Pak. J. Agri. Sci., Vol. 52(3), 701-707; 2015
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
http://www.pakjas.com.pk

ANALYSIS OF DROUGHT RESPONSIVE TRAITS IN HEXAPLOID WHEAT (*Triticumaestivum* L.)

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Dearth of water resources and fluctuating climatic conditions severely affect crop yields. In order to explore genetic diversity underlying drought tolerance, 200 genotypes of bread wheat (*Triticum aestivum* L.) were phenotyped over two seasons (2009-10 and 2010-11). Analysis of variance showed significant variation in morphological and physiological traits: days to heading (DTH), peduncle length (PL), extrusion length (EL), awn length (AL), plant height (PH), leaf rolling (LR), waxiness (WAX), relative water content (RWC), proline (PRO), thousand grain weight (TGW), grains per spike (GR/SP) and yield (Y) under normal and drought conditions over both years. Using multivariate analysis, two data sets C&S (control and stressed) and C-S/C (relative performance) were prepared to examine plant responses to drought. The first principle component (PC) accounted for 24.97% variation for the 2009-10 and 43.85% variation for the 2010-11 in the C&S dataset. For C-S/C dataset, 18.12% and 15.58% variation was observed in 2009-10 and 2010-11, respectively. The biplot based on relative performance data set showed a maximum variation for plant developmental and yield traits except GR/SP and the number of days to heading. TGW and yield vectors remained close on the biplot showing a significant association. Association among different traits identified in this study will enrich the repertoire to excel breeding programs like selection and hybridization.

Keywords: Drought, germplasm, genetic diversity, principal component analysis

Abbreviations: Days to heading (DTH), Relative water content (RWC), Peduncle length (PL), Extrusion length (EL), Awn length (AL), Plant height (PH), Leaf rolling (LR), Waxiness (WAX), Thousand grain weight (TGW), Grains per spike (GR/SP), Proline (PRO), Principal components (PCs), Fresh weight (FW), Dry weight (DW), Turgid weight (TW)

INTRODUCTION

Wheat is world's leading cereal grain and the most important food crop supplying one third of the world's population with more than a half of their calories and nearly half of their protein intake. In the current scenario of continuous increase in world's population, researchers are required to exploit the germplasm variability in order to increase production parallel to its demand. Environmental constraints like diminishing water resources for irrigation are affecting wheat production drastically due to crop yields that are rarely attained from their full genetic potential.

Wheat is mainly grown in rain fed areas of the world. About 37% of the area in developing countries is semi-arid where availability of the moisture is the primary constraint to wheat production (Dhanda *et al.*, 2004). Drought causes significant losses in growth, productivity and yield by affecting plant's morphological, physiological, biochemical and molecular processes throughout the lifecycle. Depending on the time and the intensity of drought and the presence of other biotic and abiotic stresses, yield losses may vary from 10% to 90% of its potential yield under non-stressed conditions (Reynolds *et al.,* 2004; Ahmad *et al*., 2014). The increasing occurrence of drought globally demands an immediate attention to focus on the genetic improvement for developing drought tolerant crops. Genetic basis of stress tolerance can only be predicted by screening under stress conditions (Birsin, 2004). Multivariate analytical techniques are commonly used to identify genetic diversity irrespective of the data sets (biochemical, molecular markers or morphological data; Pantheeet *al*., 2006). Among these algorithms, principal component analysis (PCA), principal coordinate analysis (PCoA), and multi-dimensional scaling (MDS) are being used by plant breeders (Sajjad *et al.,* 2011). The objective of the present study is to analyze the extent of genetic variation contributing to drought tolerance and identify the genotypes with superior performance under water stress.

MATERIALS AND METHODS

Plant material and growth conditions:A germplasm

Table 1. Average weather conditions during 2009-2011.

Months	2009-10			2010-11		
	Temp. (°C)	RH(%)	Rainfall(mm)	Temp. (°C)	RH(%)	Rainfall (mm)
November	18.2	64.7	0.7	18.8	62.3	0.0
December	14.5	64.4	0.0	13.4	70.4	0.0
January	14.0	62.0	0.0	10.1	73.4	0.0
February	15.7	62.7	11.9	14.4	73.0	20.6
March	23.5	57.5	8.8	19.8	59.8	6.8
April	29.9	36.8	1.3	24.8	47.0	20.9

population of 200 bread wheat (*Triticumaestivum* L.) including breeding lines, elite local cultivars and land races was selected from the large collection reported earlier (Sajjad *et al*., 2011). The population was grown during the years 2009-2010 and 2010-2011 at the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad (Latitude = 31° - 26' N, Longitude = 73° - 06' E, Altitude = 184.4 m). A randomized complete block design adopted with two replications in the form of 5 m long plots with 15 cm and 30 cm plant to plant and row to row distance, respectively. Sowing was performed using dibbler method manually adding 2-3 seeds per hole followed by plant thinning after germination leaving a single plant in each hole. The randomization of 200 genotypes was done using Crop Stat v7.2 software. Pre-anthesis water stress was created by withholding water after the 1st irrigation at tillering stage. The control treatment received four irrigations during the whole season. 1st growing season received no precipitation whereas, 2nd growing season received precipitation at maturity. All agronomic and plant protection measures were carried out to get healthy crop performance. Over all weather conditions during study are listed in Table 1.

***Data collection*:** Data were collected from ten randomly tagged plants from each genotype for traits related to plant development, morphology, physiology and yield. Data for days to heading (DTH), relative water content (RWC) were recorded before anthesis while peduncle length (PL), extrusion length (EL), awn length (AL), plant height (PH), thousand grain weight (TGW), grains per spike (GR/SP), and yield/plant (Yield) were recorded at maturity. RWC was calculated using the formula RWC (%) = [(FW-DW)/ (TW-DW)] x 100 as reported by (Barrs 1968)**.**Proline contents (PRO) were determined by methods suggested by (Bates *et al.,* 1973). Leaf rolling and waxiness was visually scored using a (0-6) and (3-9) scale, respectively. Genotypes showing no visible leaves rolling were scored as (0) and the genotypes with completely rolled leaves were scored as (6). Genotypes showing no deposition of wax on leaf, leaf sheath, and spike were scored as (3) while (9) indicated the highest level of wax (Izanloo *et al.,* 2008).

***Data analysis*:** Analysis of variance (ANOVA) for each genotype was calculated according to (Steel and Torrie, 1980). Phenotypic diversity was analyzed by Principal Component Analysis (PCA) according to (Panthee *et al.,* 2006). From mean data, two data sets were made according to (Ivandic *et al.,* 2000). The C&S data set denotes mean data from control (C) and stress (S) treatment while the relative performance was calculated using [(C-S)/C] x 100. This data set was used to derive principal components for identifying stress tolerance genotypes on the basis of correlation matrix. Significant PCs having eigen value more than 1 were given weightage for data explanation.

RESULTS

Analysis of variance (ANOVA) revealed significant results for all the factors i.e., genotypes, treatments and years. Year and treatment factors resulted in higher variation when compared with genotypes (Table 2). Higher levels of diversity were observed under control conditions as compared to drought except leaf rolling and waxiness. Heading was greatly influenced under drought conditions.

Table 2.Mean squares of 12 traits¶ of bread wheat (*Triticumaestivum* L.)

SoV	df	GR/SP	TGW	Yield	RWC	DTH	PL	EXL	AL	PH	LR	WAX	PRO
Blocks	1	45.3**	4.7*	3.51*	13.0**	7.4*	17.1**	0.85	2.0*	0.3	10.401**	0.23	ND
Genotype	199	190.2**	139.7**	82.85**	246.7**	97.9**	109.6**	58.15**	14.1**	231.5**	5.61*	13.27**	0.25*
Treat.	1	23919.0**	1105.0**	8177.00**	8696.0**	16518.0**	24180.0**	3409.00**	1369.0**	65016.0**	813.68**	1220.00**	25.01**
Year	1	3924.0**	1615.0**	1536.00**	4740.0**	3235.0**	1252.0**	283.00**	284.0**	2712.0**	5.18*	6.13*	ND
G*T	199	19.5**	12.7**	7.58*	55.8**	10.4**	12.7**	6.585*	2.0*	42.1**	1.30	2.06*	ND
G*Y	199	9.2*	8.3*	4.53*	34.3**	3.2*	0.3n.s	0.10n.s	0.4n.s	12.1**	0.30	0.34*	ND
T*Y	1	472.5**	2.8n.s	81.84**	351.8**	6.9*	93.2**	0.01n.s	0.9n.s	86.1**	0.05n.s	5.41*	ND
G*T*Y	199	7.9*	4.0*	2.99*	17.7**	2.4*	0.3n.s	0.07n.s	0.1n.s	7.0**	0.23n.s	0.31*	ND
Error	799	1.2	1.1	0.19	2.7	0.5	0.7	0.540	0.3	1.8	0.25	0.23*	0.02

* =significant at 0.05 **=significant at 0.01 n.s= Non significant ND=Not detected;

¶ =GR/SP=Grains/Spike, TGW=Thousand grain weight, RWC=Relative water contents, DTH= days to heading, PL= peduncle length, EXL= Extrusion length, Al=Awn length, PH=Plant height, LR=Leaf rolling, WAX= waxiness, PRO=Proline

The earliest genotype took 89 days to heading as compared to 115 days under control conditions while under drought conditions it ranged between 84 and 109 days. Plant height ranged from 60- 106cm in control conditions and 51-92 in drought conditions. In case of grain yield, it reduced from 36.18 g/plant under control conditions to 23.21 g/plant under drought.

***Principal component analysis (PCA)*:** Variances among the principal components, eigen values and eigen vectors for the first and second year are shown in (Table 3&4). During the year 2009-2010, 1st PC accounted 24.9% of the variation containing the major source of diversity for plant height and days to heading with positive eigen vectors while seed size, yield, peduncle length and peduncle extrusion length had negative eigen vectors. The 2nd PCA accounted for variation of morphological traits with highest loading values for peduncle length, peduncle extrusion length, plant height and awn length (Table 3).

Eigen values of the 1st PCA during the year 2010-2011 showed higher genetic diversity as compared to the year 2009-2010 (Table 4). Being the diverse component it contained about 43.8% of total variation of the population as

Table 3. Principal component analysis of 200 genotypes of all performance traits under normal and drought stress conditions for the year 2009-10

	PCA based on C&S data set					PCA based on (C-S/C) data set					
Variables	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**
GR/SP	-0.124	-0.103	0.506	0.699	0.280	0.282	-0.056	-0.011	0.201	0.075	-0.896
TGW	-0.802	-0.220	-0.187	-0.214	-0.103	0.040	-0.872	-0.158	0.091	-0.286	0.216
Yield	-0.862	-0.159	-0.031	0.139	-0.122	0.184	-0.908	-0.141	0.076	-0.114	-0.057
RWC	-0.219	0.212	-0.225	0.533	-0.668	-0.090	0.126	0.302	-0.578	-0.516	-0.012
DTH	0.755	0.218	0.027	0.028	-0.108	0.090	0.372	-0.531	0.175	-0.469	-0.030
PL	-0.490	0.788	-0.015	0.029	0.093	0.880	0.036	0.161	-0.045	-0.029	0.091
EXL	-0.340	0.779	-0.052	-0.028	0.209	0.771	0.126	0.104	-0.270	-0.098	0.042
AL	-0.301	0.360	0.357	-0.173	0.240	0.521	-0.131	0.225	0.040	0.392	0.179
PH	0.528	0.603	-0.146	0.145	-0.080	0.466	0.265	-0.576	0.078	-0.144	0.093
LR	0.017	-0.255	-0.442	0.392	0.536	-0.052	0.013	-0.569	-0.374	0.526	0.126
WAX	0.022	0.070	-0.758	0.019	0.212	-0.043	-0.284	-0.226	-0.679	0.047	-0.310
Eigen value	2.747	1.968	1.266	1.046	1.011	1.993	1.929	1.213	1.103	1.051	1.016
Proportion	24.971	17.887	11.505	9.505	9.194	18.121	17.536	11.027	10.033	9.557	9.234
Cumulative	24.971	42.858	54.363	63.868	73.062	18.121	35.657	46.685	56.717	66.274	75.508

¶ =GR/SP=Grains/Spike, TGW=Thousand grain weight, RWC=Relative water contents, DTH= days to heading, PL= peduncle length, EXL= Extrusion length, Al=Awn length, PH=Plant height, LR=Leaf rolling, WAX= waxiness

Table 4. Principal component analysis of 200 genotypes of all performance traits under normal and drought stress conditions for the year 2010-11

	PCA based on C&S data set			PCA based on (C-S/C) data set					
Variables	**PC1**	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**
GR/SP	0.688	0.077	-0.239	0.126	-0.175	-0.196	-0.648	-0.308	0.453
TGW	0.551	-0.638	-0.315	-0.408	-0.796	0.100	-0.025	0.086	-0.216
Yield	0.657	-0.622	-0.259	-0.369	-0.824	0.117	-0.144	-0.158	-0.030
RWC	0.771	-0.051	-0.071	0.227	0.011	0.544	-0.113	-0.446	0.016
DTH	0.568	0.576	-0.157	0.016	0.198	-0.111	-0.577	-0.094	-0.544
PL	0.868	-0.129	0.381	0.756	-0.356	0.122	-0.024	0.237	-0.031
EXL	0.655	-0.141	0.623	0.723	-0.256	0.218	0.079	0.195	-0.258
AL	0.679	-0.102	0.119	0.245	-0.356	-0.279	0.274	0.042	0.465
PH	0.761	0.280	0.254	0.530	-0.123	-0.185	-0.115	-0.228	-0.125
LR	-0.578	-0.333	0.171	0.225	-0.043	-0.341	0.284	-0.627	-0.070
WAX	-0.547	-0.214	0.313	-0.086	-0.143	-0.389	0.389	-0.253	-0.379
PRO	-0.530	-0.322	0.172	-0.081	0.098	0.660	0.271	-0.352	0.061
Eigen value	5.263	1.519	1.023	1.870	1.748	1.247	1.186	1.064	1.001
Proportion	43.858	12.658	8.523	15.584	14.563	10.395	9.886	8.869	8.340
Cumulative	43.858	56.515	65.039	15.584	30.147	40.542	50.428	59.298	67.638

¶ =GR/SP=Grains/Spike, TGW=Thousand grain weight, RWC=Relative water contents, DTH= days to heading, PL= peduncle length, EXL= Extrusion length, Al=Awn length, PH=Plant height, LR=Leaf rolling, WAX= waxiness, PRO=Proline compared to 24.9%. It accounted variation for yield, morphological and physiological traits. Except leaf rolling, waxiness and proline, all other traits represented positive eigen vectors. Seed size, grain yield and days to heading also revealed maximum diversity in 2nd PCA of the 2nd year trial. Extrusion length was found prominent in PC3 (Table 4).

PCA on the basis of (C-S/C) data set was used for identifying genotypes having stress tolerance (Table 3&4). Under (C-S/C) 1st PCA is related to morphological traits while 2nd PC accounted maximum variation for yield traits such as yield and seed size. Maximum diversity with negative eigen vectors for plant height and leaf rolling were accounted in 3rd PCA whereas, 4th and 5th PCA showed higher loading values for RWC.

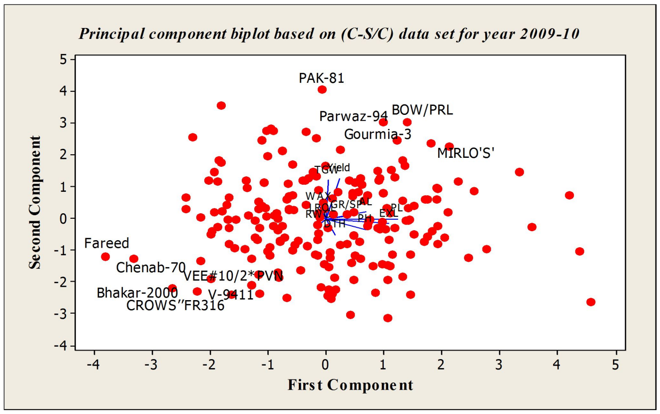

Figure1. Principal component biplot based on (C-S/C) data set for year 2010-11 showing the dispersion of genotypes on two dimensional ordinations obtained using Minitab 16.

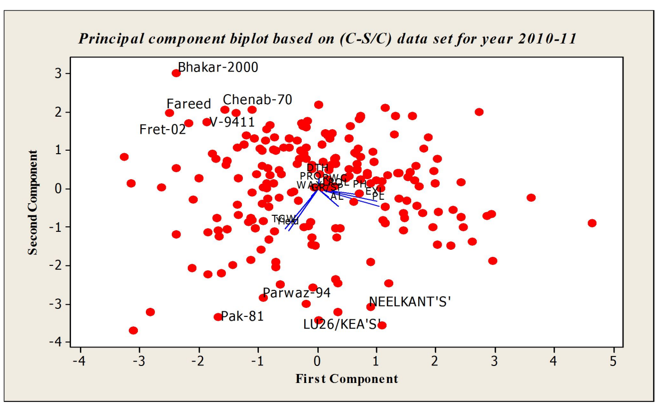

Figure 2. Principal component biplot based on (C-S/C) data set for year 2010-11 showing the dispersion of genotypes on two dimensional ordinations obtained using Minitab 16.

Data matrix prepared to identify stress tolerant genotypes during the year 2010-2011 showed 67.6% of variation in first six PCs (Table 4). Patterns of trait association in first PCA demonstrated maximum diversity for peduncle length, extrusion length and plant height while yield traits such as thousand grains weight and yield per plant also associated negatively in 1st PCA. Higher loading values for yield traits in 2nd PCA show the effect of drought stress on yield parameters. Association among different traits was graphically represented by biplots (Figs. 1, 2).

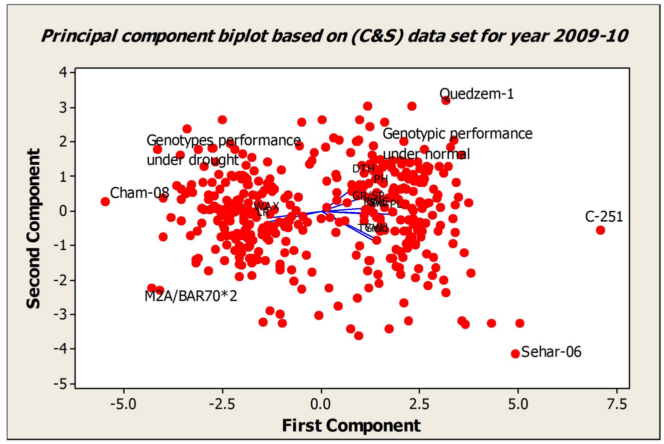

Figure 3. Principal component biplot based on (C&S) data set for year 2009-10 showing the dispersion of genotypes on two dimensional ordinations obtained using Minitab 16.

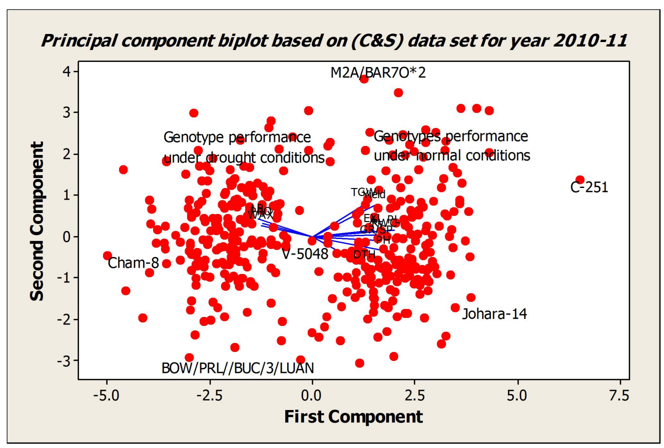

Figure 4. Principal component biplot based on (C&S) data set for year 2010-11 showing the dispersion of genotypes on two dimensional ordinations obtained using Minitab 16.

Biplot based on 1st year's analysis showed that all the traits except leaf rolling, glacousness, RWC and awn length contributed for variation. Similarly, biplot based on 2nd year's C&S data showed that all the traits contributed for variation (Figs. 3&4) but relative performance data set showed maximum variation for plant developmental and yield traits except GR/SP and number of days to heading.

Close angle between thousand grains weight and yield also indicated the contribution of this trait to plant yield.

DISCUSSION

Climate change and continuous increase in world's population have leveled off the benefits achieved through the Green Revolution of 1970s. Meeting the projected demand of more food from the decreasing and/or diminishing resources is one of the greatest challenges we face today. Drought is the major environmental stress which may reduce wheat yields by 50-90% (Dhanda *et al.,* 2004). Plant responses to drought can be studied by evaluating different morpho-physiological attributes endowing plants drought tolerance (Inou *et al.,* 2004; Noorka and da Silva, 2014).

In the present study, significant variation for genotypes, treatments and genotype-treatment interaction indicated the involvement of genetic factors behind the prevailing variations (Birsin, 2005). To estimate the contribution of various traits to drought tolerance, Principle Component Analysis (PCA) was performed (Panthee *et al*., 2006).The 1st PC using combined data set (C&S) revealed greater level of diversity as compared to C-S/C data set which suggested that increase in the degree of stress decreased the correlation among data set. Mohammadi and Prasanna (2003) suggested that 1st few PCs express maximum diversity when there is high correlation among the original data set but the extent of variation decreases when correlation among the original data set is not high. Our results also indicated that different traits show different levels of variation in different PCs. Largest loading values for yield traits (thousand grains weight and grain yield) and developmental traits (peduncle length, extrusion length, awn length, plant hight and days to heading) were observed in first two PCs. Similar results have also been observed in Barley (Ivandi*c et al.,* 2000) and in oat genotypes (Iannucci *et al.,* 2011). Our results along with the other studies mentioned above revealed that first two PCs are the major source of variation for traits related to yield and plant development (Iannucci *et al.,* 2011). Eigen values gradually decrease from 1st PC to the last PC. Therefore, it can be concluded that PCA groups the major amount of variation in the first few principle components (Leilah and Al-Khateeb, 2005).

In biplot analysis, vector length helps to select the genotypes with favorable combinations (Firincioglu *et al.*, 2009). Small vectors lines corresponding to leaf rolling, glaucousness, especially RWC indicated lower variability in their response to drought stress. Yield related traits such as grains per spike, 1000 grain weight and yield were also significantly affected by water stress. Pakistani released varieties 'Inquilab', 'Fareed-06', 'Sehar' and 'Bhakkar' performed well and appeared as most tolerant genotypes (Fig. 1, 2). Reduction in yield under drought was maximum in 'Pak-81' (47%) and 'Neelkant' (68%) indicating their sensitivity to drought (Fig. 1, 2). Grain yield is a mutual effect of morphological and physiological traits and is greatly influenced by shortage of water which reduces the translocation of photosynthates to developing grains and finally, affecting 1000-grain weight and number of grains per spike (Inou *et al.,* 2004; Villegas et al., 2007; Sajjad *et al*., 2014). El Hafid *et al.* (1998) demonstrated that lower number of grains per spike is due to reduced pollination as a result of limited water availability. High positive association between yield traits, peduncle length and peduncle extrusion length was revealed in the 1st PC during 2010-2011 (Bogale *et al.,* 2011; Villegas *et al.,* 2007).

Under drought stress conditions heading dates were shortened and sensitive genotypes headed earlier than tolerant genotypes (Majer *et al.*, 2008).In general, genotypes tended to shorten their vegetative period upon exposure to stress, but tolerant genotypes do so without scarifying yield as in this study genotype VEE#10/2*PVN' (13 days earlier) and 'Navek-4' (12 days, Fig. 1).

Extent of variability for plant height (60cm-106cm) in the germplasm was prominent. Due to reduced plant height more photosynthates becomes available for spike growth leading to more grain setting and yield (Inou*et al.* 2004; Shahzad *et al*., 201x). RWC has been used as an indicator of plant water status under stress conditions (Keyvan, 2010; Loutfy *et al.,* 2012) while the role of proline in osmoregulation is also well documented (Mostajeran and Rahimi-Eichi, 2009). In the current study, drought caused significant increase in proline content with maximum in 'V-9411' (also one of the most tolerant genotypes) and lowest in 'MH-97' (a susceptible genotype). It is well demonstrated that tolerant genotypes accumulate more proline under drought stress as compared to sensitive genotypes (Kamran *et al.,* 2009).

***Conclusion*:** The study identified the elasticity in the performance of wheat germplasm under normal and drought conditions. The performance of local cultivars was better under drought conditions and most of the tolerant genotypes belong to local germplasm, indicating the breeding trend in Pakistan. Although, tolerant genotypes sustain their yield on the expense of their vegetative growth under drought conditions but a positive association between peduncle length, extrusion length and yield suggest the role of last node in the final yield. Different genotypes showed varying patterns of trait association that enriched the repertoire, and can be used for the formulation of better hypothesis in breeding studies.

***Acknowledgement*:** We acknowledge Higher Education Commission of Pakistan for providing funds for this research under the scheme of 5000-Indeginous Scholarships.

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