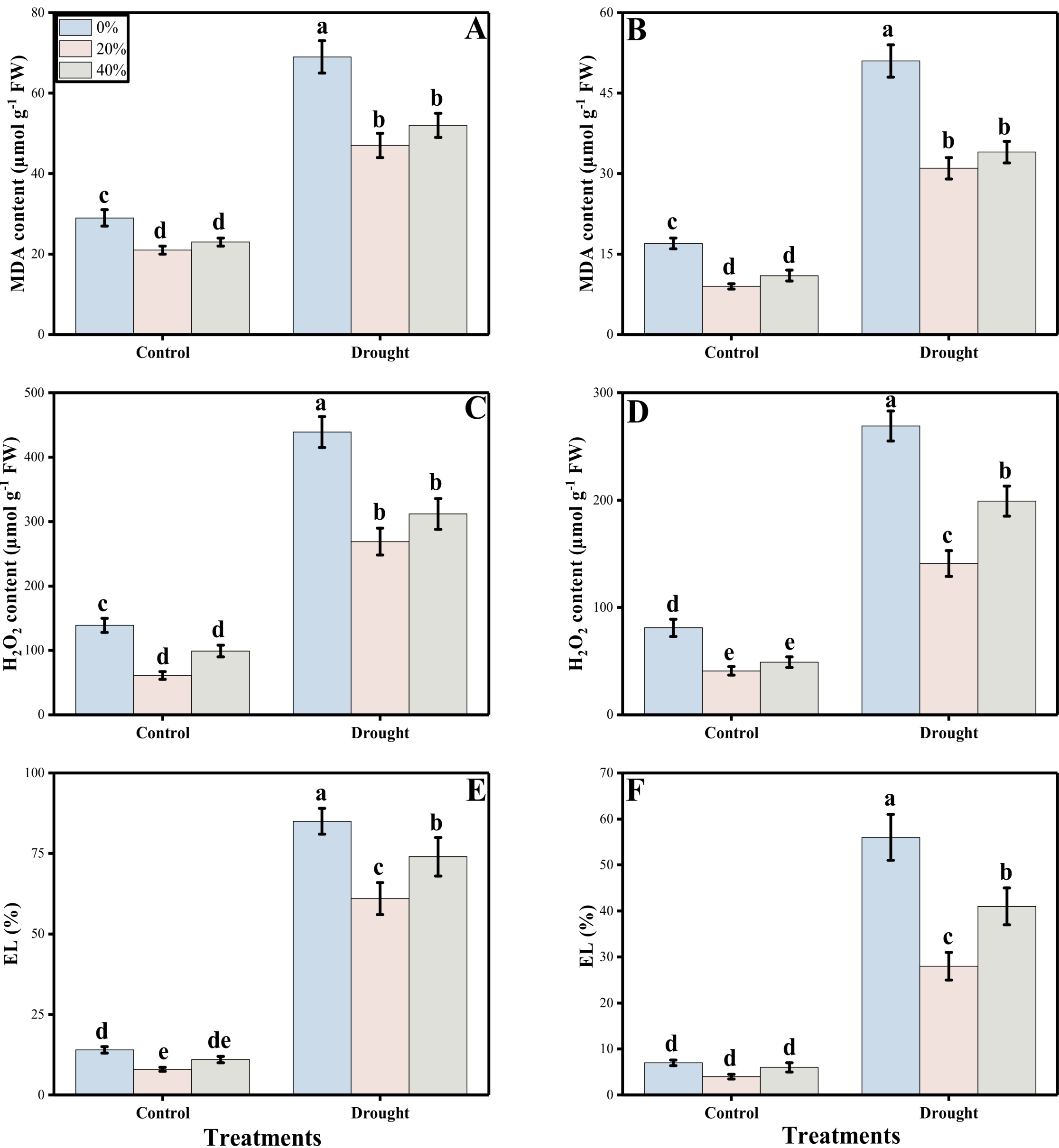

Figure 5. Effect of seed priming with *Plantago ovata* leaf extract on MDA contents in the roots (**A**), MDA contents in the leaves (**B**), H_2O_2 contents in the roots (**C**), H_2O_2 contents in the leaves (**D**), EL percentage in the roots (**E**) and EL percentage in the leaves (**F**) under water stressed and non-stressed environments in maize cultivar Cimmyt-Pak. Means sharing similar letter(s) within a column for each parameter do not differ significantly at $p < 0.05$. Data in the figures are means of three repeats ($n = 3$) of just one harvest of *Zea mays* plants ± standard deviation (SD). Different lowercase letters on the error bars indicate significant difference between the treatments. Different treatments (*P. ovata* extract) used in this study are as follows: 0% (0% *P. ovata* extract), 20% (20% *P. ovata* extract) and 40% (40% *P. ovata* extract).

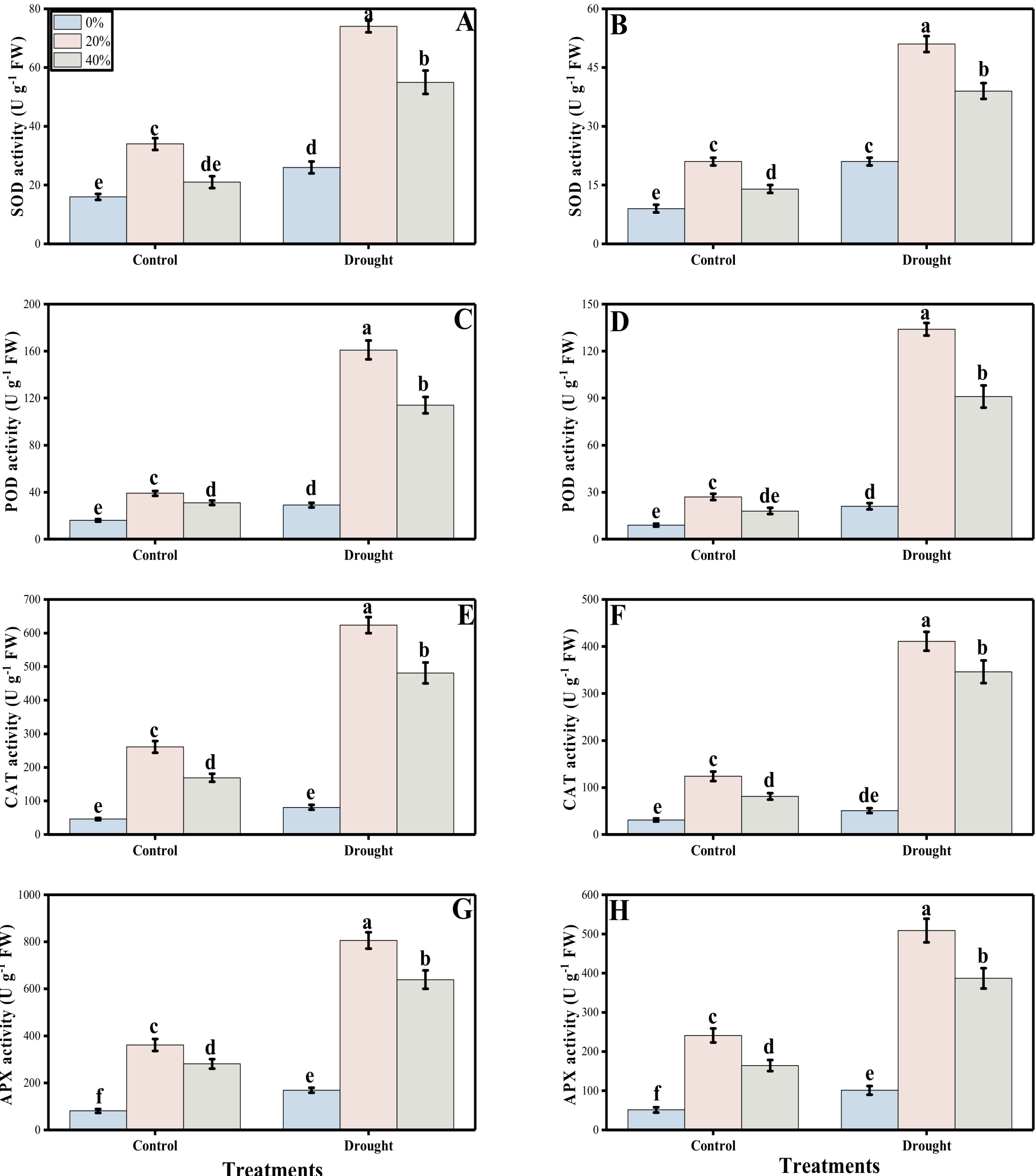

Figure 6. Effect of seed priming with *Plantago ovata* leaf extract on SOD activity in the roots (**A**), SOD activity in the leaves (**B**), POD activity in the roots (**C**), POD activity in the leaves (**D**) CAT activity in the roots (**E**), CAT activity in the leaves (**F**), APX activity in the roots (**G**) and APX activity in the leaves (**H**) under water stressed and non-stressed environments in maize cultivar Cimmyt−Pak. Means sharing similar letter(s) within a column for each parameter do not differ significantly at $p < 0.05$. Data in the figures are means of three repeats ($n = 3$) of just one harvest of *Zea mays* plants ± standard deviation (SD). Different lowercase letters on the error bars indicate significant difference between the treatments. Different treatments (*P. ovata* extract) used in this study are as follows: 0% (0% *P. ovata* extract), 20% (20% *P. ovata* extract) and 40% (40% *P. ovata* extract).

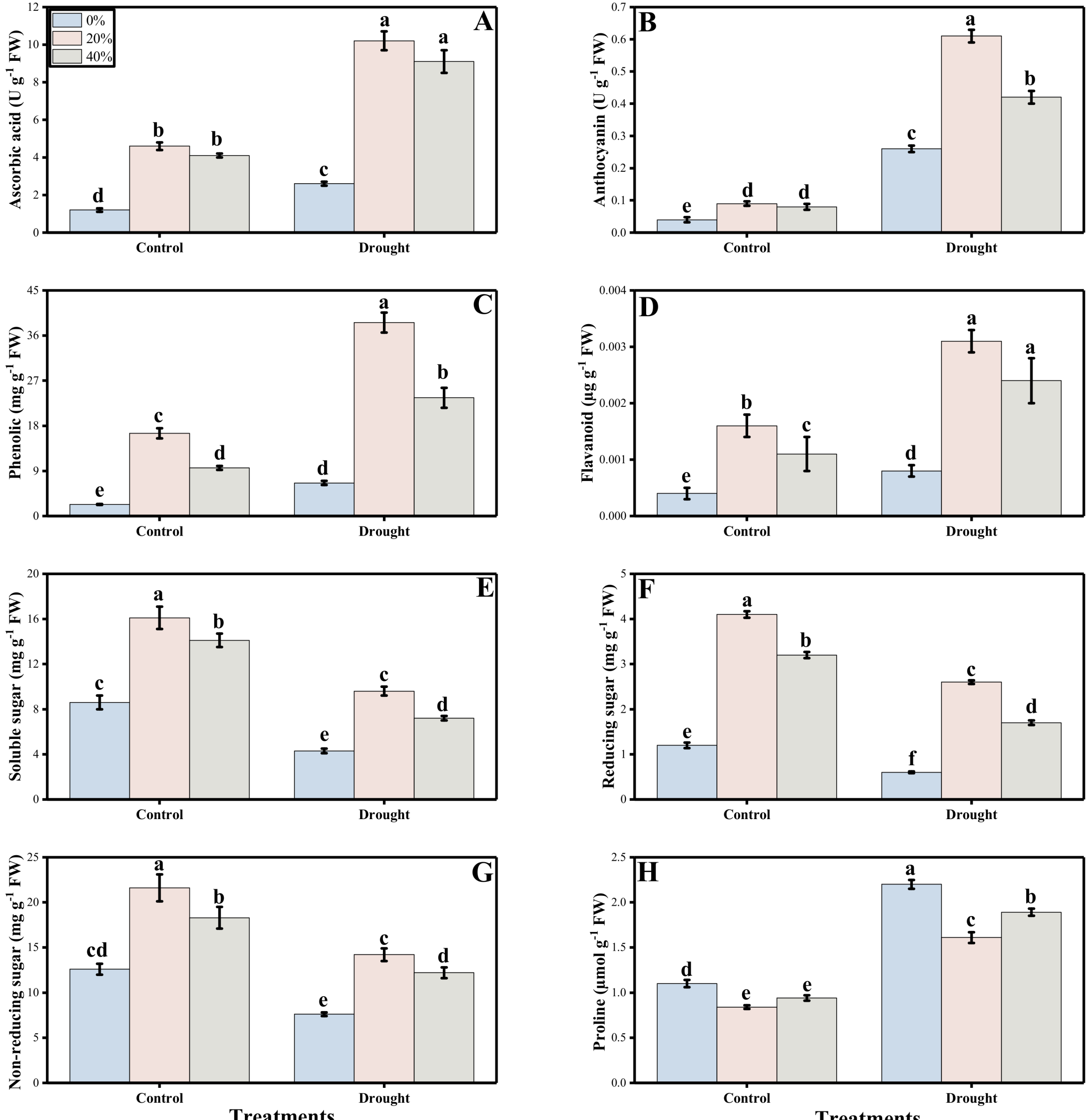

Figure 7. Effect of seed priming with *Plantago ovata* leaf extract on ascorbic acid contents (**A**), anthocyanin contents (**B**), phenolic contents (**C**), flavonoid contents (**D**), soluble sugar contents (**E**), reducing sugar contents (**F**), non-reducing sugar contents (**G**) and proline contents (**H**) under water stressed and non-stressed environments in maize cultivar Cimmyt−Pak. Means sharing similar letter(s) within a column for each parameter do not differ significantly at $p < 0.05$. Data in the figures are means of three repeats ($n = 3$) of just one harvest of *Zea mays* plants ± standard deviation (SD). Different lowercase letters on the error bars indicate significant difference between the treatments. Different treatments (*P. ovata* extract) used in this study are as follows: 0% (0% *P. ovata* extract), 20% (20% *P. ovata* extract) and 40% (40% *P. ovata* extract).

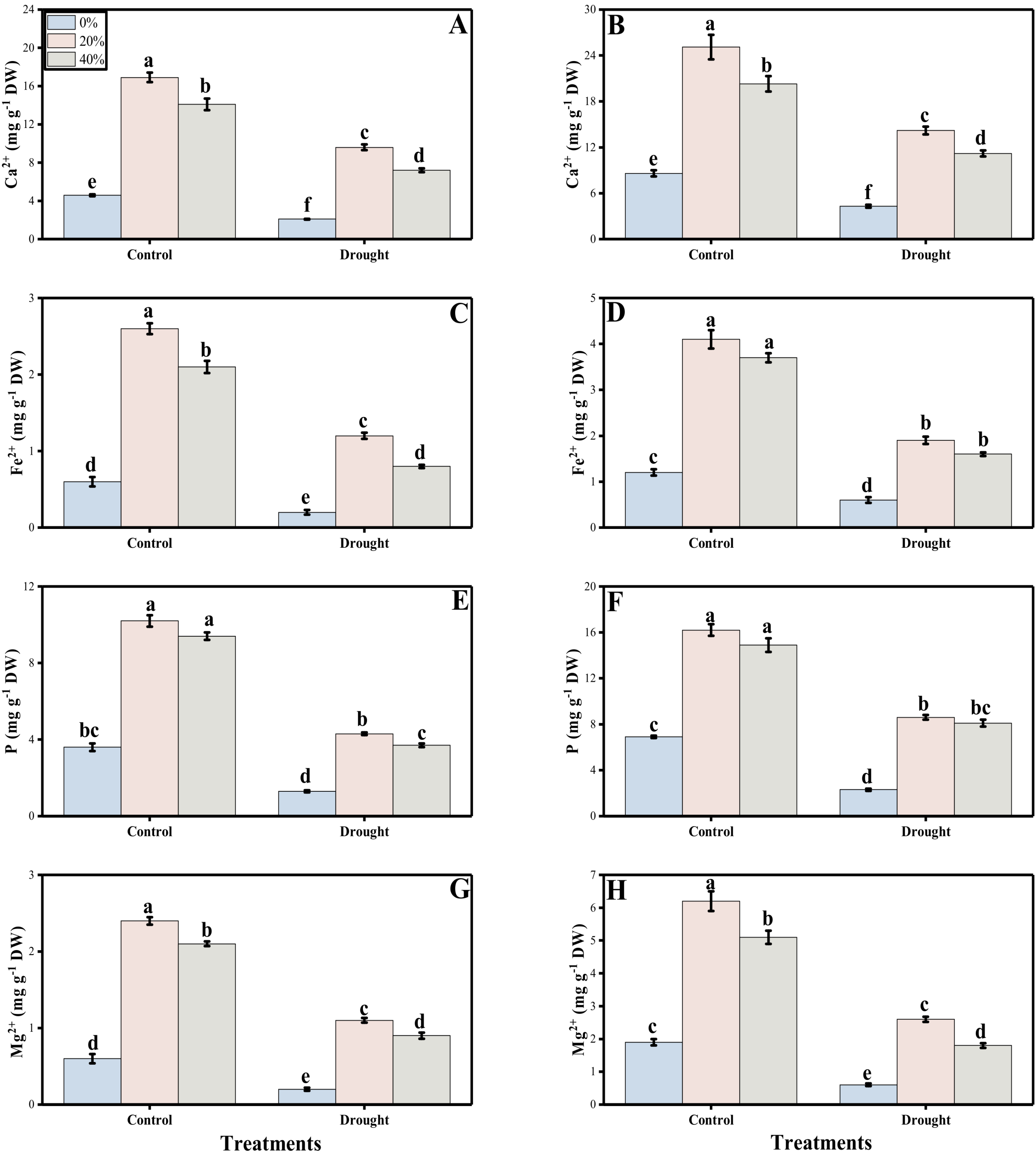

Figure 8. Effect of seed priming with *Plantago ovata* leaf extract on magnesium contents in the roots (**A**), magnesium contents in the shoots (**B**), phosphorus contents in the roots (**C**), phosphorus contents in the shoots (**D**), iron contents in the roots (**E**), iron contents in the shoots (**F**), calcium contents in the roots (**G**) and calcium contents in the leaves (**H**) under water stressed and non-stressed environments in maize cultivar Cimmyt−Pak. Means sharing similar letter(s) within a column for each parameter do not differ significantly at $p < 0.05$. Data in the figures are means of three repeats ($n = 3$) of just one harvest of *Zea mays* plants ± standard deviation (SD). Different lowercase letters on the error bars indicate significant difference between the treatments. Different treatments (*P. ovata* extract) used in this study are as follows: 0% (0% *P. ovata* extract), 20% (20% *P. ovata* extract) and 40% (40% *P. ovata* extract).

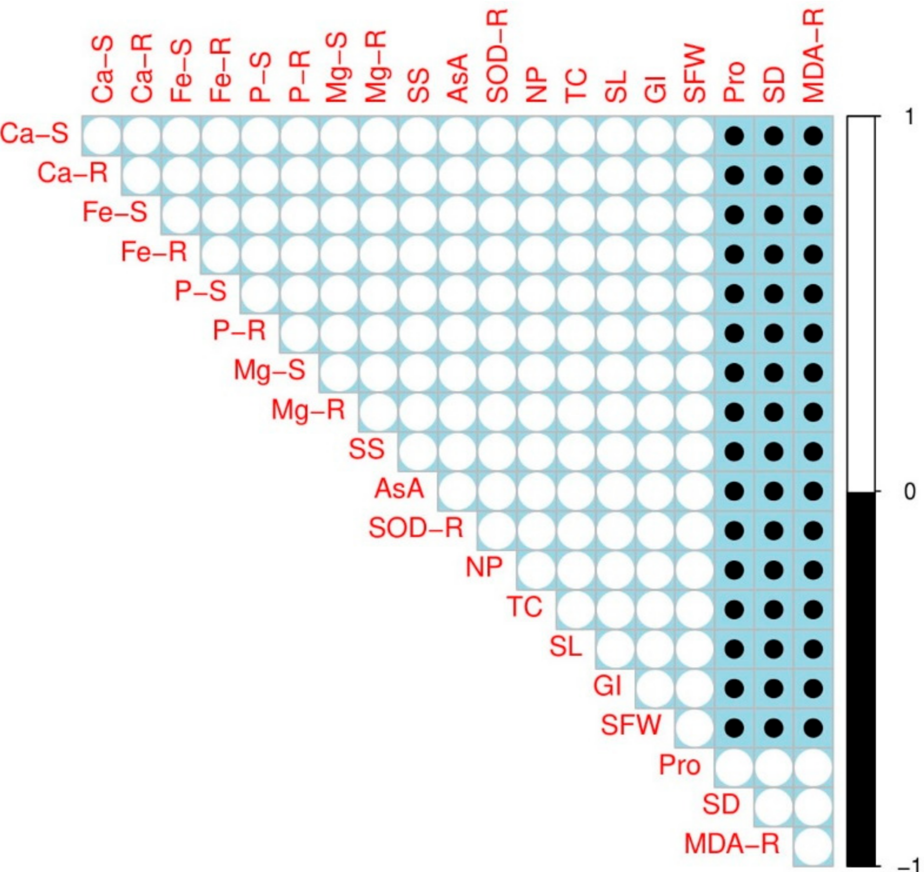

Figure 9. Correlation between different morph-physiological traits with ions uptake/accumulation in different parts of plants. Different abbreviations used are as follow: Ca-S (calcium contents in the shoots), Ca-R (calcium contents in the roots), Fe-S (iron contents in the shoots), Fe-R (iron contents in the roots), P-S (phosphorus contents in the shoots), P-R (phosphorus contents in the roots), Mg-S (magnesium contents in the shoots), Mg-R (magnesium contents in the roots), SS (soluble sugar), AsA (ascorbic acid contents), SOD-R (superoxidase dismutase activity in the roots), NP (net photosynthesis), TC (total chlorophyll contents), SL (shoot length), GI (germination index), SFW (shoot fresh weight), Pro (proline contents), SD (stomatal density) and MDA-R (malondialdehyde contents in the roots).

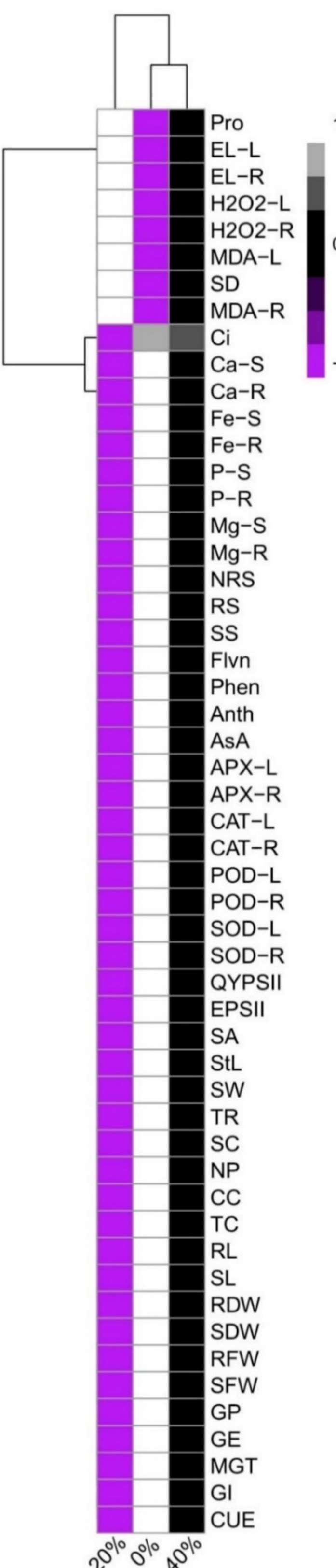

Figure 10. Heatmap histogram correlation between different studied attributes of *Zea mays grown* under water stressed and non-stressed environment Different treatments (*P. ovata* extract) used in this study are as follows: 0% (0% *P. ovata* extract), 20% (20% *P. ovata* extract) and 40% (40% *P. ovata* extract). Different abbreviations used are as follow: Pro (proline contents), EL-L (electrolyte leakage in the leaves), EL-R (electrolyte leakage in the roots), H2O2-L (hydrogen peroxide initiation in the

leaves), H2O2-R (hydrogen peroxide initiation in the roots), MDA-L (malondialdehyde contents in the leaves), SD (stomatal density), MDA-R (malondialdehyde contents in the roots), Ci (intercellular CO_2), Ca-S (calcium contents in the shoots), Ca-R (calcium contents in the roots), Fe-S (iron contents in the shoots), Fe-R (iron contents in the roots), P-S (phosphorus contents in the shoots), P-R (phosphorus contents in the roots), Mg-S (magnesium contents in the shoots), Mg-R (magnesium contents in the roots), NRS (non-reducing sugars), RS (reducing sugars), SS (soluble sugars), Flvn (flavonoid contents), Phen (phenolic contents), Anth (anthocyanin contents), AsA (ascorbic acid contents), APX-L (ascorbate peroxidase activity in the leaves), APX-R (ascorbate peroxidase activity in the roots), CAT-L (catalase activity in the leaves), CAT-R (catalase activity in the roots), POD-L (peroxidase activity in the leaves), POD-R (peroxidase activity in the roots), SOD-L (superoxidase dismutase activity in the leaves), SOD-R (superoxidase dismutase activity in the roots), QYPSII (quantum yield of PSII), EPSII (efficiency of PSII), SA (stomatal aperture), StL (stomatal length), SW (stomatal width), TR (transpiration rate), SC (stomatal conductance), NP (net photosynthesis), CC (carotenoid contents), TC (total chlorophyll), RL (root length), SL (shoot length), RDW (root dry weight), SDW (shoot dry weight), RFW (root fresh weight), SFW (shoot fresh weight), GP (germination percentage), GE (germination energy), MGT (mean germination time), GI (germination index) and CUE (coefficient of uniformity of emergence).

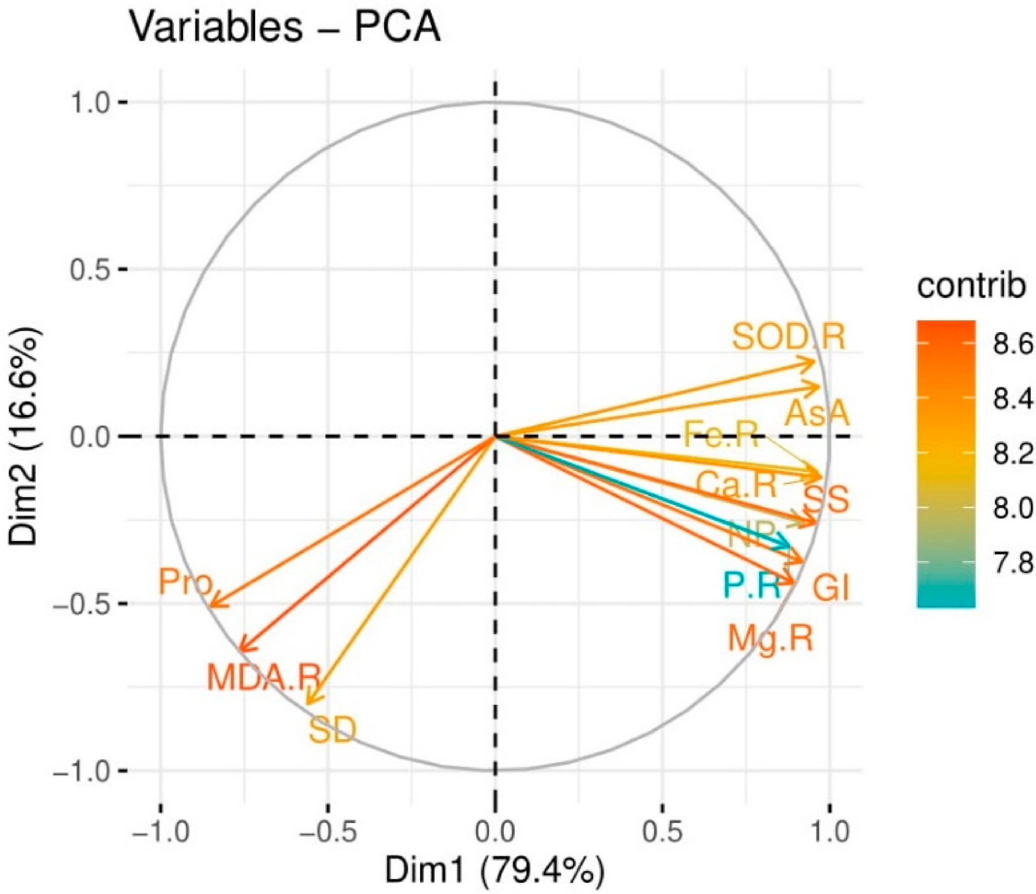

Figure 11. Loading plots of principal component analysis (PCA) on different studied attributes of *Zea mays* grown under water stressed and non-stressed environments with various application levels of *Plantago ovata* leaf extract. Different abbreviations used are as follow: Ca-R (calcium contents in the roots), Fe-R (iron contents in the roots), P-R (phosphorus contents in the roots), Mg-R (magnesium contents in the roots), SS (soluble sugar), AsA (ascorbic acid contents), SOD-R (superoxidase dismutase activity in the roots), NP (net photosynthesis), GI (germination index), Pro (proline contents), SD (stomatal density) and MDA-R (malondialdehyde contents in the roots).

4. Discussion

Plants are typically exposed to a broad myriad of biotic and abiotic stresses, including feeding from wild animals and insects, weed infestation, hail, mechanical injury, diseases, low soil fertility, drought, salinity and others that can diminish the plant photosynthetic area and, thus, the attained total plant biomass or grain yield [3,5,7]. Recent research has shown that native plant species may exhibit stronger tolerance or better and faster mechanisms to adjust to or withstand abiotic stress conditions, such as drought and salinity stress, compared to their cultivated relatives [4,16,48]. With the cultivation of indigenous crops, landscape architects not only produced vernacular ecosystems but also provided solutions to the increased air temperatures experienced at a global scale in the last 50 years and the wasteful use of water channels [1,49]. According to Fernández-García et al. [50], several taxa of native plants (both arid and semi-arid) are listed as resistant to drought, mostly based on anecdotal observations of plant performance in countryside planting. This study describes germination and growth parameters reduced in seeds given PEG-induced stress when compared with seeds provided with enough water (Figures 1 and 2). PEG-induced water deficit imparted negative effects on germination, growth, morphology, physiology and other internal mechanisms that are related to drought [3,5,9]. The decreasing plant growth trend in maize seedlings could be in response to water shortage [50,51]. Different studies have reported that a reduction in plant morphological attributes is due to drought stress arrival [1,4–6,20,52]. Water deficit conditions reduced rates of photosynthesis, slowed leaves swelling, increased thinning in stomata, reduced initial leaves catabases and reduced plant fertility [51,52]. Drought affected plants and their water relation, i.e., transpiration rate, plant leaf surface temperature, efficient use of water and its accumulation, along with the moisture content [52,53]. Photosynthetic measurements taken from maize seedlings grown in the stressed condition decreased in contrast to seeds given enough water for growth (Figure 3). Quite similar effects were observed related to gas exchange properties and the behavioral pattern of stomata (Figure 4). Net photosynthesis dropped during the regulatory processes; this may be related to a decline in the stomatal properties and chlorophylls after seedlings went under the water deficient environment [54]. Our study revealed a decrease in the photosynthetic rate, pigment content and stomatal characters under drought stress (Figures 3 and 4).

It has been observed that water deficiency reduces the rate of photosynthesis, either because of it causing a decrease in the chlorophyll synthesis or because of damage to its molecules [4,55,56]. When maize seedlings were exposed to drought stress, a similar reducing trend in the described traits was observed [21,57] in response to closing of the stomata due to water deficit conditions, as described in cowpea by Rivas et al. [58]. A reduction in transpiration rate may be due to unmaintained water field capacity in response to the transpiration rate [59]. Limited water supply closed stomata, reduced stomatal conductance and the rates of transpiration and photosynthesis in fynbos legume, as described by Lotter et al. [60].

Water deficit environments are generally known to initiate oxidative stress in plants by the production of extra reactive oxygen species (ROS) [61–63] and antioxidative enzymes that play a protective role in reducing the metal toxicity by scavenging ROS [64–66]. Previously, it was shown that drought stressed conditions increased ROS production in cells/tissues, which were then scavenged by the activities of antioxidant compounds [56,65,67]. Additional scavenging of ROS and increased plant tolerance against water deficient environments can be achieved by increasing the content of AsA and GSH, which was previously reported by [9]. This can be achieved when there is an abiotic stress environment and plant-induced oxidative damage to the membranous bounded tissues [68,69]. Moreover, non-enzymatic antioxidants also establish redox active molecules that can decrease ROS generation within the cell by participating in an ascorbate–glutathione cycle [70,71]. The increase in the activities of antioxidant enzymes was concomitant with the generation of extra ROS [62,72,73]. It was also reported that increases in the activities of various antioxidant enzymes under environmental stress conditions are due to the reduction in glutathione con-

tents [74,75]. In the current experiment, oxidative stress biomarkers were enhanced in maize seedlings cultivated in a minimal water containing environment (Figure 5), while the water deficient environment also increased the antioxidants activity in drought, with respect to the control seedlings (Figure 6). There are different mechanisms (osmotic adjustment) in plants to overcome the various abiotic stresses. The enhanced proline content and SS in water deficient seedlings (Figure 7) suggest that a developed cell osmotic change can conserve high water moisture in the cell and lead to declining EL in the plant tissues [3,13,67].

Studies have also reported that low water moisture in the environment means that plants are unable to absorb essential growth promoting nutrients from the soil [2,54,71,76,77]. This is because the plants absorbs these nutrients in ionic form and water performs a significant role for the uptake of these nutrients from the soil. However, the ability of plants to uptake these essential nutrients from the environment becomes low or even ceases when there is low water content in the environment [77,78]. Hence, a water deficient environment results in the low absorbance of these essential ions due to the availability of low water content in the environment, which reduces the root's power to absorb such nutrients. In this study, essential nutrient uptake ability also decreased in maize seedlings that were facing water scarcity (Figure 8); our results are in correlation with reported studies. Plants control many metabolic processes by up-taking a sufficient quantity of nutrients; a decline in nutrient uptake leads to the impairing of a plant's internal metabolism that ultimately minimizes the growth and yield [54,77]. In the present study, nutrient contents were negatively correlated with oxidative stress indicators and proline contents with correlation coefficient of -1 but were positively correlated with plant growth and biomass, and photosynthetic efficiency with a correlation coefficient of +1. These findings coincide with the previous study by Hameed et al. [65], when they studied carrots under a drought stress environment and controlled conditions.

Seed priming techniques are becoming popular. Priming with diverse natural extracts has led to increases in the growth, plant biomass and yield [79–82]. *Plantago ovata* Forsk is a member of family Platanaceae and is commonly known as psyllium, ispaghula or desert Indianwheat. It is very important economically and referred to as a medicinal plant. Its husk (isabgol) contains important compounds for the activation of primary and secondary metabolism [83,84]. ROS production under drought induces oxidative stress in plants, causes reduction in the plant biomass and yield [67,75]. In this study, maize seeds (primed with *P. ovata* leaf extract) antioxidant activity increased, resulting in enhancement in the plant growth and biomass by scavenging the ROS under drought conditions [83]. Very limited literature is available related to the use of *Plantago ovata* leaf extract for mitigating the effects of abiotic stresses in plants. The present study reveals the positive effects of *P. ovata* extract due to improved germination, positive growth, increases in pigment contents, activation of antioxidant mechanisms, stomatal behaviors and scavenging of ROS. High dose (40%) *P. ovata* leaf extract has reduced the described properties and mechanisms and this may be due to a high content of toxicity of extract [85].

5. Conclusions

In this study, we investigated the influence of *P. ovata* leaf extract on *Zea mays* cultivar (Cimmyt-Pak) seedlings, to study different morphological, physiological and uptake fluxes of essential nutrients from the medium when grown in well-watered (100% WFC) and water depleted conditions (60% WFC). We have found that, drought conditions caused a harsh impact on plant growth and germination, photosynthetic measurements, stomatal behaviour and induced oxidative stress, antioxidant enzymes and osmo-protectants. Seeds primed with *P. ovata* leaf extract are useful in alleviating oxidative stress by accelerating the activities of antioxidants, and increasing the content of soluble sugars, AsA and GSH, and also increasing fluxes of essential nutrients, even in the drought stress condition. Hence, we suggest that the novel application of seeds primed with *P. ovata* leaf extract offers new opportunities in the field of sciences, and plants can show a greater tolerance to drought conditions, and an enhanced capacity to adapt to future environmental challenges.

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