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Research Article

Bio-priming of seed with *Trichoderma* sp. and *Bacillus* sp. leads to improved morpho-physiological parameters related to drought tolerance in maize

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ABSTRACT

Maize (*Zea mays* L.) holds an important position as a major grain crop in Pakistan flourishing across a diverse range of environmental conditions. The maize crop encounters various severe abiotic challenges with drought being the most concerning factor leading to diminished plant growth and yield. Seed priming a well-established and widely adopted technique serves as an effective mechanism to alleviate drought stress ultimately boosting both growth and grain yield. The primary objective of this study was to evaluate the impact of diverse seed priming methods on the growth and development of maize plants. Additionally, the study sought to assess the potential of various seed priming techniques in mitigating drought stress in maize. A pot experiment was conducted using a completely randomized design under natural conditions. The aim was to scrutinize the influence of different priming agents on morpho-physiological traits associated with water deficit conditions in maize. The gathered data underwent ANOVA analysis revealing variations in all parameters for both maize varieties at a 5% probability level. Specifically, the Pak-Afgoi variety displayed notable responsiveness to the seed priming approach in alleviating drought stress in maize. Furthermore, the findings underscored the evident and substantial impact of seed priming on both maize varieties across a range of studied characteristics under drought stress conditions. In conclusion, the present study suggests that seed priming holds the potential to enhance drought tolerance in maize.

Keywords: Maize; seed priming; drought tolerance; *Trichoderma* sp.; *Bacillus* sp.

INTRODUCTION

Maize is cultivated for multipurpose, and it is referred as the 'other gold' due to its variety of applications for human consumption, component of livestock, used as poultry feed as well as it has advantageous role in many health issues. Maize plants have developed specific adaptive traits and the ability to thrive in unfavorable conditions due to their immobile nature. There may be some molecular and cellular changes which maintain the metabolism of plant like some adaptation in gene expression and activity or hormonal imbalance (Saritha *et al.*, 2020). A supplementary report discovered that when maize plants were exposed to water shortage circumstances for more than 12 days, then the grain output decreased during the grain filling and flowering stages (Monneveux *et al.*, 2006). About 25% production of maize can be boosted by providing good quality seed (Kandel 2021). Water is an important for the reproductive stages of maize and water scarcity may cause 25 to 50% reduction in maize grain output (Shah *et al.*, 2020). Maize has been found to be more resistant to water logging and is highly susceptible to drought stress



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than the other grain crops (Satyavathi *et al.*, 2019; Mendoza-Grimon *et al.*, 2021). Water stress caused yield loss in maize hybrids between 22.6 to 26.4%, which in turn decreased the grain kernels weight (Pandey *et al.*, 2000). It is observed from previous study that priming of maize with CaCl_2 would lower the drought stress ability and increased the maize yield (Mustafa *et al.*, 2017). Many researchers and biotechnologists are producing drought-tolerant varieties by breeding, drought-responsive genes/QTLs and genetic engineering of most superior plants. These approaches are complicated and need technical expertise to cope drought situations (Jisha *et al.*, 2013). The best proved strategy is treating seeds with natural and synthetic substances by which plant activates its defense response from infections and other abiotic stresses.

Seed priming induces many physiological, morphological, and biochemical modifications in plants for stimulating stress-responsive genes in water scarce condition (Beckers and Conrath 2007). Primed seeds have more potential for growth and yield and are substantially more productive and consistent in comparison to non-primed seeds (Ahmadvand *et al.*, 2012). It is reported that in *Zea mays* caryopses germination and seedling emergence were both boosted up about 43% and 29% when copper and zinc primed seeds were planted and allowed to grow (Jisha *et al.*, 2013). Under drought circumstances, it's observed that GA3 primed rye seedling up-regulates the antioxidants and enhance germination efficiency (Nakaune *et al.*, 2012; Tayyab *et al.*, 2020). The germination of seeds takes place in three stages. First is the imbibition phase when seed rapidly absorb water, in second stages seed water content changes and the final stage is radical emergence phase (Ahmadvand *et al.*, 2012).

The goal of seed priming is to avoid seed from expanding during lag phase and prevent it from absorbing enough water to cause dramatic emergence. The germination process is controlled by water content and temperature level of seed. During priming of seeds various stress tolerance genes activated depending on the conditions that are given to seeds (Mustafa *et al.*, 2017). By keeping these reports in mind, the present study was designed to study the effect of different seed priming treatments such as *Trichoderma* sp., *Bacillus* sp., CaCl_2 and distilled water on the plant growth and development as well as to estimate the potential of seed priming approaches for mitigating the drought stress in maize.

MATERIALS AND METHODS

The experiment was managed to investigate the effect of different seed priming treatments on morpho-physiological parameters of plants under drought conditions on two maize varieties.

Experimental material and treatments

A pot experiment was performed in experimental area of plant pathology field in University of Agriculture Faisalabad (UAF) Pakistan to study the effect of seed priming on the morpho-physiological parameters of maize plant under drought stress. The experiment was planned at experimental area of department of plant pathology, UAF. The seeds of two maize varieties (Pak-Afgoi Sg-2002 and Af-5101) was stored in plastic bags to keep its strength till experimentation and labeled them properly. The pots were filled with 5kg saturated soil. *Trichoderma* fungal specie (T_1), *Bacillus* bacteria specie (T_2), Calcium chloride (T_3), Distilled water (T_4), Control (T_5) were the treatments used in this research study and one drought level was applied at fifth leaf stage.

Preparation of Nutrient Broth (NB) and Potato Dextrose Broth (PDB) media

For the preparation of nutrient broth media, 6.5g of powdered nutrient broth were dissolved in 500ml of distilled water and confirmed complete dissolution using autoclave at 121°C temperature and 15psi pressure for 20 minutes. *Bacillus* sp. was cultured in Nutrient Broth (NB) media.

Preparation of media

For the preparation of PDB, 100g of sliced potatoes were boiled in one liter of water for 40 minutes. The resulting broth was filtered discarding potatoes then, 20g of glucose were added along with distilled water and make up volume up to 1L. *Trichoderma* sp. was cultured on Potato Dextrose Broth (PDB). Both cultures were placed on shaker for the growth of fungus and bacteria. The growth of cultures was monitored over a period of 24 to 48 hours. Once the growth was observed, the cultures were used for seed priming.

Preparation of priming agents and sowing of seeds

In this protocol two agents *Bacillus* specie and *Trichoderma* fungal specie were used as bio-priming material. The solution of 2.2% of calcium chloride was prepared by taking 2.2g of powdered calcium chloride and dissolving into 80ml distilled water and make up the volume up to 100ml. The distilled water was also used as a treatment. Maize seeds were primed and soaked for 18 hours with priming solutions and was placed in incubator at 120°C optimum temperature.

After priming, seeds were dried at room temperature and were sown in pots. The drought stress was applied at the

fifth leaf stage. Plants were harvested after applying stress and whole plant sample was collected from each pot in paper envelopes and different parameters were studied.

Morpho-physiological characters

The following morpho physiological characters of the plants were observed and data was collected. Leaf area (cm²) was calculated by using this formula: Leaf area= LxW where L is length and W is the average width (Hidangmayum *et al.*, 2022). The chlorophyll content was determined with the help of portable chlorophyll meter (SPAD) early in the morning. The dry weight (DW) of the leaf was measured in grams with the help of electrical balance. Shoot length and root length of the plants were determined in centimeters (cm) with the help of measuring scale. Fresh mass (g) of the plant shoot and root was taken immediately after sampling with the help of electrical balance. The dry weight (g) of root and shoot was measured after drying the samples in oven for 2-3 days with the help of weight machine.

The residual transpiration (g H₂O/min/cm²/10⁵) was determined according to the given formula: $RT = (W_1 - W_2) / L.A$ where W₁ is the weight of leaves after half an hour of excision and W₂ is the weight of leaves after 3hours, leaf area (LA) was measured by same formula as earlier (Ali *et al.*, 2009). Relative water content of leaf samples was determined by using this formula: $RWC (\%) = (FW - DW) / (TW - DW) * 100$ whereas FW = Fresh weight, DW = Dry weight and TW = Turgid weight (Hidangmayum *et al.*, 2022). Relative dry weight was calculated by using formula: $RDW = DW / (TW - DW)$ (Ali *et al.*, 2009). For the determination of cell membrane stability, the plant samples were first weighed (0.4g) and then placed in beakers containing 20ml of deionized water. First reading (L1) was taken after 2hours of incubation with electrical conductivity meter and second reading (L2) was measured after autoclaved the samples at 121°C for 20 minutes and CMS was calculated by using following formula: $CMS (\%) = [1 - (L1 / L2)] * 100$ (Ali *et al.*, 2009).

Statistical analyses

For statistical analysis completely randomized design was used. The data were analyzed through analysis of variance (ANOVA). The means of different maize parameters were compared by using Least Significance Difference (LSD) method at 95% level of confidence through the statistical software Statistix version v.8.1 and correlation of all parameters were determined.

RESULTS

The results presented in Table 1, indicating F-statistics in the analysis of variance (ANOVA). The findings reveal greater variation among sample means compared to within the sample means. This disparity is evident in the highly significant variations observed in leaf area (cm²), chlorophyll content (%), dry leaf weight (g), relative water content (%), relative dry weight (g) and cell membrane stability (%). These variations were identified across treatments, drought conditions, varieties * treatments, treatments * drought conditions, and varieties * treatments * drought conditions. On the other hand, dry root weight (g), residual transpiration (%), fresh shoot weight (g), dry shoot weight (g) and root length (cm) exhibited more pronounced variation demonstrating highly significant differences in varieties, treatments and drought conditions. The elevated average mean values for leaf area (79.367), shoot length (61.550), relative water content (62.815) and cell membrane stability (88.125) indicated that various priming treatments (*Trichoderma*, *Bacillus*, CaCl₂, distilled water) have induced drought tolerance in both maize genotypes.

The mean comparison following least significance difference (LSD) test at 5% probability level showed variation between varieties, treatments, and conditions in both maize genotypes Pak-Afgoi Sg-2002 and Af-5101. Drought stress significantly diminished morpho-physiological parameters in both maize cultivars. However, the application of priming treatments (*Trichoderma*, *Bacillus*, CaCl₂, distilled water) substantially alleviated the impacts of drought stress on all growth attributes in both maize cultivars. This mitigating effect is particularly pronounced when comparing well-watered and drought conditions. The Pak-Afgoi Sg-2002 genotype exhibited high resistance to drought stress, as evidenced by increased leaf area (122.16, 78.3, 104.36), chlorophyll content (35.500, 38.500, 34.00), dry leaf weight (0.2673 in CaCl₂), shoot length (67.00, 61.00), fresh root weight (1.84), relative water content (69.535, 59.030, 66.683) and relative dry weight (0.4213, 0.2731, 0.3222) when primed with *Bacillus*, *Trichoderma* and CaCl₂ compared to the normal water condition. Similarly root length, fresh shoot weight, dry shoot weight, dry root weight, residual transpiration and cell membrane stability exhibited varying results with different priming treatments under drought stress compared to well-watered conditions as depicted in Table 2.

During drought stress, the Af-5101 maize variety demonstrated the highest values for residual transpiration (5.2503, 2.6203), relative water content (85.125, 88.953, 75.86), relative dry weight (0.3226, 0.1943, 0.13413), cell membrane stability (96.033, 94.947) and dry shoot weight (5.0667, 4.7333, 5.0333) when treated with *Trichoderma*, *Bacillus* and

Table 1. Analysis of variance for different maize characters under different drought conditions and treatments.

Source of variance	LA (cm ²)	CC (%)	DLW (g)	SL (cm)	RL (cm)	FSW (g)	DSW (g)	FRW (g)	DRW (g)	RT (g H ₂ O/mi n/cm ² /1 05)	RWC (%)	RDW (g)	CMS (%)
Varieties (V)	108.3	11.267*	0.0329*	18.15	0.6615	30.788**	23.238**	0.21361	0.3300**	3.7507	53.32	0.0145**	56.784**
Treatments (T)	1941.5**	55.969**	0.0372**	110.68**	8.6531**	16.664**	5.6774**	0.18154*	0.19268**	1.0605**	347.36**	0.00257*	13.82
Conditions (C)	10748**	29.4**	0.0634**	5.4	20.068**	14.8205*	0.068	1.2327**	0.4051**	6.7306**	9408.5**	0.0675**	21.087
V × T	498.5**	104.07**	0.026**	19.931*	0.2344	7.7433	1.0344	0.12492	0.04745	1.8006	264.54**	0.0106**	122.482**
V × C	22.1	163.35**	0.0098	2.4	1.0935	0.8736	0.2968	0.6202**	0.00104	4.4808	910.14**	0.0189**	268.267**
T × C	2712.9**	18.16**	0.01972*	69.619**	0.4669	5.2558	0.3664	0.03739	0.01208	1.2106	371.32**	0.0028*	25.418**
V × T × C	942.7**	37.985**	0.01215	87.619**	0.5506	3.5291	0.5903	0.05304	0.00326	2.5506	1083.1**	0.0106**	58.644**
Grand Mean	79.367	32.083	0.2692	61.550	7.9850	8.7273	3.9477	1.8313	0.8605	3.3803	62.815	0.2621	88.125
CV	12.18	4.47	29.24	4.10	12.64	22.51	23.16	14.63	18.92	30.66	7.84	11.68	2.76

*= Significant and **= highly significant at 5% probability level, LA=leaf area, CC= chlorophyll content, DLW=dry leaf weight, SL= shoot length, RT= root length, FSW= fresh shoot weight, DSW= dry shoot weight, FRW= fresh root weight, DRW= dry root weight, RT= residual transpiration, RWC= relative water content, RDW= relative dry weight, CMS= cell membrane stability (Mohan *et al.*, 2018)

Table 2. Interaction of maize genotypes on the basis of morpho-physiological characters related to drought tolerance.

Condition/ variety	Treatment	LA (cm ²)	CC (%)	DLW (g)	SL (cm)	RL (cm)	FSW (g)	DSW (g)	FRW (g)	DRW (g)	RT (g H ₂ O/min/c m ² /105)	RWC (%)	RDW (g)	CMS (%)
Watered condition														
Pak-Afgoi	Trichoderma spp.	59.81 i-l	32.333 c-f	0.2693 b-d	58.500 ef	8.7 a-c	8.167 b-f	3.1233 f-h	1.5367 de	0.9333 b-d	2.8303 d-g	43.060 j	0.2332 e-i	86.740 c-e
Pak-Afgoi	Bacillus spp.	72.0 f-i	29.500 fg	0.3807 b	58.500 ef	9.2667 ab	9.333 a-e	3.2900 d-h	1.8367 b-d	0.7967 c-f	2.7903 d-g	63.573 e-g	0.2131 g-i	87.920 cd
Pak-Afgoi	CaCl ₂	53.82 j-l	28.000 fg	0.2313 c-e	70.500 a	9.9 a	8.733 b-f	4.6000 b-f	2.1167 a-c	0.9733 b-d	1.5103 g	52.445 hi	0.2010 hi	89.160 cd
Pak-Afgoi	Distilled water	65.38 h-k	30.000 e-g	0.2103 c-e	62.500 b- e	7.8333 b-e	6.943 d-f	3.2000 e-h	1.7967 b-e	0.8000 c-f	3.8703 b-e	40.951 j	0.2187 f-i	95.733 a
Pak-Afgoi	Control	75.22 e-i	31.000 d-g	0.2357 c-e	60.000 c-e	7.2667 c-f	9.967 a-d	2.9333 gh	1.7800 b-e	0.8600 c-f	4.7803 a-c	66.195 d-g	0.2657 c-f	89.743 bc

Drought condition															
Pak-Afgoi	Trichoderma spp.	78.3 e-h	38.500 b	0.202 c-e	61.000 e	b-7.7 b-f	6.733 d-f	2.6867 h	1.3800 e	0.6100 f	3.1903 c-g	59.030 g-i	0.2731 e	b-85.680 d-f	
Pak-Afgoi	Bacillus spp.	122.16 b	35.500 bc	0.2840 bc	67.000 a-e	6.6667 ef	10.833 ab	2.8867 gh	1.8400 b-d	0.6300 ef	4.0303 a-d	69.535 c-e	0.4213 a	76.800 g	
Pak-Afgoi	CaCl ₂	104.36 cd	34.000 c-e	0.2673 b-d	62.000 e	b-8.7 a-c	7.367 c-f	4.6667 a-e	1.8300 b-d	0.8100 c-f	2.0003 fg	66.683 d-g	0.3222 b	83.310 ef	
Pak-Afgoi	Distilled water	81.76 e-g	34.500 d	0.2650 b-d	61.500 e	b-6.1667 f	5.833 f	2.9667 gh	1.7933 b-e	0.7100 d-f	4.0503 a-d	83.940 ab	0.3080 bc	89.723 bc	
Pak-Afgoi	Control	67.35 g-j	31.833 c-g	0.1113 e	59.500 de	6.6 ef	6.2 ef	2.9000 gh	1.8067 b-e	0.7400 c-f	5.5903 a	73.312 cd	0.3202 b	86.710 c-e	
Watered condition															
Af-5101	Trichoderma spp.	73.98 e-i	31.000 d-g	0.5543 a	58.500 ef	8.7667 a-c	10.833 ab	4.8000 a-c	2.1200 a-c	0.9533 b-d	2.1703 e-g	31.343 k	0.2223 f-i	88.763 cd	
Af-5101	Bacillus spp.	47.84 i	27.500 g	0.2657 b-d	57.000 ef	8.4667 a-d	10.433 a-c	4.2333 b-g	1.8733 b-d	0.8633 c-f	3.8103 b-e	22.647 l	0.1855 i	87.943 cd	
Af-5101	CaCl ₂	86.88 ef	46.000 a	0.225 b-d	68.500 ab	9.8667 a	12.133 a	4.1333 c	2.3267 a	1.3600 a	1.6303 fg	71.150 c-e	0.3138 bc	85.400 d-f	
Af-5101	Distilled water	45.12 i	30.000 e-g	0.2053 c-e	56.500 ef	8.0667 b-e	7.333 c-f	3.6333 b-h	2.1833 ab	0.8867 c-e	3.3303 c-f	60.080 f-h	0.1921 hi	87.340 cd	
Af-5101	Control	79.78 e-h	28.500 fg	0.289 bc	51.500 f	7.5 c-f	8.367 b-f	3.8667 b-h	2.1767 ab	1.0000 bc	3.7703 b-e	51.484 i	0.2402 e-h	88.433 cd	
Drought condition															
Af-5101	Trichoderma spp.	49.91 ki	28.500 fg	0.296 bc	64.000 e	a-7.8 b-f	10.7 ab	5.0667 ab	1.7233 c-e	0.7500 c-f	5.2503 ab	85.125 a	0.3226 b	86.740 c-e	
Af-5101	Bacillus spp.	139.51 a	39.000 ab	0.3003 bc	67.500 a-c	7.8 b-f	9.803 a-d	4.7333 a-d	1.6667 de	0.7367 c-f	2.6903 d-g	88.953 a	0.1943 hi	96.033 a	
Af-5101	CaCl ₂	110.86 bc	34.500 b-d	0.26 b-d	71.500 a	8.4667 a-d	9.233 a-e	5.0333 ab	1.8400 b-d	1.1667 ab	2.6203 d-g	75.860 bc	0.3413 e-h	94.947 a	
Af-5101	Distilled water	84.40 ef	30.500 d-g	0.2273 c-e	57.500 ef	7.1667 c-f	8.6 b-f	3.4333 c-h	1.5633 de	0.7933 c-f	3.8003 b-e	67.950 c-f	0.2541 d-g	82.043 f	
Af-5101	Control	88.83 de	31.000 d-g	0.2527 de	63.500 e	a-7 d-f	7 d-f	4.7667 a-d	1.4367 de	0.8367 c-f	3.9803 a-d	82.987 ab	0.2994 d	b-73.333 bc	

LA=leaf area, CC= chlorophyll content, DLW=dry leaf weight, SL= shoot length, RT= root length, FSW= fresh shoot weight, DSW= dry shoot weight, FRW= fresh root weight, DRW= dry root weight, RT= residual transpiration, RWC= relative water content, RDW= relative dry weight, CMS= cell membrane stability

CaCl₂ in comparison to normal conditions. The Af-5101 genotype exhibited higher values in leaf area (139.51, 110.86), chlorophyll content (3.900), dry leaf weight (0.3003, 0.26) and shoot length (67.500, 71.500) when treated with *Bacillus* sp. and CaCl₂. Additionally, in certain morpho-physiological parameters like dry leaf weight and relative water content, distilled water also showed dominant results under drought conditions as presented in table 2. In the control experiment, well-watered plants demonstrated higher agronomic characteristics for both maize genotypes compared to plants under drought stress. In summary, the overall findings indicate that the Pak-Afgoi Sg-2002 genotype outperformed Af-5101 in all growth parameters under drought stress.

Bi-plot analysis

In this study bi-plot analysis showed the clusters of different traits depending upon the similarities among them. Under drought conditions, fresh root weight (FRW), shoot length (SL), relative dry weight (RDW) and chlorophyll content (CC) exhibited a collective negative relationship. Meanwhile, leaf area (LA), dry leaf weight (DLW), fresh root weight (FRW), dry shoot weight (DRW) and residual transpiration (RT) were grouped together with leaf area (LA) showing a particularly strong association with dry leaf weight (DLW) across various priming treatments. This indicated that a larger leaf area led to a higher dry leaf weight in maize plants. This entire group displayed a positive correlation as illustrated in bi-plot Figure 1(A).

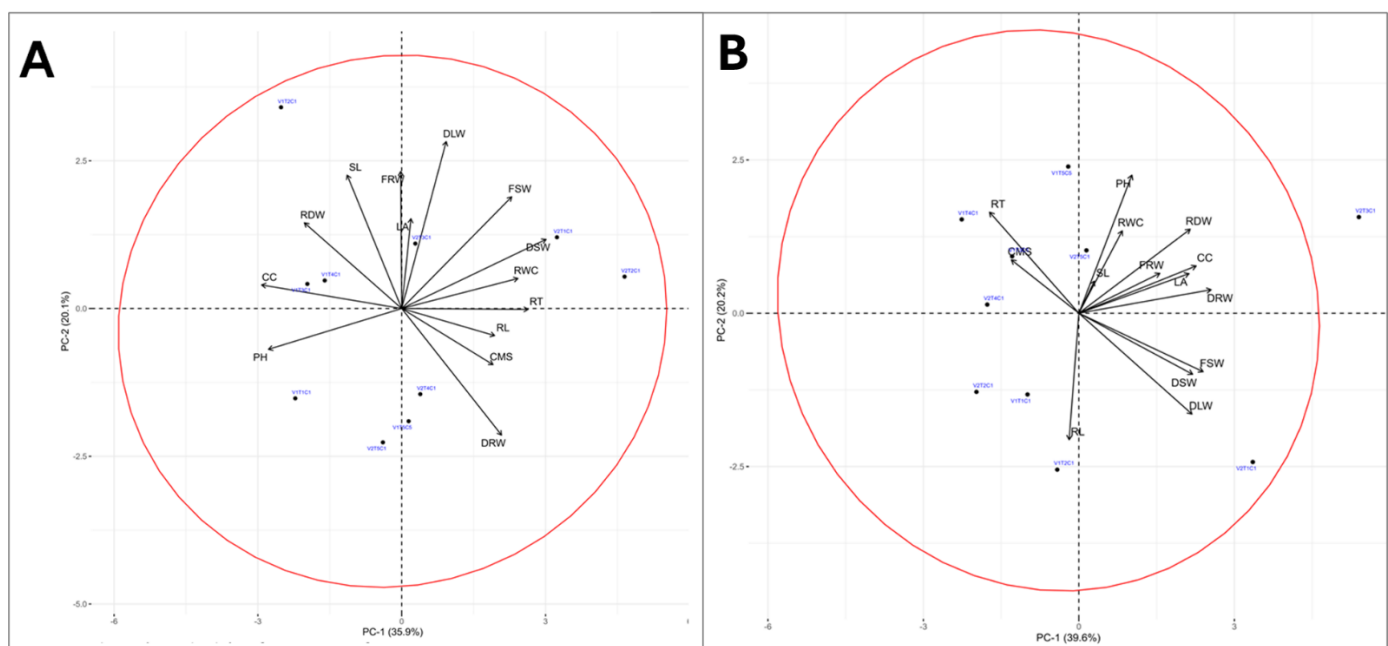


Figure 1. Bi-plot analysis of different morpho-physiological parameters of maize under drought and irrigated conditions

Under normal watered condition, plant height (PH), relative water content (RWC), shoot length (SL), relative dry weight (RDW), chlorophyll content (CC), leaf area (LA) and dry root weight (DRW) formed a closely knit cluster indicating a stronger interrelation among these parameters. In contrast, residual transpiration (RT) and cell membrane stability of maize plants demonstrated a negative correlation under different treatments as depicted in bi-plot Figure 1(B).

Correlation analysis

A correlation analysis was conducted to investigate the interrelationships between various morpho-physiological parameters under both drought stress and irrigated conditions. In drought stress, under different priming treatments chlorophyll content (CC) exhibited a significant positive association with plant height (PH) but conversely it displayed a negative correlation with residual transpiration and relative water content. Additionally, dry root weight (DRW) showed a highly significant negative correlation with chlorophyll content (CC). Furthermore, there was a significantly positive correlation between relative dry weight (RDW) and shoot length (SL) as well as with cell membrane stability (CMS) and relative water content (RWC) as represented in fig. 2(A).

The given Figure 2 depicted the response of different priming treatments (*Bacillus* sp. *Trichoderma* sp. CaCl₂ and distilled water) on the interaction of various morpho-physiological parameters in maize under well-watered conditions. In these conditions, dry shoot weight (DSW) demonstrated a highly significant positive correlation with fresh shoot weight (FSW). Moreover, a significant positive association was observed between relative dry weight (RDW),

chlorophyll content (RWC) and leaf area (LA). On the other hand, dry leaf weight (DLW) exhibited a highly significant negative relationship with residual transpiration (RT) and root length (RL) displayed a negative correlation with plant height across different treatments as demonstrated in Figure 2(B).

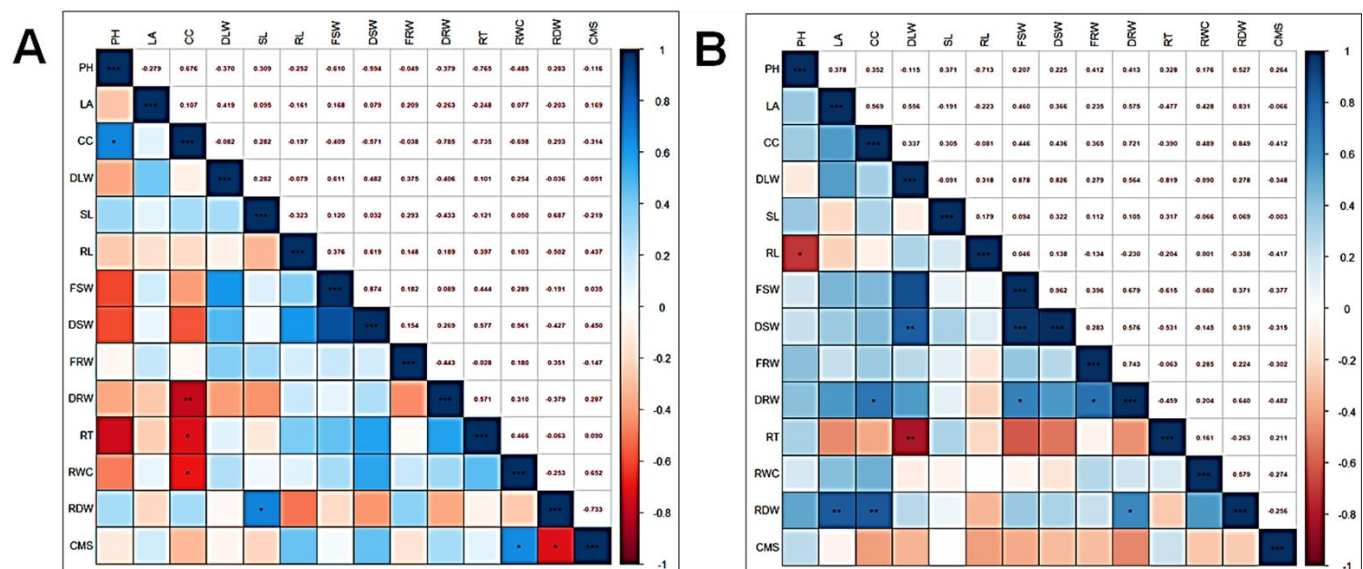


Figure 2. Correlation analysis between different morpho-physiological traits of maize under irrigated (A) and drought stress conditions (B).

DISCUSSION

In drought stressed situation plants indicated slow growth and development and there were also disruptions in oxidative system and photosynthetic activity of plants. This may be caused by the alteration in plant metabolic processes, inadequate water supply and irregular functioning of chloroplast (Parveen *et al.*, 2019). In past research, an experiment was carried out in which results showed that when osmotic concentration is increased water comes out of the cell's interior, reducing its turgidity as well as volume of cell (Sabagh *et al.*, 2018). It has been reported that priming seeds resulted in an improvement in the final germination percentage and germination rate ($1/t_{50}$, where t_{50} is the time to 50% germination) under saline conditions in canola. Likewise, plants derived from seeds primed with GA3 exhibited significantly higher leaf area, leaf water content (RWC), and chlorophyll content (Jamil *et al.*, 2009).

In the current study, analysis of variance for chlorophyll content, leaf area, relative dry weight, cell membrane stability and relative water content revealed highly significant differences among the varieties, treatments, drought conditions and interactions between these variables. However, some other parameters like root and shoot length, fresh weight of root and shoot, dry weight of root and shoot, residual transpiration indicated both significant and non-significant results. Previous literature indicated that drought stress significantly reduces shoot and root length as well as the fresh and dry weight of shoot and root in pearl and malka cultivars of maize. However, the application of silicone priming has shown positive effects. On average across the two cultivars, 6mM Si increased maize shoot length, root length, shoot dry weight and root dry weight under control and drought stress conditions respectively when compared with no Si application (Parveen *et al.*, 2019). In the present experiment, the data regarding root length, fresh shoot weight, dry shoot, and root weight, showed variation in the results when treated with different priming treatments (*Trichoderma* sp., *Bacillus* sp. and CaCl_2). Additionally, it has been observed that maize seedlings experience a significant decrease in both fresh weight and relative water content under osmotic stress during drought conditions (Moharramnejad *et al.*, 2015). Another study reported that when maize plants opposed water scarcity for more than 12 days there was a reduction in grain yield and production as well (Monneveux *et al.*, 2006).

In the present investigation, the chlorophyll content and cell membrane stability of the Pak-Afgoi Sg-2002 variety exhibited higher resistance against drought stress environment when treated with bioprimering and osmoprimering as compared to Af-5101 genotype. According to prior literature, the reduction in chlorophyll a, chlorophyll b and total chlorophyll contents varies based on the duration of drought faced by plants. The detrimental effects of drought stress have been mitigated through the application of osmoprimering with PEG, particularly in the cases of 7 days and 14 days of drought (Uddin *et al.*, 2021). In this study, treating seeds with *Bacillus* sp. and CaCl_2 conferred resistance against drought in the Af-5101 genotype. Previously, Dehghanipoodeh and colleagues noted that the application of silicone

enhanced photosynthetic activity and growth characteristics in strawberry cultivars under both normal and water-deficit conditions (Dehghanipoodeh *et al.*, 2018).

Earlier studies demonstrated that drought stress significantly impacted agronomic parameters leading to reductions in fresh weight, dry weight, leaf area and total biomass during 7 and 14 days of drought. Nevertheless, osmoprimed (PEG) plants exhibited resistance to induced drought stress showcasing mitigation in the mentioned attributes (Uddin *et al.*, 2021). This outcome corresponds with the current findings, as the elevated average mean values for leaf area (79.367), shoot length (61.550), relative water content (62.815) and cell membrane stability (88.125) indicate that various priming treatments have induced drought tolerance in both maize genotypes (Pak-Afgoi Sg-2002 and Af-5101). The analysis indicated that for cell membrane stability, sorghum genotypes FJSS-1 and FJSS-3 exhibited the highest values, ensuring robust cell stability. In contrast, varieties FJSS-6 and FJSS-15 showed minimal results indicating lower resistance to drought and significant instability (Ali *et al.*, 2009).

In this study, data pertaining to CMS (Cell Membrane Stability) was examined in both maize varieties. The findings reveal that *Trichoderma* sp., *Bacillus* sp. and CaCl₂ induce increased stability in the cell membranes of genotype Af-5101 under drought conditions compared to normal water conditions. It has also been addressed in earlier literature that the evaluation of drought tolerance, measured by an increase in Cell Membrane Stability (CMS) under water deficit conditions, exhibited clear distinctions between cultivars maize. Additionally, it demonstrated a strong correlation with a decrease in relative growth rate under stress (Premachandra *et al.*, 1991). As per certain researchers, leaves undergo a significant decrease in water potential under drought conditions. However, it is crucial to emphasize that relative water content (RWC) exhibited a notable increase during the rehydration phase surpassing its initial level across all hybrid varieties (Goodarziyan Ghahfarokhi *et al.*, 2015). The outcomes of this study are consistent with our present findings, demonstrating an augmentation in Relative Water Content (RWC) under drought conditions. The Af-5101 variety when subjected to various priming agents such as *Trichoderma* sp., *Bacillus* sp. and CaCl₂ exhibited a higher Relative Water Content (RWC) compared to the control indicating its enhanced ability to withstand drought stress.

CONCLUSION

The present investigation delves into the underlying morphological and physiological factors contributing to the drought tolerance in maize plant. Through statistical analysis, it was observed that primed seeds from Pak-Afgoi Sg-2002 and Af-5101 varieties exhibited robust performance and demonstrated resilience to drought. Notably, calcium chloride and *Trichoderma* these two treatments emerged as the most effective and outperforming the control group in both maize genotypes Pak-Afgoi Sg-2002 and Af-5101. Additionally, the study highlighted significant improvements in the maize genotype Af-5101 across various treatments, effectively countering the adverse effects of drought conditions. Investigating the potential of the priming technique is crucial to ensure successful germination and initial growth of maize in challenging environmental circumstances.

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AUTHOR CONTRIBUTIONS

AI mainly conducted the research, UA helped in data analysis, AJ and MS assisted in data recording and SA reviewed the manuscript.

COMPETING OF INTEREST

The authors declare no competing interests.

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