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EVALUATION OF DROUGHT TOLERANCE IN MAIZE (*ZEA MAYS* L.) USING PHYSIOLOGICAL INDICES

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Abstract

Drought is a threat to global food security. To develop drought tolerant high yielding maize varieties effective screening techniques for drought tolerance are required. In the current study, an effort is being made to screen maize cultivars for drought tolerance. Twelve maize cultivars were assessed for drought tolerance under laboratory conditions using physiological indices like germination stress tolerance index (GSI), shoot length stress tolerance index (SLSI), root length stress tolerance index (RLSI), fresh and dry biomass stress tolerance index (FWSI, DWSI). The data so collected was combined to various multivariate techniques which includes correlation and cluster analysis to evaluate the diversity for drought tolerance in maize cultivars. Positive and highly significant correlations were found between GSI, and DWSI. Cluster analysis classified 12 cultivars into three divergent groups. The members of first cluster (Malika, Sahiwal gold YY-15, MMRI yellow, Pop-Corn YPC-15) showed adequate degree of drought tolerance based on different physiological stress tolerance indices, whereas, cluster-2 included cultivars (Sweet Corn YSC-15, R1 czp, Pearl, Gohar 786) with moderate level of drought tolerance and cluster-3 included maize cultivars (Salman, Sahiwal 2002, Sadaf and Akbar) with lowest level of drought tolerance and did not give good performance. The results and scores achieved indicated that for drought tolerance in maize, physiological indices can be used as a screening tool.

Key words: Maize screening, Drought stress, Germination, Tolerance indices.

Introduction

Growth and development of crop plants are at a great risk due to water stress menace globally (Jaleel *et al*., 2009). Drought stress accounts for 40% reduction in maize productivity worldwide (Daryanto & Jacinthe, 2016). It is a primary abiotic constraint affecting crop production, due to shortage of fresh water. Drought stress on plants occurs when the available water lags continuous plant loss of water by transpiration. Plants normal growth and regular life cycle is disturbed due to water stress (Moussa & Aziz, 2008; Younas *et al*., 2022). The prime effect of water stress on crop plants is reduced germination and imperfect development (Harris *et al*., 2002). Water stress is a worldwide constraint to crop productivity (Waraich *et al*., 2011). Tolerance to water stress is involuted attribute including a large number of morpho-physiological characteristics (Tuberosa *et al*., 2002).

Maize (*Zea mays* L.) is the fourth major cereal crop cultivated in Pakistan. It is widely grown throughout the world in a wide range of agro-ecological environments. Being a C4 crop, maize utilizes moisture and sunlight efficiently to produce high yield and total dry matter (Khan *et al*., 2016; Badr *et al*., 2020). Moreover, it is used for food, feed and commercial purposes (Tri-da *et al*., 2006). The early stages of maize growth are more sensitive to water stress which decreases its yield (Farooq *et al*., 2015). Insufficient available soil water weakens the metabolic activity of maize, lessen its biomass accumulation, and decreases its photosynthetic rate by reducing the chlorophyll content in leaves, eventually leading to a decline in yield (Song *et al*., 2019). However, plants can tolerate water stress only up to a certain limit (threshold level) and beyond that limit, there is a severe drop in yield (Zafar *et al*., 2015). Hence, tolerance of crop plants to water stress can be enhanced by using selection criteria of morpho-physio characteristics at early growth stages (Blum, 2011; Comas *et al*., 2013). At germination and seedling stage of crop plants, physiological indices can be used to examine variation in genotypes (Hakim *et al*., 2010; Kausar *et al*., 2012). It is useful for selecting the tolerant varieties, which can be used economically for cultivation on water stressed lands. The objective of present study was to assess maize genotypes under drought stress to determine the response of these genotypes. Our study is an effort to compare the efficacy of various stress indices for finding the genotypes which shows better performance at various water stress levels.

Materials and Methods

The study was performed under laboratory conditions using PEG 6000 levels i.e., 10%, 15% and 20% to examine the drought tolerance potential of 12 maize genotypes using physiological indices. The seeds were obtained from Maize and Millet Research Institute, Yousafwala, Sahiwal, Pakistan. The experiment was conducted at Plant Stress Physiology Lab, Government College University, Faisalabad, Pakistan. The maize grains were sterilized with 10% sodium hypochlorite solution and washed with distilled water. The grains of each genotype were placed in Petri-plates having filter papers moistened with above-mentioned solutions prepared using PEG 6000 and placed in a Growth Chamber (Sanyo-Gallenkamp, UK) running at 28±2°C and 10 h photoperiod with 80 μM S^{-1} m^{-2} light intensity was maintained. Germination was noted when the radical length was 5 mm.

Physiological indices: To observe the germination stress tolerance index (GSI), the promptness index (PI) was calculated, using following formulae (Ashraf *et al*., 2008):

$$\textbf{PI} = nd1\,(1.00) + nd2\,(0.75) + nd3\,(0.50) + nd4\,(0.25)$$

where: nd1-nd4, showed the number of seeds germinated on the consecutive days. The germination stress tolerance index (GSI) was observed in terms of percentage as follows:

$$\textbf{GSI} = \frac{\textbf{PI}\text{ of stressed seeds}}{\textbf{PI}\text{ of control seeds}} \times 100$$

After fourteen days of the study, the length of shoot and root and fresh biomass was recorded. The plant samples were dried at 75°C for two days in an oven and their dry biomass was noted. Physiological stress tolerance index of root and shoot length (RLSI, SLSI) and fresh weight and dry weight stress tolerance indices (FWSI, DWSI) were calculated according to the formula given below:

$$\textbf{PHSI} = \frac{\text{Plant height of stressed plants}}{\text{Plant height of control plants}} \times 100$$

$$\textbf{SLSI} = \frac{\text{Shoot length of stressed plants}}{\text{Shoot length of control plants}} \times 100$$

$$\textbf{RLSI} = \frac{\text{Root length of stressed plants}}{\text{Root length of control plants}} \times 100$$

$$\textbf{FWSI} = \frac{\text{Fresh weight of stressed plants}}{\text{Fresh weight of control plants}} \times 100$$

$$\textbf{DWSI} = \frac{\text{Dry weight of stressed plants}}{\text{Dry weight of control plants}} \times 100$$

Statistical analysis: The data was analyzed statistically using analysis of variance (ANOVA) (Steel *et al*., 1997) and comparison of means was completed using Least Significant Difference Test at 5% probability. The coefficient of variation and cluster analysis and was performed using the MStatC and Minitab-6. The experiment was performed using completely randomized design with three replications.

Results

The polyethylene glycol induced water stress significantly inhibited seed germination in all maize cultivars (Table 1) which exhibited significant ($p \le 0.05$) differences in GSI. All the PEG concentrations significantly affected maize cultivars. Overall, GSI gradually decreased with the increase in PEG concentration and it was found to be 89.53, 78.33 and 68.05% under 10, 15 and 20% PEG-6000, respectively (Table 1). Maize cultivars, Sahiwal gold YY-15 (100%), Pop-Corn YPC-15 (100%) and Malika (96.11%) exhibited maximum and Akbar showed minimum GSI (75%) values at 10% PEG-level. At 15% PEG-6000 level Pop-Corn YPC-15 showed 95% and Sahiwal gold YY-15 showed 93.33% GSI, while it was least in Sahiwal-2002 (63.33%), however at 20% PEG-6000 level MMRI Yellow showed 89.44% GSI, while it was the least in Salman 53.33 %. The overall genotypic means and ranking for GSI indicated that Sahiwal gold YY-15 was at first, Pop-Corn YPC-15 at second and MMRI Yellow at third position while Salman was at 12th position.

The various PEG concentrations significantly influenced shoot length of maize genotypes (Table 2), the shoot length stress tolerance index (SLSI) declined with a surge in PEG application and it exhibited 58.55, 22.35 and 13.32% values under 10, 15 and 20% PEG-6000, respectively. All the maize cultivars showed significant differences at 10% PEG-6000, however, the highest SLSI (90%) was exhibited by Pop-Corn YPC-15 closely followed by Malika (86.73%), minimum SLSI was observed in Salman (29.69%). At 15% PEG-6000 concentration, Pop-Corn YPC-15 (40.76%) maintained the highest SLSI closely followed by Malika (26.98%), while minimum was reported by Akbar (13.32%). Under the highest PEG level (20% PEG-6000), the genotype Malika showed the highest (19.55%) SLSI followed by Pop-Corn YPC-15 (16.26%) and the least values were demonstrated by Akbar (7.91%) and Sadaf (9.01%). On the basis of means, Pop-Corn YPC-15 ranked at first, Malika at second and Gohar 786 at third position, while Salman was at 12th position.

Table 1. Germination stress tolerance index (GSI) of various maize cultivars.

Cultivars	Water stress levels (PEG 6000 %)				Ranking
	10	15	20	Means (GSI %)	
Malika	96.11	86.66	70	84.25 ab	5
POP-Corn YPC-15	100	95	78.33	91.66 a	2
Sahiwal gold YY-15	100	93.33	73.33	91.66 a	1
MMRI Yellow	91.66	91.66	89.44	90.92 a	3
Sweet Corn YSC-15	86.66	73.33	66.66	75.55 abc	7
Gohar 786	80	65	56.66	67.22 bc	10
Pearl	95	78.33	65	79.44 abc	6
R1 czp	93.33	81.66	80	85 ab	4
Salman	76.66	70	53.33	66.66 c	12
Sahiwal-2002	86.66	63.33	61.66	70.55 bc	9
Sadaf	83.33	75	63.88	74.07 abc	8
Akbar	75	66.66	58.33	66.66 bc	11
Mean	89.53a	78.33b	68.05c		

Note: Means sharing similar letter did not differ significantly ($p>0.05$) in row and column

Table 2. Shoot length stress tolerance index (SLSI) of various maize cultivars.

Cultivars	Water stress levels (PEG 6000 %)				Ranking
	10	15	20	Means (SLSI %)	
Malika	86.73	26.98	19.55	44.42 b	2
POP-Corn YPC-15	90.00	40.76	16.26	49.01 a	1
Sahiwal gold YY-15	59.79	25.80	13.75	33.11 d	4
MMRI Yellow	55.51	23.07	11.99	30.19 d	6
Sweet Corn YSC-15	53.38	21.34	15.23	29.98 d	7
Gohar 786	81.24	19.02	15.97	38.74 c	3
Pearl	55.40	24.56	15.34	31.77 d	5
R1 czp	39.04	17.16	13.16	23.12 ef	10
Salman	29.69	19.53	10.12	19.78 f	12
Sahiwal-2002	43.50	20.50	11.57	25.19 e	9
Sadaf	40.81	16.16	9.01	21.99 ef	11
Akbar	67.54	13.32	7.91	29.59 d	8
Mean	58.55a	22.35b	13.32c		

Note: Means sharing similar letter did not differ significantly (p>0.05) in row and column

Table 3. Root length stress tolerance index (RLSI) of various maize cultivars.

Cultivars	Water stress levels (PEG 6000 %)				Ranking
	10	15	20	Means (RLSI %)	
Malika	81.52	65.50	16.83	54.62 a	5
POP-Corn YPC-15	91.14	43.75	30.47	55.12 a	1
Sahiwal gold YY-15	92.91	43.94	28.03	54.96 a	2
MMRI Yellow	87.69	47.47	12.54	49.23 b	6
Sweet Corn YSC-15	92.59	41.90	29.81	54.77 a	4
Gohar 786	92.93	40.40	31.13	54.82 a	3
Pearl	37.04	33.81	22.55	31.14 c	9
R1 czp	71.99	49.25	21.03	47.42 b	7
Salman	37.38	20.98	9.20	22.52 d	11
Sahiwal-2002	77.20	11.12	7.48	31.93 c	8
Sadaf	42.69	5.71	3.83	17.41 e	12
Akbar	46.81	17.96	7.71	24.16 d	10
Mean	70.99a	35.15b	18.38c		

Note: Means sharing similar letter did not differ significantly (p>0.05) in row and column

Root length stress tolerance index (RLSI) of all maize cultivars was significantly affected by water stress (Table 3); it was reduced with increase in PEG level 70.99, 35.15 and 18.38% under 10, 15 and 20% PEG-6000 levels, respectively. Variations among all PEG concentrations were significant for RLSI. At 10% PEG-6000, the minimum value for RLSI (37.04%) was examined in case of Pearl and Salman (37.38%) while maximum was exhibited by Gohar 786 (92.93%) closely followed by Sahiwal gold YY-15 (92.91%). Under 15 % PEG-6000, the highest RLSI (65.50%) was exhibited by Malika closely followed by R1 czp (49.25%) and the lowest value (5.71%) was recorded for Sadaf, closely followed by Sahiwal-2002 (11.12%). The cultivar Gohar 786 showed the highest RLSI (31.13%) under 20% PEG-6000, followed by Pop-Corn YPC-15 (30.47%), while the minimum was exhibited by Sadaf (3.83%) closely followed by Sahiwal-2002 (7.48%). On the basis of cultivar means, Pop-Corn YPC-15 and Sahiwal gold YY-15 were ranked as 1st and 2nd and Sadaf 12th.

Fresh weight stress tolerance index (FWSI) was significantly influenced by water stress (Table 4), it decreased gradually (67.89, 38.07 and 23.37%) with increase in (10, 15 and 20%) water stress induced by PEG-6000. Under 10 % PEG-6000, Pop-Corn YPC-15 (91.59%) and Sahiwal gold YY-15 (85.69%) maintained the highest FWSI, while minimum FWSI was in Sahiwal-2002 (34.63%) and Akbar (12.19%). Akbar and Sadaf exhibited poor performance for FWSI (15.12% and 19.32%, respectively) at 15% PEG-6000. At the highest PEG-6000 concentration, maximum FWSI was recorded for Pop-Corn YPC-15 (62. 91%).Under the highest PEG level (20% PEG-6000), maize cultivars sadaf (10.73%) followed by Akbar (7.98%) showed the poor performance for FWSI. Overall, cultivar means indicated that Pop-Corn YPC-15 and Sahiwal gold YY-15 scored maximum points for FWSI and ranked as 1st and 2nd while Akbar was at 12th position.

PEG induced stress significantly ($p \leq 0.05$) influenced dry biomass stress tolerance index (DWSI). Significant differences in DWSI was exhibited by all the treatments (Table 5), and decreased by the increase in stress levels (59.92, 22.18 and 5.08% under 10, 15, and 20% Peg-6000). At 10% PEG-6000, maximum DWSI was recorded for Pop-Corn YPC-15 (94.26%) followed by Sahiwal gold YY-15 (91.49%), while the lowest value for DWSI (4.66%) was noted for Akbar. Under 15% PEG-6000, the highest DWSI was estimated for R1 czp (64.24%) closely followed by Pop-Corn YPC-15 (53.62%) and it was the lowest in Akbar (3.62%), which was statistically at par with Sadaf (3.95). At 20% PEG-6000, maximum value for DWSI was noted for Pop-Corn YPC-15, while the minimum was in Sadaf and Akbar. On the basis of overall means cultivar Pop-Corn YPC-15 and R1 czp ranked first, second and Akbar on 12th position.

Table 4. Fresh weight stress tolerance index (FWSI) of various maize cultivars.

Cultivars	Water stress levels (PEG 6000 %)				Ranking
	10	15	20	Means (FWSI %)	
Malika	82.57	36.57	13.45	44.20 c	9
POP-Corn YPC-15	91.59	62.91	23.13	59.21 a	1
Sahiwal gold YY-15	85.69	48.23	24.84	52.92 b	2
MMRI Yellow	80.75	34.87	25.33	46.99 c	6
Sweet Corn YSC-15	79.74	44.34	30.61	51.56 b	5
Gohar 786	70.83	36.45	24.80	44.03 c	8
Pearl	80.54	32.82	28.24	47.20 c	4
R1 czp	82.50	45.14	31.04	52.89 b	3
Salman	54.71	50.58	33.12	46.14 c	7
Sahiwal-2002	34.63	30.48	27.21	30.77 d	10
Sadaf	58.87	19.32	10.73	29.64 d	11
Akbar	12.19	15.12	7.98	11.77 e	12
Mean	67.89a	38.07b	23.37c		

Note: Means sharing similar letter did not differ significantly (p>0.05) in row and column

Table 5. Dry weight stress tolerance index (DWSI) of various maize cultivars.

Cultivars	Water stress levels (PEG 6000 %)				Ranking
	10	15	20	Means (DWSI %)	
Malika	66.29	18.52	4.68	29.83d	6
Pop-corn YPC-15	94.26	53.62	11.12	53a	1
Sahiwal gold YY-15	91.49	42.61	6.28	46.79b	3
MMRI Yellow	71.26	25.61	7.02	34.63c	4
Sweet corn YSC-15	51.49	17.64	4.65	24.59e	8
Gohar 786	70.66	6.65	2.76	26.69e	7
Pearl	78.62	7.74	7.14	31.16d	5
R1 czp	78.78	64.24	4.12	49.04b	2
Salman	30.72	13.79	4.58	16.36g	10
Sahiwal-2002	49.6	8.18	4.04	20.60f	9
Sadaf	31.21	3.95	2.03	12.39h	11
Akbar	4.66	3.62	2.54	3.60i	12
Mean	59.92a	22.18b	5.08c		

Note: Means sharing similar letter did not differ significantly (p>0.05) in row and column

Table 6. Correlation among different screening techniques.

Techniques	GSI	SLSI	RLSI	FWSI	DWSI
GSI	1				
SLSI	0.446NS	1			
RLSI	0.607*	0.679*	1		
FWSI	0.647*	0.351NS	0.685*	1	
DWSI	0.844**	0.461NS	0.725**	0.844**	1

** = Significant (p<0.01); SI = Stress tolerance index; G= Germination; SL= Shoot length; RL= Root length; SI stress tolerance index; SI stress tolerance index; Stress tolerance index; FW = Fresh weight; DWSI = Dry weight

The analysis of correlation showed notable and positive correlations between GSI and SLSI, RLSI, FWSI and DWSI; same was the case with SLSI and RLSI, FWSI and DWSI. Significant and positive correlations were also obtained between RLSI and FWSI and DWSI, and relationships between FWSI and DWSI were also positive (Table 6). The data indicated that the cultivars with high GSI, SLSI, RLSI, FWSI and DWSI were tolerant to water stress.

The cluster analysis based on full linked correlation coefficient distance was performed in the current research that separated the twelve maize genotypes into three clusters (Fig. 1). Cluster 1 showed PEG induced stress tolerant cultivars i.e. Malika, Sahiwal gold YY-15, MMRI yellow, Pop-Corn YPC-15; cluster 2 showed medium tolerant cultivars and cluster 3 showed sensitive cultivars. The results showed that for morpho-physiological markers that promote stress tolerance in maize on the basis of genetic variability is vital for development of water stress tolerant cultivars at initial and late periods of growth.

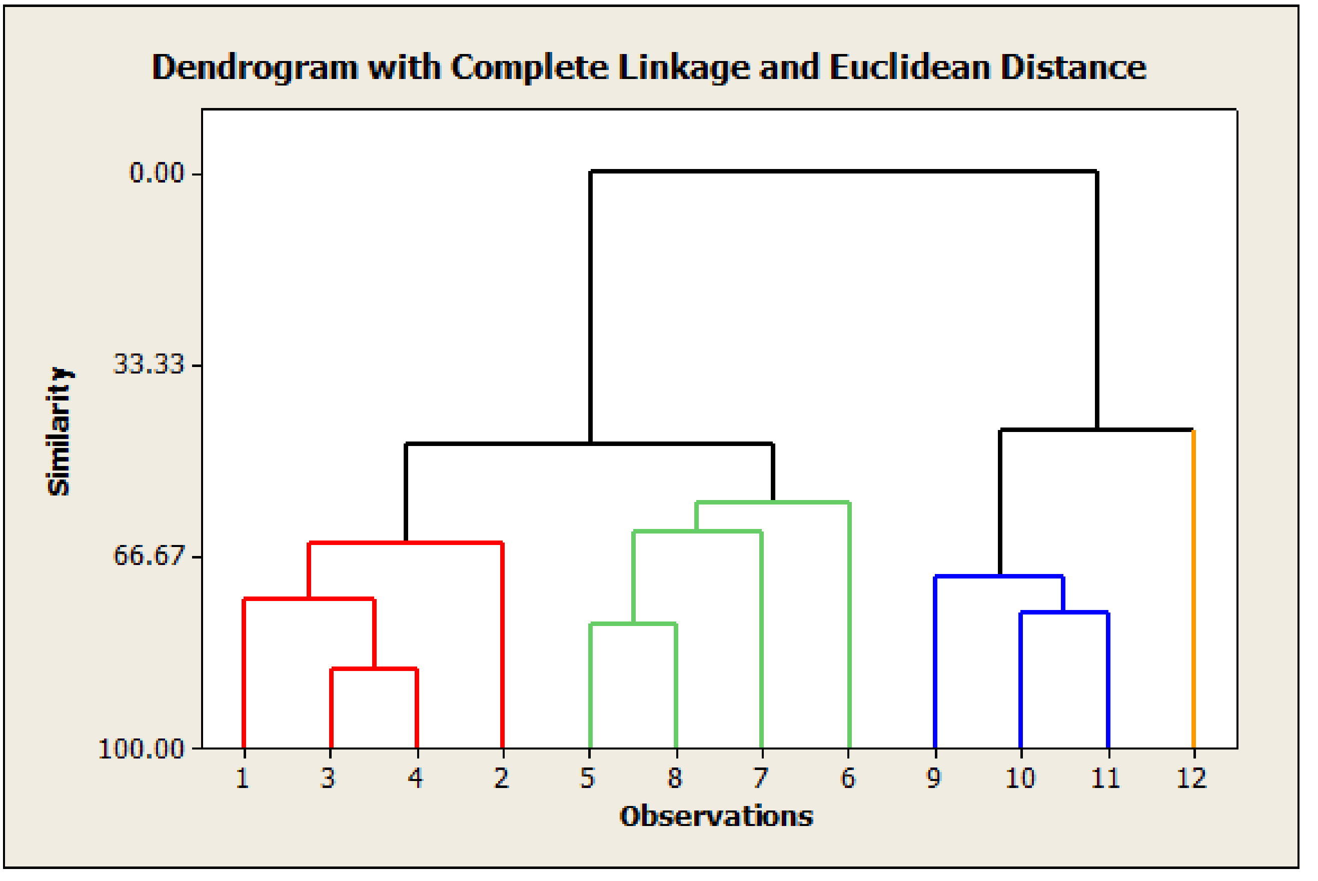

Fig. 1. Dendrogram from cluster analysis for drought tolerance in different maize cultivars based on physiological indices: a screening tool. Clusters detail; Cluster 1 includes 1; Malika, 3; Sahiwal gold YY-15, 4; MMRI yellow, 2; Pop-Corn YPC-15; Cluster 2: 5; Sweet Corn YSC-15, 8; R1 czp, 7; Pearl, 6; Gohar 786, Cluster 3: 9; Salman, 10; Sahiwal 2002, 11; Sadaf and 12; Akbar

Discussion

Polyethylene glycol (PEG) creates osmotic stress and could be used to examine the effect of water stress on seed germination (Hellal *et al*., 2018). In a water deficit environment, development of a crop mainly depends on the germination of seeds and the establishment of seedlings (Zafar *et al*., 2015). The germination process consists of enzymatic hydrolysis of stored food and the formation of new tissues (Akram *et al*., 2010; Alvi *et al*., 2022). Therefore, it is necessary to develop effective screening criteria at early growth stages to get the maximum yield (Zafar *et al*., 2015). It is an established fact that tolerance at maturity is demonstrated by the tolerance at immature stage of plant. This fact is exploited in maize (Khan *et al*., 2003a), wheat (Ali *et al*., 2002; Khan *et al*., 2003 b), cotton (Azhar & Ahmad, 2000), sorghum (Kausar *et al*., 2012) and soybean (Kamal *et al*., 2003).

Drought stress induced by applying polyethylene glycol (PEG_{6000}) reduced the water potential of all maize genotypes and in turn reduced germination (Kulkarni & Deshpande, 2007; Taiz & Zeiger, 2010). Results of present study showed that stress tolerance indices are valuable to explain some of the mechanisms indicating tolerance to drought.

The data showing stress tolerant indices GSI, SLSI, RLSI FWSI and DWSI showed that they can be used to screen the maize cultivars for drought tolerance (Okcu *et al.*, 2005). The cultivar POP-Corn YPC-15 score highest for the stress tolerant indices followed by Sahiwal gold YY-15, Malika, and MMRI Yellow, and are grouped as cluster 1 in dendrogram (Fig. 1) are categorized as tolerant ones. While, maize genotypes Akbar, Sadaf, Sahiwal 2002 and Salman maintained scores below average, they are grouped in 3rd cluster (Fig. 1) and are categorized as sensitive or non-tolerant ones. Magar *et al*., (2019) also observed the same variations in the physiological responses of maize cultivars subjected to the PEG induced water stress. So, the screened maize cultivars have a genetic potential for drought tolerance and POP-Corn YPC-15 can be cultivated on drought-affected soils.

It is suggested by some researchers that in maize plants the screening criteria for abiotic stress tolerance is germination stress tolerance index (GSI) (Ahmad *et al*., 2015; Saensee *et al.*, 2012). PEG induced water stress caused a reduction in plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI) and dry matter stress tolerance index (DMSI). This reduction in stress indices is favored by the reduction in biomass accumulation and plant growth (Li *et al*., 2009). Under water deficit conditions the decrease in plant height is due to the reduction in cell expansion (Okcu *et al*., 2005). As a result of water stress, signal transduction pathways, imbalance the production of hormones like abscisic acid (ABA) and indole-3-acetic acid (IAA) causing several morphological changes. These changes are more commonly observed in drought sensitive cultivars as compared to drought tolerant ones (De-Micco & Aronne, 2012; Jiang *et al.*, 2012, 2013).

The correlation analysis exhibited some major associations between germination and stress tolerant indices. A significant and positive correlation was noted between GSI, FWSI and DWSI, which showed that these stress tolerant indices can be used to screen the cultivars for drought tolerance. Khan *et al*., (2010), Kausar *et al*., (2012) and Zafar *et al*., (2015) noted a positive correlation among GSI and stress tolerance in brassica, sorghum and wheat and suggested that GSI is a good screening tool for drought tolerance. The reproducibility of differences between genotypes and the correlation shown by these physiological indices suggested their authenticity, uniformity and a reliable method for determining drought tolerance potential in maize germplasm.

Many researchers have used cluster analysis and grouped germplasm into clusters based on similar characteristics in a group (Nookra & Khaliq, 2007). The closely related genotypes were grouped in same cluster. The Cluster-I consisted of four cultivars out of twelve, all performed well as compared to others for all observed indices and are considered as drought tolerant. Cluster-II consisted of four cultivars and are considered as medium tolerant due to similarities among the tested indices. Whereas, cluster-III consisted of four cultivars that did not perform up to mark and showed least similarity to other genotypes for tested indices. Therefore, they were considered as non-tolerant or sensitive. It is emphasized in the literature to screen genotypes using cluster analysis technique (Noorifarjam *et al*., 2013). The cultivars selected could be helpful in further breeding programs for drought tolerance.

Conclusion

The results showed that physiological indices can be used to screen maize germplasm for drought tolerance. Significant correlations between various indices and grouping of maize cultivars on the basis of cluster analysis also proved that cultivars screened on the basis of stress tolerant indices are drought tolerant. Tolerant maize cultivars can be cultivated on drought affected soils and can be used to produce high yielding drought tolerant maize cultivars.

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