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EVALUATION OF MAIZE ACCESSIONS UNDER LOW TEMPERATURE STRESS AT EARLY GROWTH STAGES

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ABSTRACT

Maize is a very important cereal and grown globally across wide range of altitude and latitude. Among abiotic stresses, low temperature is a sever threat for spring maize in Pakistan during early growth stages. This study was planned to sort out tolerant and susceptible maize accessions by evaluation of available maize germplasm against low temperature stress and assessment of genetic diversity among them. At low temperature stress, early emergence of maize and improved photosynthetic performance are the good indicators of early seedling stand. The objectives of this study were (i) to characterize maize accessions for early emergence and photosynthesis related traits at 10/08˚C (day/night), (ii) to explain relationship between different low temperature related traits and (iii) to identify best performing accessions under low temperature to be used in hybridization to evolve chilling tolerant hybrids. The effect of low temperature stress was studied on emergence potential, root/shoot ratio, chlorophyll *a*, chlorophyll *b*, β-carotenoids and ascorbic acid contents. Significant differences were observed among maize accessions for studied traits under low temperature stress. Using biplot analysis, genetically distant accessions showed variable responses to low temperature stress. Maize accessions; 2(EV-7004Q), 5(F-150), 23(F-118), 26(B-308), 46(EV-338), 34(POP-2007), and 18(EV-329) showed superior performance for emergence whereas, 69(EV-134), 59(B-304), 56(F-151), 58(B-313), 73(F-143) and 63(F122) performed well regarding post emergence parameters by maintaining higher values for subjected traits. Chlorophyll contents reflected photosynthesis efficiency of any plant and showed significant positive association with emergence of plants therefore proved a good indicator against low temperature stress. Selection on the basis of combination of emergence related traits and chlorophyll contents provided a sound base to identify early emergence and healthy seedling development.

Key words: Maize, Low temperature stress, Physiological evaluation, Correlation analysis, Biplot analysis.

INTRODUCTION

Maize is a valuable ingredient in manufactured items which feed large population of the world and contribute considerable portion in world's economy. It is third important cereal after wheat and rice. Biotic and abiotic stresses are main threat for agricultural crops (Aslam *et al*., 2013a; Aslam *et al*., 2013b; Aslam *et al*., 2013c; Aslam *et al*., 2014; Naveed *et al*., 2014). Like other crops maize is facing the threat of different biotic and abiotic stresses (Mustafa *et al*., 2013; Naveed *et al*., 2014). In Pakistan maize is grown in two seasons, namely spring and autumn. Different hybrids have specificity for spring and autumn seasons. Per unit area production of spring maize is more than that of autumn crop due to more area under hybrid cultivation in spring maize. In spring crop temperature becomes high at reproductive stage in late May and during June, which is not desirable. Exposure of plants to high temperature around 38°C during reproductive stage promotes pollen desiccation resulting in yield losses (Wahid *et al.*, 2007). To resolve this problem early planting is required to avoid high temperature during anthesis and seed maturity. Therefore, by planting spring maize during early January instead of February the aforesaid result can be achieved, this practice demands hybrids with ability to germinate rapidly and good seedling stand under low temperature stress.

Maize is sensitive to low temperature stress at early growth stages with variable degree of chilling injury among different varieties and differences in the response to low temperature stress is due to variable antioxidant systems (Zaidi *et al*., 2010). Temperature below 12-15°C may damage the young maize plants causing chilling stress (Hola *et al.*, 2003). It requires relatively high temperature (30-35°C) for better germination, growth and development (Henselová *et al*., 2012), and under 10°C, its germination is badly affected (Janowiak *et al.*, 2002). In case of early sowing in spring maize seedling emergence is delayed due to low temperature. Suboptimal temperature causes growth inhibition, chlorosis, wilting, necrosis and sometimes plant death (Janowiak *et al.*, 2002). Due to low temperature, crop growth rate is reduced while growth duration is prolonged. Hence seedlings are weakened and grain yield is reduced.

Optimal conditions are recommended for germination to obtain better early crop development (Leipner, 2009). Seedling establishment at suboptimal temperature is affected due to damage of macromolecules and cellular structure, caused by production of reactive oxygen species (Apel and Hirt, 2004). Cell membrane, photosynthetic apparatus and enzymes undergo severe changes at suboptimal temperature (Lukatkin, 2003). This results in reduction of food assimilates consequently vegetative growth is suppressed and yield reduces. The photosynthetic apparatus of maize is very sensitive to chilling-induced photo inhibition. Some structural changes in photosynthetic development are caused by low temperature and persist for longer times. Hence photosynthetic rate of plant developed under chilling stress remains lower even after restoration of normal temperature. Leaf development is delayed due to low temperature stress. Suboptimal temperature results in prolong cell cycle and decrease in cell production (Rymen *et al.*, 2007).

In chilling susceptible lines total chlorophyll content decreases, reducing the photosynthetic rate and ultimately yields (Aroca *et al.*, 2001). Therefore suboptimal temperature is threat for maize yield in areas of low temperature. In Pakistan temperature during maize vegetative and reproductive growth is not low except early spring season temperature which is not supportive for seedling emergence. Maize shows a poor seedling establishment at low temperature during early spring planting (Ahmad *et al.*, 2012). There is an urgent need of low temperature tolerant germplasm having good emergence and early vigor to have early maize crop. Maize cultivars with good early vigor are desirable to enhance establishment in areas where low temperature retards early growth. For selection of better accessions biplot graphical analysis is easiest, convenient and reliable approach because it considers all parameters for a given accessions under certain environmental conditions at a time (Golabadi *et al*., 2006). The present study was planned to characterize maize accessions for early emergence and photosynthesis related traits at low temperature, to explain relationship between different low temperature related traits and to identify best performing accessions under low temperature to be used in heterosis breeding to evolve chilling tolerant hybrids.

MATERIALS AND METHODS

Experiment was conducted in screenhouse of the Department Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan (latitude; 31º 25‘N, longitude 73º, 90‘E) during 2012. Seeds of 76 maize accessions were surface disinfected with 0.02% (w/v) of $HgCl_2$ and sown in polythene bags (6"×9") filled with soil (pH=6.5 & EC=0.0ds/m) in a screenhouse at 8-10°C, 70% RH, 10/14 hrs (day/night) photoperiod. Standard approved agronomic practices were followed to raise the seedlings. Seedling emergence was daily counted for emergence potential studies. Samples were collected at 4 leaf stage (after 25 days of sowing) to study the effect of low temperature on mean emergence time (MET), emergence index (EI), energy for emergence (EE%), energy for final emergence percentage (FE%), root/shoot ratio, chlorophyll *a*, chlorophyll *b*, beta carotenoids and ascorbic acids contents (A.A). Chlorophyll contents were estimated by using methodology used by Nagata & Yamashita (1992) and ascorbic acid contents were estimated by following Kampfenkel (1995).

Seedling emergence: The numbers of emerged seeds were counted daily according to the seedling evaluation Handbook of Association of Official Seed Analysis (1990) until the constant count was achieved.

Mean emergence time (MET) was calculated according to the following equation of Ellis and Roberts (1981).

$$MET = \frac{\sum Dn}{\sum n}$$

Where, n is the number of seeds, which were emerged on given day, and D is the number of days counted from the beginning of emergence.

Emergence index (EI) was calculated as described in Association of Official Seed Analysis (1990).

$$EI = \frac{\text{No. of emerged seeds}}{\text{Days of first count}} - + \frac{\text{No. of emerged seeds}}{\text{Days of final count}}$$

Energy of emergence (EE) [%]: Energy of emergence was recorded at 4^{th} day after planting. It is the percentage of emerged seeds 4 days after planting relative to the total number of seeds tested (Farooq *et al*., 2006).

Final emergence percentage (FEP): Final emergence percentage was taken at the end of total emergence. It represented the ratio, in percentage, of number of emerged seeds to total seeds planted.

Seedling growth: On 25th day after sowing, the seedling was tested for vigor after careful uprooting. Five plants were carefully uprooted from each entry, shoot and root length of every seedling was measured with scale and average was calculated to get mean shoot and root ratio.

Statistical Analysis: Analysis of variance (ANOVA) was conducted by following Steel *et al*. (1997). Association among traits was computed by subjecting the data to Pearson Correlation coefficient analysis proposed by Goulden (1952). Data was analyzed by Principle component analysis (PCA; Adam *et al*., 1999) and GGE Biplot analysis model recommended by Gabriel (1971) for agricultural data analysis.

RESULTS

Analysis of variance (ANOVA) showed high level of genetic diversity among the maize accessions for all the parameters under study (Table-2). Results presented in Table-2 depicted that chlorophyll *a* (Chl. *a*) has highest variability followed by chlorophyll *b* (Chl. *b*) and carotenoid contents (Cart.) because of higher CV values respectively. According to distribution analysis around mean all the traits have right skewed distribution of data except emergence index (EI%) and final emergence (FE%).

Association of Chl. *a* with Chl. *b*, A.A and MET was positive and highly significant whereas with FE% value was significant but not strongly positive (Table-3). It showed negative and significant correlation with EE% and RSR. Chl. *a* was positively and non-significantly related with CART but negatively and non-significantly with EI. Chl. *b* was positively and significantly correlated with FE% whereas had highly significant association with CART and MET (Table-3). Association of Chl. *b* with EI was significantly negative but with EE% and RSR was non-significantly negative. Relationship between Chl. *b* and AA was positive and significant. EI, EE%, FE% and MET showed positive and non-significant association with CART. AA was negatively and significantly related with FE%, negatively and highly significantly with EI, positively and highly significantly with RSR, positively and non-significantly with EE% and MET. Association of EI with EE% and FE% was positive and highly significant but with MET negative and highly significant. EI and RSR were negatively and non-significantly related. EE% showed positive and highly significant, negative and highly significant and positive and significant relatedness with FE%, MET and RSR respectively. FE% associated positively and non-significantly with MET and negatively and non-significantly with RSR. MET and RSR showed negative and non-significant relatedness with each other (Table-3).

Eigen values of four PCs (PC1, PC2, PC3 and PC4) were greater than one. Cumulatively these four PCs were contributing 79.79% variation in total variability. Rest of the PCs did not contributed significantly in variability. Individually PC1, PC2, PC3 and PC4 contributed 32.02%, 21.70%, 14.95 % and 11.12% of total variation respectively (Table-4). In PC1, all the parameters were contributing negatively except EI, EE% and RSR whereas, in PC2, all the parameters were contributing positively except AA, MET and RSR. FE% and MET contribution was negative in PC3 but all other parameters contributed positively. In PC4 positive contribution was from Chl. *a*, AA, EI, EE%, and FE% whereas, negative contribution was from Chl. *b*, MET and RSR.

Biplot Graphical analysis is multifunctional biometrical technique. In this typical research experiment biplot analysis was used for the recognition of most low temperature stress tolerant and susceptible accessions under imposition of suboptimal temperature stress based on certain discriminating parameters.

Biplot of PC1 and PC2: PC1 (32.0%) and PC2 (21.70%) were used for biplot analysis being highly variable PCs (Fig-1). Both the PCs contributed 53.70% variability. Traits were represented with vectors and length of vectors was proportional to the trait variability. In this biplot, graphic presentation of two groups with discriminating traits was formed. First group consisted of the Chl. *a*, Chl. *b* and FEP while second group consisted of EI, EE and MET as discriminating traits. Among studied maize accessions, 2 (EV-7004Q), 5 (F-150), 23 (F-118), 26 (B-308), 46 (EV-338), 34 (POP-2007), 18 (EV-329), 21 (MT-2), 6 (B-326), and 67 (B-327) performed very well under low temperature stress for emergence related parameters. Accessions ranking on the basis of emergence related discriminating traits was represented in Table-5. Accessions; like 69 (EV-134), 59 (B-304), 56 (F-151), 58 (B-313), 73 (F-143), 63 (F122), 62 (E-337), 61 (BF-238), 74 (EV-310), and 2 (EV-7004Q) maintained higher chlorophyll contents under cold stress which is helpful for maintaining the higher photosynthetic activity.

Biplot for PC1 and PC3: In this typical biplot graph (Fig-2) PC3 (14.9%) had less variation as compared to PC2 (21.7%). On the basis of their vector length FEP and Chl. *a were* proved as discriminating traits for accessions performance. Under low temperature stress, accessions like 59 (B-304), 56 (F-151), 58 (B-313), 69 (EV-134), 70 (F-134), 71 (UAF-1), 73 (F-143), 61 (BF-238), 62 (E-337), 49 (BS-2), 60 (F-105), and 66 (UAF-5) showed tolerant behavior.

Biplot for PC1 and PC4: This biplot contributed less variation (43.1%) as compared to biplots of PC1 with PC2 and PC3 independently (Fig-3). In this typical biplot Cart and FEP were proved as discriminating traits due to their longer vector length. Biplot of PC1 and PC4 declared, 70 (F-134), 71 (UAF-1), 60 (F-105), 68 (UAF-3) and 66 (UAF-5) accessions as tolerant on the basis of discriminating traits.

Biplot for PC2 and PC3: Biplot for PC2 and PC3 (Fig-4) sorted out the accessions under cold stress contributing 36.60% variation. A.A and RSR were discriminating parameters of this biplot on the basis of which accessions 50 (EV-336) and 29 (B-316) were proved cold tolerant.

Biplot for PC2 and PC4: Biplot presented in Fig-5 screened the accessions for cold tolerance by exploiting 32.80% variation. Cart was proved most discriminating trait followed by EI, FEP, Chl. *a*, and A.A. Accessions, 70 (F-134), 76 (EV-330), and 71 (UAF-1) were proved as cold tolerant.

Biplot for PC3 and PC4: This biplot was product of the least variable (26.0%) PCs (Fig-6) among all selected PCs. Cart was most discriminating trait for this biplot and low temperature stress tolerant accessions were 70 (F-134), 71 (UAF-1), and 76 (EV-330).

Table 1. Descriptive Statistics for the studied biochemical and morphological trait

	Chl. *a* (mg/100ml)	Chl. *b* (mg/100ml)	Cart (mg/100ml)	A.A (µgm/ml)	EI (Seeds/day)	EE%	FE%	MET (Days)	RSR
N	76	76	76	76	76	76	76	76	76
Mean	2.088	2.529	0.982	1.836	3.520	12.680	77.924	20.137	2.221
SD	1.710	1.955	0.751	1.046	0.975	4.3485	14.906	1.037	1.298
Variance	2.926	3.822	0.564	1.095	0.951	18.909	222.2	1.075	1.685
SE Mean	0.196	0.224	0.086	0.120	0.112	0.499	1.709	0.119	0.149
CV	81.94	77.28	76.51	56.97	27.70	34.295	19.129	5.148	58.43
Mini.	0.0004	0.0008	0.0008	0.101	0.629	11.00	33.333	18.69	0.733
Max.	7.418	8.474	4.800	4.667	6.161	33.333	100.00	23.93	8.533
Skewness	1.157	1.179	3.012	0.515	-0.038	2.669	-0.291	1.138	2.283
Kurtosis	1.216	1.021	11.831	0.026	0.194	6.712	-0.124	1.331	7.222

Abbreviations; Chl. *a* = Chlorophyll *a*, Chl. *b* = Chlorophyll *b*, Cart = Beta-Careteniods, MET = Mean Emergence Time, EI = Emergence Index, EE% = Energy for Emergence Percentage, FEP = Final Emergence Percentage, RSR= Root Shoot Ratio.

Table 2. Analysis of variance (ANOVA) for studied biochemical and morphological traits.

SOV	d.f	Chl. *a*	Chl. *b*	Cart	A.A	EI	EE%	FE%	MET	RSR
Accessions	75	11.45**	8.84**	1.707**	1.62**	2.79**	2.88**	180.83**	666.44**	10.04**
Error	152	1.29	1.11	0.75	0.82	21.75	2.26	30.43	104.14	1.78
Total	277									

Level of significance $p<0.05=$ *, $P<0.01=$ **

Table.3. Estimation of Pearson Correlation Analysis among studied traits.

	Chl. *a*	Chl. *B*	CART	A.A	EI	EE%	FE%	MET	RSR
Chl. *a*	0.00								
Chl. *b*	0.82**	0.00							
CART	0.05ns	0.48**	0.00						
A.A	0.11**	0.13ns	-0.02ns	0.00					
EI%	-0.08ns	-0.15*	0.01ns	-0.27**	0.00				
EE%	-0.003*	-0.05ns	0.02ns	0.009ns	0.45**	0.00			
FE%	0.27*	0.25*	0.09ns	-0.17*	0.52**	0.15**	0.00		
MET	0.39**	0.47**	0.18ns	0.05ns	-0.70**	-0.36**	0.08ns	0.00	
RSR	-0.24*	-0.17ns	-0.01ns	0.28**	-0.05ns	0.23*	-0.18ns	-0.18ns	0.00

Level of significance $p<0.05=$ * $P<0.01=$ **

Table.4. Principle Component Analysis (PCA)

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Standard deviation	1.698	1.398	1.159	1.001	0.873	0.777	0.558	0.278	0.252
Proportion of variance	0.320	0.217	0.149	0.111	0.085	0.067	0.046	0.009	0.007
Cumulative proportion	0.320	0.537	0.687	0.798	0.882	0.949	0.984	0.993	1.000
Chlorophyll a	-0.40	0.30	-0.22	0.41	-0.27	0.27	-0.12	-0.16	-0.59
Chlorophyll b	-0.46	0.29	-0.33	-0.04	-0.17	0.14	-0.19	0.25	0.67
Caretenoid contents	-0.18	0.19	-0.29	-0.82	-0.03	-0.23	0.02	-0.05	-0.33
Ascorbic Acid contents	-0.09	-0.26	-0.57	0.34	0.13	-.0.67	0.07	-0.10	0.02
Emergence index	0.35	0.54	-0.04	0.03	0.09	-0.11	-0.25	-0.67	0.22
EE%	0.41	0.20	-0.36	0.03	-0.35	0.18	0.71	0.08	0.06
FE%	-0.07	0.55	0.09	0.14	0.66	-0.11	0.25	0.38	-0.11
Mean emergence time	-0.51	-0.15	0.16	-0.05	0.22	0.14	0.54	-0.54	0.18
Root shoot ratio	0.18	-0.26	-0.51	-0.08	0.52	0.57	-0.17	-0.06	-0.03

Table.5. Ranks of accession based on different discriminating traits following trait biplot analysis of PCA1 and PCA2 as axis of biplot.

(A) **Sorting of accessions based on chlorophyll *a*, chlorophyll *b* and final emergence %age.**					**(B) Sorting of accessions based on emergence %age, mean emergence time and emergence index**				
Ranking of Accessions	**Accession Name /Number**	Chl. *A*	Chl. *b*	**MET**	**Ranking of Accessions**	**Accession Name / Number**	**FE%**	**EI**	**EE**
1	EV-134 (69)	7.40	7.80	20.70	**1**	EV-7004Q (2)	100.00	6.20	33.30
2	B-304 (59)	7.40	7.70	21.80	**2**	F-150 (5)	100.00	5.10	11.10
3	F-151 (56)	6.60	6.80	21.60	**3**	F-118 (23)	100.00	4.70	11.10
4	B-313 (58)	5.70	6.00	21.90	**4**	B-308 (26)	100.00	4.70	00.00
5	F-143 (73)	4.80	5.20	22.00	**5**	EV-338 (46)	100.00	4.70	00.00
6	F122 (63)	4.70	5.30	20.30	**6**	POP-2007 (34)	100.00	4.30	00.00
7	E-337 (62)	4.70	5.10	21.80	**7**	EV-329 (18)	100.00	4.10	00.00
8	BF-238 (61)	4.50	5.00	20.90	**8**	MT-2 (21)	100.00	4.00	00.00
9	EV-310 (74)	4.40	4.70	20.40	**9**	B-326 (6)	100.00	3.80	00.00
10	EV-7004Q (2)	4.00	4.20	19.00	**10**	B-327 (67)	100.00	3.50	00.00
11	BS-2 (49)	3.90	4.10	20.70	**11**	UAF-2 (64)	100.00	3.40	11.10
12	F-127 (47)	3.80	3.90	20.30	**12**	B-313 (58)	100.00	2.50	00.00
13	BF-212 (45)	3.60	3.70	19.00	**13**	F-143 (73)	100.00	2.50	00.00
14	B-305 (17)	3.50	3.70	19.80	**14**	F-114 (1)	88.90	5.20	22.20
15	F-140 (65)	3.50	3.70	19.70	**15**	B-306 (54)	88.90	4.90	22.20
16	F-150 (5)	3.40	3.60	19.80	**16**	EV-324 (4)	88.90	4.70	22.20
17	ISLA-GOLD (8)	3.10	3.50	18.90	**17**	B-314 (19)	88.90	4.40	11.10
18	EV-347 (75)	3.00	3.70	20.30	**18**	F-127 (47)	88.90	4.40	11.10
19	UAF-5 (66)	3.00	3.60	22.60	**19**	B-305 (17)	88.90	4.00	11.10
20	E-322 (15)	3.00	3.40	19.50	**20**	EV-340 (27)	88.90	3.90	11.10
21	Marghla (30)	0.004	0.00	18.90	**21**	UAF-3 (68)	33.30	0.60	0.00
22	MT-1 (10)	0.004	0.10	18.70	**22**	E-343 (35)	44.40	2.60	0.00
23	SWL-2002 (14)	0.004	0.20	19.80	**23**	Rakaposhi (24)	55.60	1.80	0.00
24	EV-342 (11)	0.004	1.10	19.40	**24**	SWL-2002 (14)	55.60	2.50	11.10
25	UAF-4 (72)	0.10	0.90	19.80	**25**	Sawan-3 (31)	55.60	2.50	11.10
26	B-316 (29)	0.10	1.20	19.30	**26**	EV-334 (28)	55.60	2.80	11.10
27	EV-336 (50)	0.20	0.00	19.50	**27**	Sh-139 (16)	55.60	3.00	11.10
28	Rakaposhi (24)	0.20	1.00	21.00	**28**	EV-335 (39)	55.60	3.10	11.10
29	B-96 (22)	0.30	0.10	19.60	**29**	Margla (30)	55.60	3.30	11.10
30	Sh-139 (16)	0.30	0.60	19.20	**30**	EV-323 (51)	66.70	2.50	11.10
31	EV-1097 (7)	0.30	0.70	20.10	**31**	F-113 (25)	66.70	2.70	0.00
32	EV-340 (27)	0.30	1.10	19.50	**32**	F-142 (36)	66.70	2.70	0.00
33	F-111 (43)	0.50	0.50	19.20	**33**	VB-51 (32)	66.70	2.70	11.10
34	EV-334 (28)	0.50	0.90	19.20	**34**	F-128 (53)	66.70	2.80	11.10
35	UAF-3 (68)	0.50	1.00	23.90	**35**	EV-134 (69)	66.70	2.90	11.10
36	EV-335 (39)	0.60	0.60	18.90	**36**	EV-310 (74)	66.70	2.90	11.10
37	329 (18)	0.60	0.60	19.80	**37**	EV-347 (75)	66.70	3.00	11.10
38	B-312 (3)	0.60	0.90	19.30	**38**	EV-79 (38)	66.70	3.20	0.00
39	B-314 (19)	0.70	1.00	19.20	**39**	F-134 (70)	66.70	3.20	0.00
40	UAF-2 (64)	0.70	2.30	21.10	**40**	EV-342 (11)	66.70	3.30	11.10

Fig. 1-6. Graphic representation of biplot analysis for PCAs with eigen value >1.

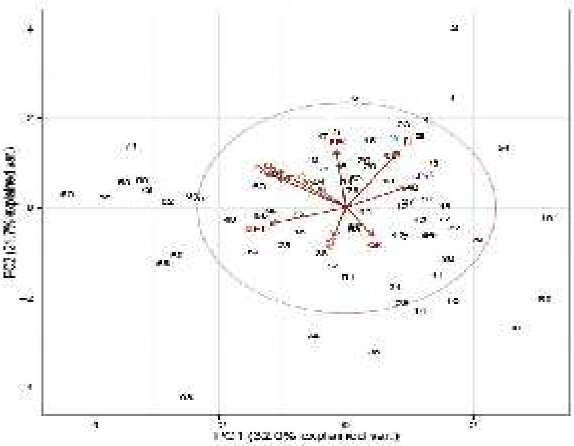

Figure.1. Biplot Graph by PC1 and PC2

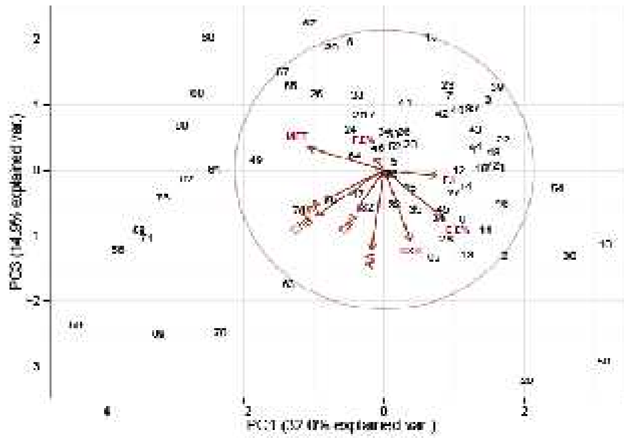

Figure.2. Biplot Graph by PC1 and PC3

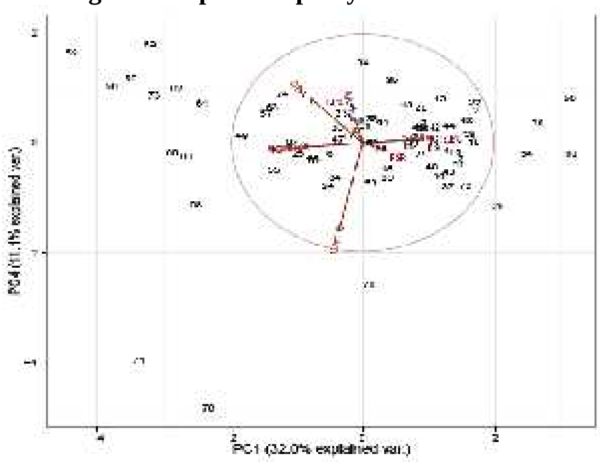

Figure.3. Biplot Graph by PC1 and PC4

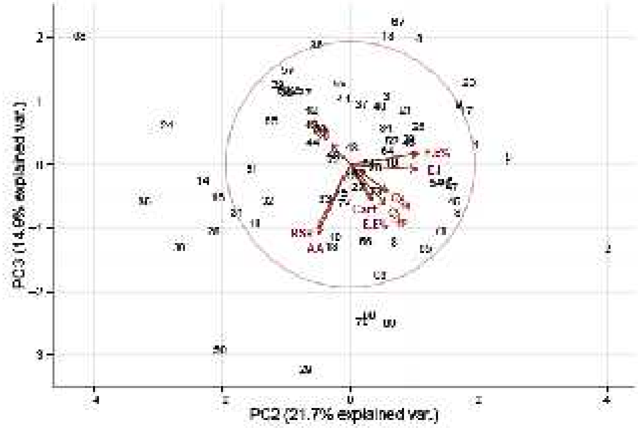

Figure.4. Biplot Graph by PC2 and PC3

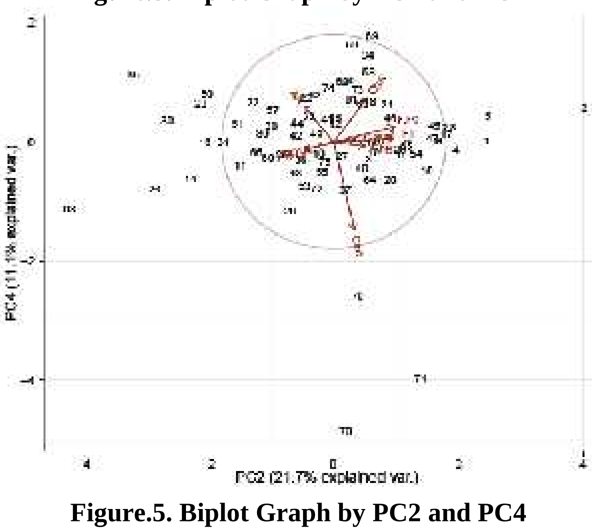

Figure.5. Biplot Graph by PC2 and PC4

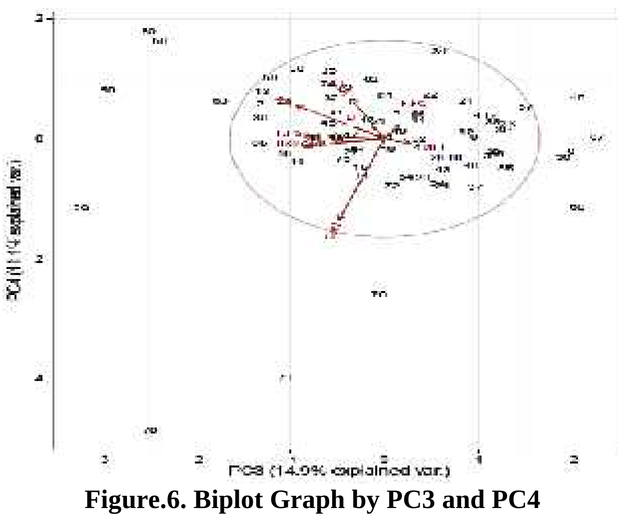

Figure.6. Biplot Graph by PC3 and PC4

DISCUSSION

Highly significant differences among accessions for all the traits validated the data for further biometrical analysis and proved that there was a provision to improve sub optimal temperature stress tolerance by selecting best performing genetic material and its utilization in breeding program. Among all parameters, Chl. *a* showed greater variability providing maximum option of selection to improve low temperature stress tolerance. Chlorophyll contents reflect photosynthesis efficiency of any plant. The photosynthetic apparatus of maize is very much sensitive to low temperature stress and leaves developed at suboptimal temperature have poor photosynthetic capacity (Ying *et al*., 2002; Zaidi *et al*., 2010). Among all studied maize accessions; EV-134 and B-304 were found best accessions which showed high chlorophyll contents under low temperature stress at early growth stage. Improved chlorophyll contents and tolerance of photosynthesis apparatus to suboptimal temperature may contribute towards better performance of maize against early growth chilling stress (Morroco *et al*., 2005). Yellow and old leaves due to loss of chlorophyll lose their photosynthetic power (Mahajan and Tuteja, 2005). Considering the close relationship between leaf chlorophyll contents with success rate of plant for photosynthesis and resistance to environmental stresses, selecting accessions (cultivars) with greater chlorophyll content can be useful in breeding programs. Correlation analysis proved that there was positive and significant association between chlorophyll contents and most of the germination related parameters. Chl. *a* and Chl. *b* were associated positively and significantly with MET, FE%, CART and AA.

Talebi *et al*. (2013) found that chlorophyll and higher carotenoids were associated with stress tolerance in plants. Carotenoids act as photoprotective pigments by avoiding the generation of singlet oxygen and chlorophyll photooxidation (Dillard & German, 2000). RSR which is indicator of early seedling establishment has positive and highly significant relationship with AA and EE% respectively.

PCA is a data reduction method and it changes total variance into some unrelated variables. Each biplot for PCAs is independent of all others and reveals different portion of variation, in this way whole variation is partitioned into different components (Mohammadi and Prasanna, 2003). Eigen values of four PCs (PC1, PC2, PC3 and PC4) were greater than one and contribute most of the variability exhibited. Therefore, it is more beneficial to concentrate on these four PCs for the selection of accessions. For the selection of better accessions under different environmental conditions, biplot graphical analysis on the basis of highly variable PCs is easiest, convenient and reliable approach because it considers all parameters for a given accession under certain environmental conditions at a time (Golabadi *et al.*, 2006). Biplot of PC1 and PC2 was highly variable among PCs so, accessions selected through this typical biplot were more reliable with high level of variability as compared to the accessions selected through any other biplot graph. In this typical case two groups of discriminating traits were formed. First group consisted of chlorophyll content and FE% as discriminating traits while second group of emergence related traits. Chlorophyll content is important indicator for survival of maize accessions after emergence under cold stress whereas, emergence related discriminating parameters were important for the selection of maize accessions which have high mergence establishment under cold stress. To overcome the problems of early stage low temperature or late growth stage heat stress related problem; the performance of the EV-7004Q was outstanding for emergence establishment. Under chilling stress, EV-134, B-304 and F-151 were outstanding accessions to maintain the higher chlorophyll contents and helpful for maintaining the higher photosynthetic activity. According to biplot graph among highly variable PCs, EV-7004Q, F-150, B-305, and B-313 showed good performance for emergence and post-emergence related discriminating parameters. These accessions can be a source for breeding program when selection for greater emergence establishment, higher chlorophyll content retention and higher photosynthetic activity is to be devised.

In this comparative study of maize accessions, genetic differences were evident among accessions in chilling tolerance for chlorophyll contents (photosynthetic apparatus) in leaves developed under suboptimal temperature (8-10˚C). High level of variation was observed among accessions regarding different physiological and morphological parameters, which indicated that alleles are available which can improve low temperature stress tolerance. As cold tolerance is complicated phenomenon because multiple genes are involved to control chilling stress (Morroco *et al*., 2005) therefore, a multidisciplinary approach which may include physiology, genetics and molecular biology will be the best way to understand responses of maize to low temperature stress at emergence stage.

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