Pak. J. Bot., 53(1): 227-239, 2021. DOI: http://dx.doi.org/10.30848/PJB2021-1(29)

MULTIVARIATE ANALYSIS FOR YIELD AND PROLINE CONTENT IN WHEAT UNDER LAB AND FIELD CONDITIONS

MIRZA FAISAL QASEEM[1], RAHMATULLAH QURESHI[1*], HUMAIRA SHAHEEN[2] AND ABDUL WAHEED[1]

[1]Department of Botany PMAS Arid Agriculture University Rawalpindi, Pakistan
[2]Department of Biosciences COMSATS University Islamabad Pakistan
Corresponding author: rahmatullahq@yahoo.com, rahmatullahq@uaar.edu.pk

Abstract

Drought is major constraint to wheat yield and globally causes significant yield losses. Increasing wheat tolerance to drought by screening diverse germplasm and incorporation of tolerant genes is an important goal of wheat breeding program. The present study was aimed at screening drought tolerant genotypes from CIMMYT bread wheat nurseries using agro-traits and proline content. A panel of one hundred and eight comprising mixture of CIMMYT advanced wheat lines along with local high yielding varieties were evaluated for phenological and yield traits along with proline content under glasshouse and field conditions during two consecutive cropping years (i.e. 2014-15 and 2015-16). Results revealed that proline content of genotypes was increased under drought stress and had a weak correlation with yield and yield components. Using multivariate analysis technique, genotypes having higher yield and yield components under both stressed and non-stressed conditions were identified and 15 high yielding varieties were identified and recommended for future research. Thus, we can contemplate that it can be used as criteria for selecting tolerant genotypes under stressed condition. These can be incorporated in local breeding program for developing drought tolerant varieties.

Key words: CIMMYT, Drought, Proline content, Tolerant genotypes, Phenological traits, Wheat yield.

Introduction

Wheat is cultivated over 9180 thousand hectares of land with a global production of 768.49 million metric tons by 2020. A 0.55% increase in global wheat is predicted in 2021 with an addition of 4.17 million tons to the last year production (Anon., 2020). It is grown in diverse range of climatic conditions with altitude range from 15° to 60° N to 15° to 45° S and thus have to encompass great alteration in temperature, rainfall and range of other biotic and abiotic stresses (Braun *et al*., 2010; Lobell *et al*., 2011; Semenov & Shewry, 2011). Effects of stress on plants are mainly related to severity, duration of exposure and plant growth stage. Booting, anthesis and grain filling duration in wheat are highly sensitive to heat and drought stress (Barnabás *et al*., 2008; Slafer *et al*., 2014). Major effects of drought stress on wheat include impairment of membrane integrity, extension of root length, effect on opening and closing of stomata, inhibition of photosynthesis decrease in chlorophyll content, reduction in transpiration, growth inhibition, hormone composition, protein changes (Yordanov *et al*., 2000; Lawlor & Cornic 2002; Praba *et al*., 2009), pollen sterility, grain loss, accumulation of abscisic acid in spikes of drought-susceptible wheat genotypes, and abscisic acid synthesis genes in the anthers (Liu *et al*., 2010; Gyugos *et al*., 2019).

Phenotyping has major role in strengthening plant breeding program as genomic data alone cannot explain complexity of traits. Besides, phenotyping also offers an easy way of visualization and selection of favorable traits (Dudley, 2008). Selection of traits by adapting phenotypic screening had improved the wheat yield over the years. Reduction in phenological traits like days to heading and days to maturity is considered to be an avoidance mechanism in plants against drought and heat stress (Lopes *et al*., 2012). Similarly, reduced plant height after incorporation of reduced height gene in wheat had improved the crop yield under stressful conditions, since genotypes are capable to maintain their yield components such as grain per spike, spike length under stress condition. Among different stress indices used to screen genotypes, stress tolerance index is useful in selecting genotypes having higher yield under stress and non-stressed conditions ultimately helpful in wheat improvement (Shah *et al*., 2020). Plant respond to abiotic stresses by accumulating compatible solutes like sugars, antioxidant enzymes and proline (Vendruscolo *et al*., 2007). All these compounds help in survival of plants to abiotic and various studies reported the role of proline in stress tolerance in wheat (Hong-Bo *et al*., 2006; Wu *et al*., 2014; Harsh *et al*., 2016; Mwadzingeni *et al*., 2016). Proline is involved in osmotic adjustment, membrane stabilization and gene signaling to activate anti-oxidizing enzymes that scavenge reactive oxygen species (ROS) (Zivcak *et al*., 2009; Hayat *et al*., 2012; de Carvalho *et al*., 2013; Zadehbagheri *et al*., 2014; Qaseem *et al*., 2019; Liu *et al*., 2020). Apart from its importance, its correlation with grain yield is either poorly known or inconsistent. The present study was hypothesized that proline content may have positive correlation with grain yield and its components and may serve as screening tool for selection of tolerant genotypes. Keeping this in view, this study was aimed to: 1) evaluate the effects of drought on wheat yield and associated traits and identification of drought tolerant and sensitive genotypes among studied genotypes and 2) study the role of proline content in drought tolerance and investigate its association with grain yield.

Materials and Methods

Plant materials: A diverse panel comprising of one hundred and eight genotypes was evaluated against drought stress for two cropping seasons. Ninety-eight genotypes belonged to CIMMYT heat and drought nurseries while ten were high yielding varieties from Pakistan (Qaseem *et al*., 2018). The selection of genotypes was done on the basis of their high 1000 kernel weight under rainfed conditions and their parentage diversity. The lines were evaluated under greenhouse and

field conditions during 2014/15 and 2015/16 at the National Agriculture Research Center (NARC), Islamabad, Pakistan. A total eight environment were created Pot_2015 (Control and Drought), Pot_2016 (Control and Drought), Field_2015 (Control and Drought) and Fied_2016 (Control and Drought).

Experimental design and crop establishment: Parallel experiments were carried out under glass house and field conditions for two cropping seasons. Glass house experiments were carried in plastic pots (30cm ×40 cm) filled with loamy soil i.e. soil having equal quantity of sand silt and clay. Six seeds of each genotype were grown in a single pot and after germination three healthy plants were kept for data collection. The field experiments were planted in lattice design (3 rows, 2m long) with two replications in tunnel. One-meter-deep ditch was also dig around brick lined tunnel to prevent seepage of rainwater inside the tunnel. Sowing was done with small hand drill with row spacing of 22 cm. The same experiments both under field and pot/glass house conditions were repeated for two cropping seasons.

Stress treatments

Pot experiment: The drought stress was imposed by withholding water after heading till maturity. Pots were rehydrated after 2 days with 400ml water to prevent permanent wilting. The moisture content of pots was maintained at 30% of the total available water capacity using Time domain refractometer (TDR). After maturity pots were shifted back to normal conditions with optimal agronomic practices. Respective control (without any treatment) pots were grown at normal conditions with all standard agronomic practices.

Field experiment: Stress was induced after heading using two different water levels i.e. Control with normal irrigation throughout crop cycle and Drought no irrigation after heading till maturity. The non-stress treatment (Control) set was kept open and irrigation was supplied when required the stressed (Drought) plants were prevented from rain by covering the tunnel with polythene sheath during rainy days. Furthermore, fungicide was also applied after intervals to prevent disease spreading due to moist conditions inside the tunnel. After maturity stress was removed, and data was recorded for agronomic traits. Grain yield was estimated from seven plants from glass house and field experiment grown in 1m row with 30 plants in each row for each genotype. From the glass house experiment grain yield was extrapolated based on thirty plants to agree with field data.

Data collection: Data for following traits was estimated: awn length (AL), Plant above ground biomass (DW), days to anthesis (DA), days to maturity (DM), flag leaf length (FLL), flag leaf width (FLW), grains per spike (GPS), grain yield (GY), harvest index (HI), leaf area (Area), peduncle length (PL), peduncle extrusion (Pext), plant height (PH), spikelets per plant (SPL), spike length (SL), and tillers per plant (Till).

Proline content (PC): Proline content was determined by using method developed by Bates *et al*., (1973) with slight modifications Ábrahám *et al*., (2010). Briefly, flag leaf samples were cut from both non stressed and stressed plant and were immediately stored at -20°C. Leaves were cut into same size pieces (approximately 0.2g in weight) and crushing was done in 3% sulfosalicylic acid. Two milliliters of extract were mixed with 2 ml glacial acetic acid and 2 ml of ninhydrin reagent. The mixture was heated at 100^{o}C in water bath for one hour and was cooled at room temperature. Finally, four milliliters of concentrated toluene were added to cooled samples and absorbance was measured at 520nm using UV Spectrophotometer. Concentration of proline was determined using a standard curve.

Stress tolerance index: Stress tolerance index was used as criteria for section and ranking of genotypes based on their performance under both stress and non-stress conditions. Stress tolerance index was estimated using following formula given by (Fernandez, 1992).

$$\text{STI} = \frac{\text{Yp} \times \text{Ys}}{(\text{Xp})^2}$$

Ys is yield of a genotype under stressed environment; Yp is yield of a genotype under non-stressed environment; Xp represents average yield of genotypes under non-stressed environment.

Data analysis: The identification of trait correlations and summary statistics were performed using routine implements in the *R* package (www.r-project.org/). Effects of treatment, genotype and environment and significance of differences between treatments means (Tukey's test) was determined by using "*agricolae*" package in *R* package (www.r-project.org/). Principal component analysis (PCA) was performed on mean data from all the treatment and environments. The PCA was also executed using *FactoMinor* package while all graphs were drawn using *ggplot* package in R software.

Results

Effects of genotype, treatment and environment on agro-traits and proline content: Drought stress significantly affected all agronomic and yield traits, the mean values of all studied traits were significantly reduced under drought stress in pot/glasshouse as well as field conditions (Fig. 1A-I). The mean days to anthesis was 112.55 and 104.06 under control and drought stress treatment with earliest genotypes being EB 18 and ES 24 respectively. The mean grain yield under all the tested environments was 312.45g and both optimum and stress treatments of pot experiment conducted during 2016 (Pot_2016) were most productive among all of pot experiment conducted during 2016 (Pot_2016). Genotype EB 18 and EB 3 have highest yield under non stressed and stressed treatment respectively while WY 34 and ES 12 had

least value for grain yield under control and drought treatment respectively. The average plant height across all studied environment was 85.8 with control treatment of Field_2016 having tallest plants and drought treatment of Pot_2016 having shortest plants. EB 18 and EB 7 were tallest under all stresses while NARC and ZH 37 were shortest under drought and non-stress treatment respectively. The average days to maturity were lower under stressed environments with EB7 and WY 19 took shortest time to mature, EB 12 took (149.83 days) to mature under optimum condition and ES 25 took (140.17 days) to mature under drought stress. The mean value for spike length across all environments was 11.47 while the value of spikelets per spike was 18.26 (cm). The average number of grains per spike across all environments was 68.66 with control and drought treatments of Pot_2016 having highest number of grains per spike than all other environments and treatments. Thus, mean values for all yield and agronomic traits were reduced by drought stress (Fig. 1A-I). The percent reduction (50%) was higher for grain yield in Field_2015 environment followed by Pot_2016 (33%). Similarly, grains per spike and spike length undergo higher reduction during Field_2015 environment while Field_2016 was adverse environment for spikelets per spike (Fig. 2). Among other studied traits harvest index and were severely affected by drought stress while plant height was least affected trait. To access the performance of genotypes under both stressed and non-stress conditions stress tolerance index (STI) values were used as criteria for selection. Genotypes having higher value of STI were tolerant while other having lower values were regarded as sensitive one. Mean value of STI for present panel was 0.81 with 45.6% of total genotypes have their STI values above this average. Top ranked fifteen genotypes and five genotypes with least STI rank are listed in Tables 1a and 1b. Proline content significantly changed across all environments with its higher accumulation during Field 2016. The mean value for proline content under all stress and non-stress environments was 0.50 mg/g while overall there was 85% increase in proline content among all environments during drought stress treatment. Highest proline content 1.15 was recorded for lines EB18 and ES 25 under drought stress (Fig. 3).

Results from combined analysis of variance showed that all the genotypes, treatments and their interactions had significant effects on all studied traits. Combined analysis of variance for agro-morphological, phenological traits and proline content is summarized in Table 2. Highly significant differences were observed among the main effects of genotype, environment and treatment except interaction of genotype and environment which had non-significant effects on tillers per plant and grains per spike. Similarly, interaction of genotype and treatment had a non-significant effect on grain per spike. Proline content, days to anthesis and days to maturity were also significantly affected by treatment genotypes and their interactions (Table 2).

Table 1a. Summary of mean values yield and yield components of 15 tolerant genotypes based on STI rank under non-stressed of drought stress treatments.

Treatment	Genotype	Tolerant genotypes													
		4503	EB15	EB18	EB19	EB3	EB4	EB5	EB7	ES19	ES31	NARC	WY19	WY34	ZH1	ZH7
Control	GPS	55±0.6	65±0.67	57±0.65	59±0.65	58±0.56	49±1.45	43±1.21	54±1.54	44±1.22	54±0.86	52±1.65	44±1.82	48±0.45	39±0.45	69±1.23
Drought		80±0.9	69±0.99	65±0.55	64±0.51	83±0.67	82±1.32	78±0.96	79±1.34	79±1.65	79±0.55	77±2.1	79±1.54	72±0.67	64±0.99	90±1.3
Control	GY	130±1.2	160±1.32	152±0.98	110±1.21	116±0.55	123±0.87	118±0.67	141±1.54	113±1.33	128±0.99	122±1.2	124±0.98	83±1.34	139±0.44	117±1.3
Drought		150±0.7	127±1.1	122±0.87	101±1.32	217±0.63	151±0.51	175±0.49	169±0.67	162±1.21	148±1.4	176±0.98	191±1.34	80±1.4	189±0.34	156±0.34
Control	HI	43±0.89	38±0.34	61±0.44	38±0.5	39±0.76	40±0.87	40±0.87	48±0.43	39±1.87	43±1.6	40±0.56	40±0.78	50±0.98	44±0.76	40±0.88
Drought		77±7.87	61±0.54	60±0.24	55±0.89	68±0.88	52±0.43	66±1.43	58±0.98	52±0.42	73±0.98	76±1.42	59±0.86	69±0.7	65±0.92	61±1.98
Control	PC	0.69±0.5	0.53±0.52	0.83±0.65	0.60±0.54	0.62±0.94	0.66±0.56	0.65±0.90	0.73±0.67	0.61±1.34	0.68±0.45	0.65±0.77	0.66±0.81	0.57±0.78	0.73±0.22	0.63±1.3
Drought		0.42±.99	0.28±0.45	0.27±0.56	0.40±0.11	0.30±0.79	0.44±0.99	0.47±0.78	0.46±1.29	0.46±1.43	0.41±0.42	0.48±0.40	0.40±0.69	0.45±0.54	0.50±1.2	0.45±1.3
Control	SL	9±1.2	9±0.99	9±0.99	8±0.54	10±0.89	9±1.09	8±0.56	9±1.33	8±1.34	8±1.3	8±0.46	9±0.77	9±0.14	10±0.23	9±1.65
Drought		12±0.63	11±1.1	10±1.32	10±0.78	13±0.32	12±1.02	13±0.45	13±0.99	12±1.6	12±1.44	13±0.34	11±0.45	12±0.76	11±0.45	12±0.45
Control	SLP	20±0.78	21±0.4	20±0.67	16±0.11	20±0.19	18±0.13	21±0.33	20±0.87	20±0.11	20±0.47	19±0.37	20±0.55	20±0.46	18±0.55	20±0.12
Drought		23±1.11	23±0.23	21±0.45	22±0.09	22±0.11	23±0.43	23±0.77	23±0.12	22±0.43	23±0.21	23±0.32	23±0.56	20±0.5	20±0.32	22±0.34

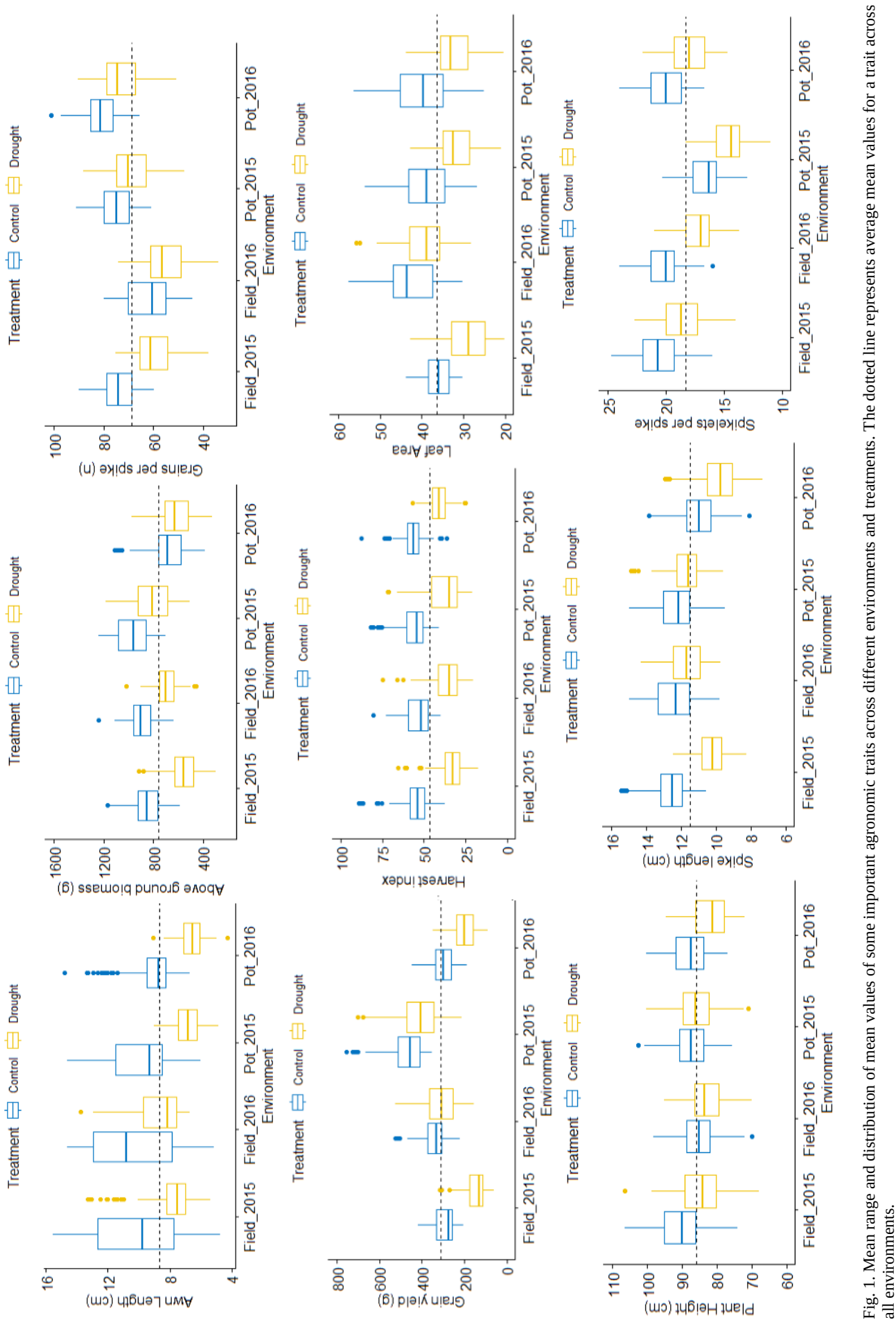

Fig. 1. Mean range and distribution of mean values of some important agronomic traits across different environments and treatments. The dotted line represents average mean values for a trait across all environments.

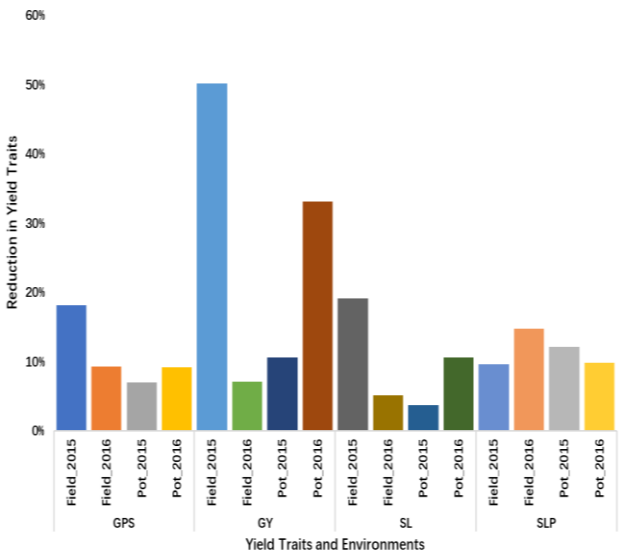

Fig. 2. Percent reduction in yield traits caused by drought stress during different environments.

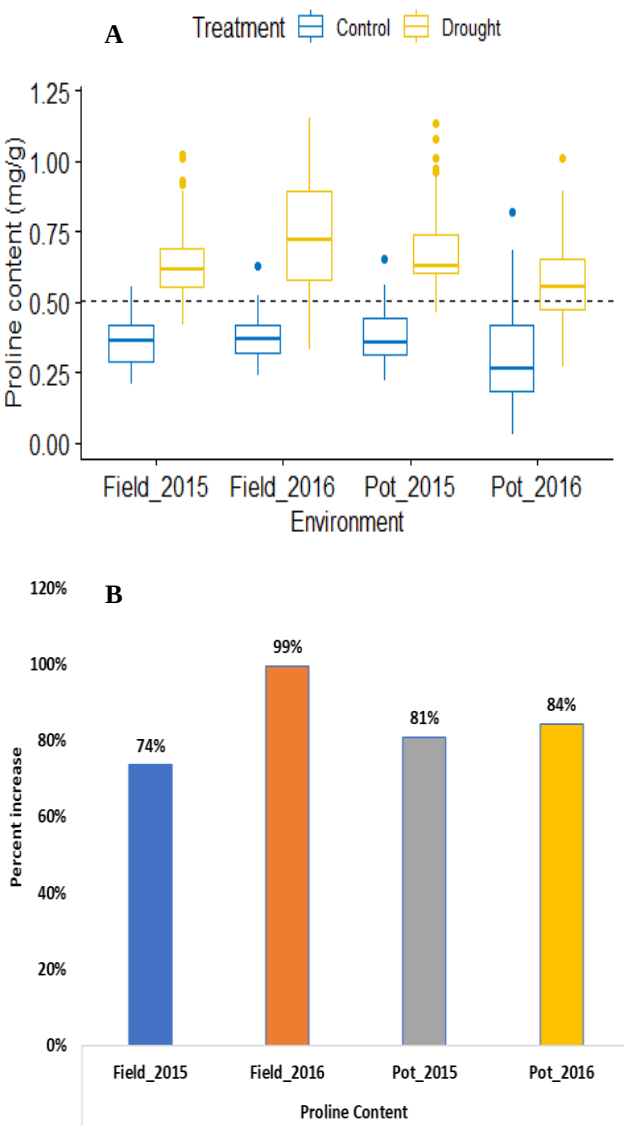

Fig. 3. Proline content **A)** Effect of environments and treatments on proline content **B)** Percent increase in proline content during drought stress over different planting environments.

Correlation analysis: Grain yield showed significant and positive association with all traits except DA and DM under both stressed and non-stress treatment. Under well water treatment grain yield was positively associated with most of the traits except DA, DM, SPLS and TILL while under stressed environment it was positively associated with DA, DM, FLL, GPS, HI, PH and SL. Days to maturity had strong positive correlations with DTH under both stressed and optimum conditions, but with negative correlations with almost all other traits. Plant height was significantly correlated ($r > 0.5$, $P < 0.05$) with all traits except DA well-watered conditions while under stressed condition it had positive association with all traits except DA. DM HI and AL under well-watered treatment peduncle length were positively associated with all traits except DA and DM while it was negatively associated with DA, FLW, Pext and DM under drought stress. Pext was positively associated with all traits except spikelet per spike in well water treatment while under drought stress it was significantly and positively associated with DA and FLW under drought stress. All significant correlations under non stress and stressed conditions are shown in Figure 4A and 4B. The non-significant association either positive or negative was kept blank in heatmap.

Principal component analysis (PCA): The rotated component matrix Table 3 shows the proportion of total variance explained by different principal components and their correlations with variable traits. From drought treatment, five principal components were important having eigen value more than one, contributing 67.5% of the total variation observed. The first two principal components were the most influential with a cumulative contribution to the total variation of 46.2%. FLL, PH and GY were three major contributors to first principal component, while SPL, SPLPS and Pext were major contributors to second principal component (Fig. 3A-D).

All traits except DM, DA and Pext had positive loading into the first principle component while AL, DM, FLL, HI, PC, PL and TILL has positive loading for the second principal component. Likewise, AL, FLL, FLW GPS, SLP, PL and Tillers had high positive loading into the third principal component and AL, Area, DM GPS, HI, PC, Pext, PL and SL had high positive loading into the fourth principal component. Similarly, six principal components having Eigen value more than one were important under optimum conditions, accounting for 73.3% of the total variation of which 42.9% was accounted for by the first three components. All traits except DA and DM had positive loading into the first principal component while SL, SLP, Area, FLW, Tillers and GPS had negative loading into the second principal component. Three major contributors to first principal component under drought stress were GY, STI and Biomass while SPL, FLW and PL were major contributors to second component (Fig. 6A-D).

Principal component biplot analysis: The relationships between the different variables and genotypes with respective principal components are

further illustrated by the principal component biplots in Figs. (5E and 6E) for both stress and non-stress conditions respectively. Smaller angles between dimension vectors in the same direction indicated high correlation of the variable traits in terms of discriminating genotypes. Genotypes excelling in a particular trait were plotted closer to the vector line and further in the direction of that particular vector, often on the vertices of the convex hull. Under stress, most of the genotypes were scattered in the positive side of the first principal component, with genotypes such as HT25, WY 37 and EB 7excelling towards yield which was contributed mostly by their high tiller numbers and GPS, as well as optimum values for other yield components. Based on STI value genotypes were classified into high, moderate and sensitive. Of total penal 24 genotypes were tolerant, while 56 and 28 numbers of genotypes were moderate and sensitive respectively. Under optimum conditions, the genotypes were also more concentrated on the positive side of the first principal component with genotype. Based on performance of genotypes i.e. having higher yield under non stress treatment genotypes were classified as High performers (having yield more than 140g), Moderated performers (having yield more than 110g but less than 140g)and Low performers (having yield less than 110g). Altogether 24% genotypes have high yield and were regarded as high performers while 44 and 32% genotypes were moderate or low performers respectively.

Table 1b. Summary of mean values of 5 sensitive genotypes based on STI rank under non-stressed of drought stress treatments.

Treatment	Genotype	Sensitive genotypes				
		6006	ES12	ES24	ES25	ZH37
Control	GPS	66 ± 0.45	74 ± 0.87	73 ± 0.43	63 ± 0.4	61 ± 0.4
Drought		57 ± 0.34	75 ± 1.9	74 ± 0.79	74 ± 1.34	62 ± 0.5
Control	GY	73 ± 1.34	91 ± 1.56	77 ± 0.88	84 ± 1.3	90 ± 0.43
Drought		73 ± 0.98	53 ± 1.65	73 ± 0.97	62 ± 1.1	60 ± 1.76
Control	HI	37 ± 0.65	42 ± 1.43	25 ± 0.64	38 ± 1.9	36 ± 0.43
Drought		59 ± 0.72	64 ± 1.45	61 ± 0.23	59 ± 1.43	52 ± 1.2
Control	PC	0.32 ± 0.65	0.29 ± 1.43	0.39 ± 1.34	0.36 ± 0.34	0.37 ± 0.87
Drought		0.57 ± 0.33	0.51 ± 1.78	0.70 ± 1.67	0.70 ± 0.98	0.60 ± 0.54
Control	SL	12 ± 0.25	11 ± 0.88	12 ± 1.3	12 ± 1.33	9 ± 0.54
Drought		12 ± 0.59	13 ± 0.77	12 ± 1.98	12 ± 1.34	9 ± 0.5
Control	SLP	20 ± 0.34	20 ± 0.18	21 ± 0.34	19 ± 0.33	19 ± 0.67
Drought		22 ± 0.23	22 ± 0.11	21 ± 0.17	21 ± 0.19	18 ± 0.08

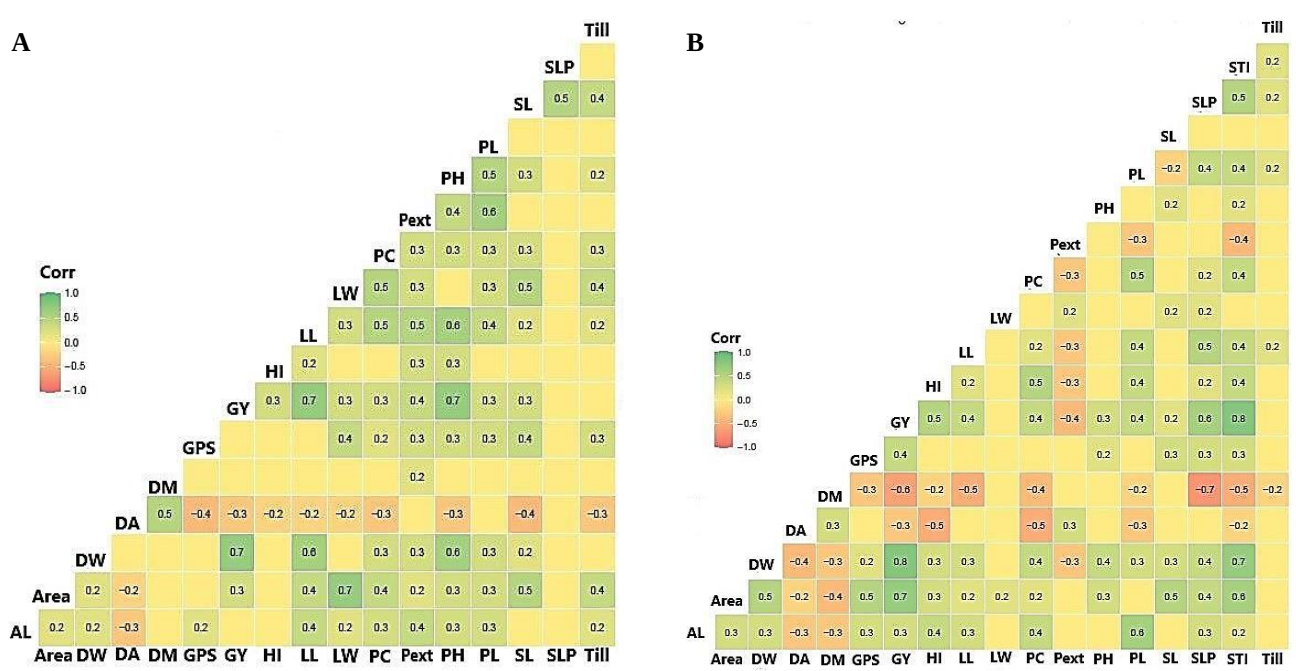

Fig. 4. Correlation analysis for Agro traits and proline content: a) correlation among traits under optimum condition b) correlation among traits under drought Stress.

Fig. 4. Correlation analysis: A) Correlation among studied traits under non stress conditions. B) Correlation among studied traits under drought stress conditions. The blank squares in both heatmaps represent non-significant correlation.

Table 2. Combine analysis of variance (ANOVA) table for agro traits estimated during two cropping seasons.

Traits	Geno.	Env.	Treat.	Geno × Env.	Geno × Treat.	Env. × Treat.	Geno. ×Env. ×Treat
Degree of freedom	107	7	1	749	107	7	749
Awn length (cm)	13.057***	3997.514***	5876.912***	8.065***	11.936***	3415.691***	8.083***
Plant above ground dry weight (g)	5323***	2956***	114860***	2021***	1638***	68945***	634***
Days to anthesis	4.62***	238.77***	549.94***	0.315ns	18.523**	780*	1398ns
Days to maturity	4.812***	566.563***	31.298***	0.515ns	18.816***	769*	1980ns
Flag leaf length (cm)	7.445***	102.975***	1.245**	3.608***	1.427**	46.958***	1.49***
Flag leaf width (cm)	6.618***	170.721***	446.139***	4.462***	1.996***	421.486***	1.398***
Grains per spike (n)	11.253***	111.56***	2273.493***	0.507ns	0ns	11.722***	1.324***
Grain yield (g)	2850.1***	2023.9***	1075***	1019.5***	1318.9***	142502.1***	533.3***
Harvest index (%)	16.684***	32.855***	44.792***	7.508***	10.302***	81.897***	6.21***
Leaf area	5.754***	131.955***	68.486***	3.717***	1.697***	143.244***	1.482***
Peduncle extrusion (cm)	19.887***	145.76***	269.935***	1.671***	11.096***	248.255***	1.75***
Plant height (cm)	20.527***	254.18***	2.687**	4.066***	5.429***	143.607***	4.503***
Peduncle length (cm)	11.07***	278.012***	1314.234***	1.905***	6.598***	566.049***	1.683***
Proline content	4.237***	138.129***	1519.487***	1.93***	1.948***	457***	765***
Spike length (cm)	12.654***	286.922***	221.343***	2.042***	4.589***	919.933***	1.465***
Spikelets per spike (n)	24.095***	42.678***	197.676***	5.138***	8.681***	361.936***	2.915***
Tillers per plant (n)	16.485***	78.041***	79.867***	0.915ns	2.497***	162.33***	2.867***

Geno.: = Genotype, Env. = Environment , Treat. = Treatment

*** Significant at alpha 0.001; ns Non-significant

Table 3. Principal component analysis of sixteen phenotypic traits and proline content of 108 wheat genotypes evaluated in four test environments under stressed and optimum conditions.

Traits	Control/Non-stress treatment						Traits	Drought stress treatment				
	PC1	PC2	PC3	PC4	PC5	PC6		PC1	PC2	PC3	PC4	PC5
AL	0.500	0.145	0.188	0.216	0.195	0.243	AL	0.525	0.247	0.151	0.560	0.025
Area	0.623	-0.295	0.271	-0.407	-0.044	-0.247	Area	0.695	-0.451	-0.224	0.247	0.010
Biomass	0.609	0.182	-0.498	-0.238	-0.162	0.161	Biomass	0.759	-0.148	-0.294	-0.194	-0.022
DA	-0.463	0.451	0.196	-0.235	-0.560	-0.130	DA	-0.464	-0.458	0.271	-0.034	0.407
DM	-0.118	0.433	0.575	0.162	-0.434	-0.118	DM	-0.664	0.093	-0.412	0.045	-0.105
FLL	0.766	0.207	-0.216	-0.127	-0.134	-0.088	FLL	0.585	0.106	0.411	-0.287	0.280
FLW	0.628	-0.289	0.431	-0.213	-0.021	-0.313	FLW	0.129	-0.544	0.356	-0.147	-0.145
GPS	0.480	-0.223	0.289	0.491	0.004	0.278	GPS	0.381	-0.490	0.029	0.489	-0.051
GY	0.734	0.143	-0.471	-0.064	-0.118	-0.160	GY	0.906	-0.188	-0.130	-0.086	0.057
HI	0.293	0.251	-0.019	0.527	0.336	-0.598	HI	0.572	0.378	-0.192	0.278	-0.066
PC	0.638	0.007	0.194	-0.298	0.225	-0.103	PC	0.587	0.462	-0.116	0.069	-0.073
Pext	0.578	0.543	0.241	0.172	0.012	0.054	Pext	-0.387	-0.382	0.439	0.365	-0.257
PH	0.752	0.229	-0.357	0.110	-0.121	0.115	PH	0.296	-0.351	-0.297	-0.285	-0.492
PL	0.561	0.424	0.207	0.102	-0.107	0.211	PL	0.605	0.503	0.125	0.108	0.075
SL	0.539	-0.634	0.072	0.168	-0.272	-0.111	SL	0.107	-0.690	-0.412	0.121	0.089
SLP	0.097	-0.536	-0.226	0.422	-0.533	-0.038	SLP	0.680	-0.247	0.436	-0.030	0.116
Tillers	0.465	-0.262	0.386	-0.198	0.040	0.380	Tillers	0.245	0.131	0.487	-0.127	-0.594
							STI	0.810	-0.142	-0.050	-0.278	0.095
Eigenvalue	5.2	2.1	1.7	1.3	1.1	1.0	**Eigenvalue**	5.8	2.5	1.7	1.2	1.0
Variance (%)	30.7	12.2	10.2	7.8	6.8	5.7	**Variance (%)**	32.1	14.1	9.2	6.6	5.5
Cumulative variance (%)	30.7	42.9	53.1	60.8	67.6	73.3	**Cumulative variance (%)**	32.1	46.2	55.5	62.0	67.5

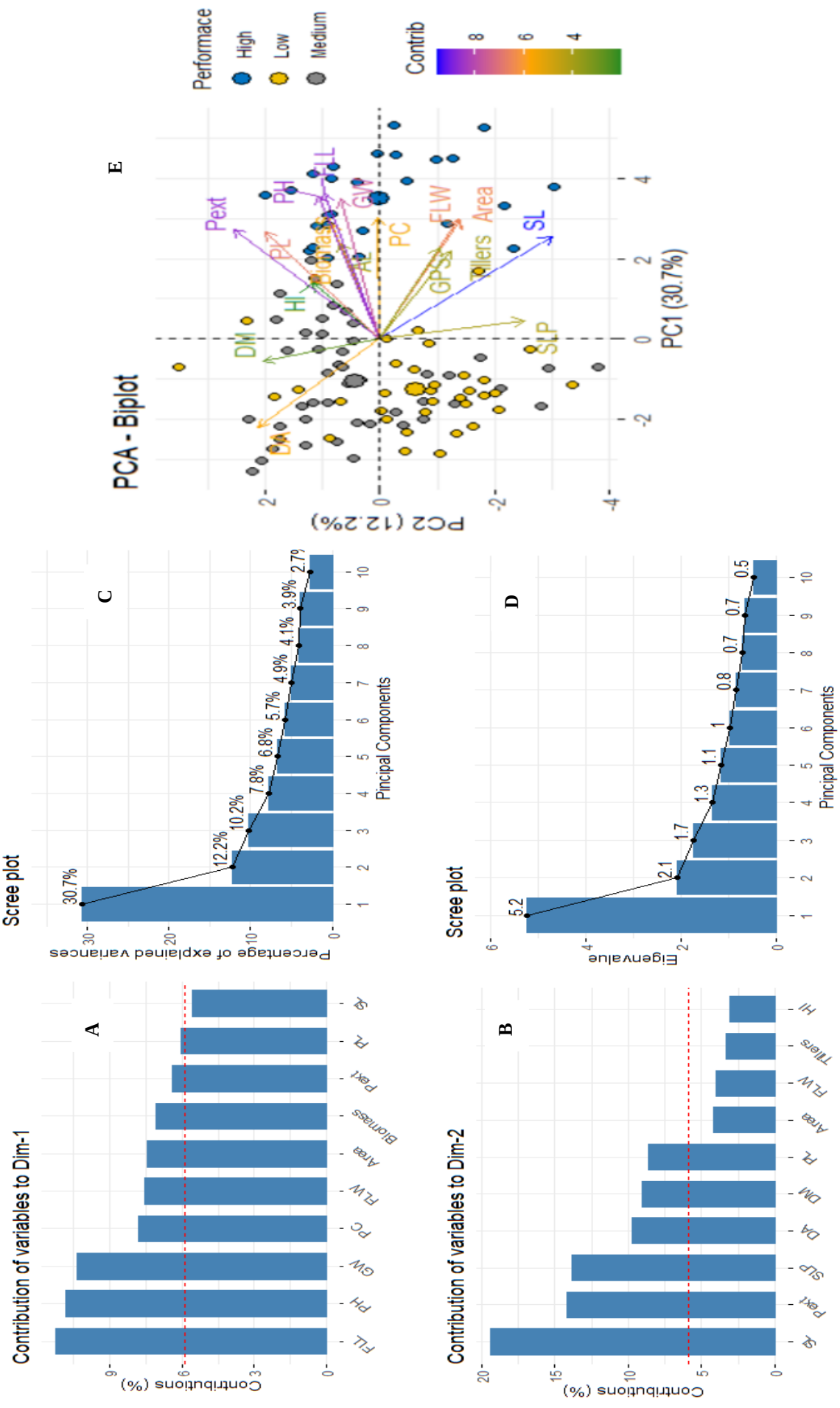

Fig. 5. Principal component analysis under non stress/ control condition: A) Contribution of studied traits to first principal component dotted line represents mean contribution of all traits to first principal component. B) Contribution of studied traits to second principal component dotted line represents mean contribution of all traits to secondprincipal component. C) Percent variation accounted by each principal component in data D) Eigen values of principalcomponents under non stress condition. E: Principal component analysis biplot showing ranking of different genotypes based on their performance and colors of vectors indicate contribution of studied traits to data.

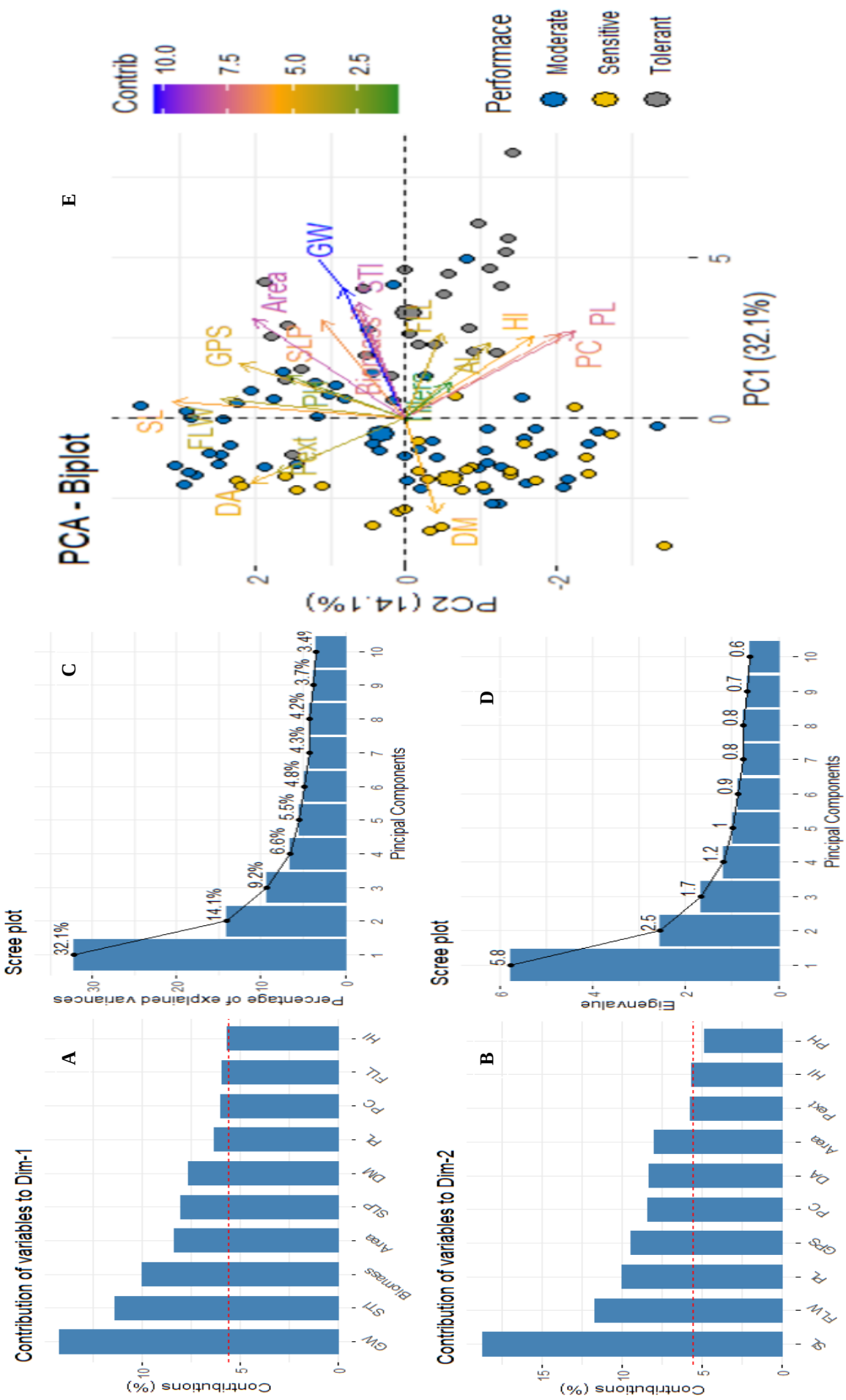

Fig. 6. Principal component analysis under drought stress condition: A) Contribution of studied traits to first principal component dotted line represents mean contribution of all traits to first principal component. B) Contribution of studied traits to second principal component dotted line represents mean contribution of all traits to second principal component. C) Percent variation accounted by each principal component in data D) Eigen values of principal components under drought stress condition. E: Principal component analysis biplot showing ranking of different genotypes based on their STI score and colors of vectors indicate contribution of studied traits to data.

Discussion

Development of drought tolerant germplasm and improvement of wheat through incorporation of tolerant varieties is ultimate goal of wheat breeding. Screening of genotypes through managed drought stress is an effective way of selecting material to improve breeding program. In the present study, the significant differences among genotypes for measured trait showed that germplasm used in present study was highly diverse and are a good source for diversity in breeding program. The differential responses of genotypes to different water regimes were helpful in identifying genotypes having high tolerance to drought stress. This diversity in response of genotypes to treatments, environments and their interactions is due to diversity in parentage among genotypes and high heritability of studied traits. Selecting genotypes able to maintain higher yield under stressed and non-stressed conditions can perform well in either of treatment. In present study genotypes which had higher yield under stress condition had also higher yield under drought stress and these finding are in accordance with studies by (Foulkes *et al.,* 2007). In present study genotype WY 37, EB 18, ES 8, EB 14, EB 7, EB 11, ZH 1, HT 19, EB 27 and 4503 had higher yield under both stress and non-stress conditions, all these lines were advance from CIMMYT and had better performance than local varieties. The better performance might be attributed by the fact that these were developed by CIMMYT for cultivation in heat and drought prone areas and were better adapted to summer planting, and thus may as good source of diversity for spring cultivation. The positive and significant association of traits with grain yield shows direct contribution of these traits in grain yield. In the present study, seven studied traits namely Area, Biomass, HI, PC, PH, PL and SL had positive association with grain yield suggesting that improvement in these traits could lead to yield enhancement and these traits must be targeted during selection (Dodig *et al.,* 2012; Mwadzingeni *et al.,* 2016). Under non stress treatment awn length, biomass, flag leaf length, and leaf area had maximum contribution to grain yield and could be targeted for further enhancement of yield under such conditions. Among the yield components those genotypes which had higher value of grains per spike, spikelets per spike and had more surface leaf area had higher yield which can be justified by more grain number per plant and eventually could compensate reduction in grain weight under stress (Fig. 2) (Slafer *et al.,* 2014).. Similarly, those accessions which take more time to mature and have broader leaves and tall stems under optimum or non-stressed conditions had more reserves to be utilized during grain filling duration. Such genotypes are ideal for selection to get higher yield under optimum conditions (Shavrukov *et al.,* 2017). In present study all these traits had high to moderate correlation with grain yield under optimal growing conditions. But under stress conditions the days to maturity had a negative relation with yield implying the avoidance mechanism of genotype i.e. genotypes use most of their resources to cope with the stress and manage to complete its life cycle prior to stress (Blum, 2011). This cause reduction in grain filling duration and genotypes having high rank under optimum conditions fall to lower ranks under stress. Genotypes with higher stress tolerance index (STI) value had higher grain yield under optimum and stress conditions showing reliability of this index in selecting tolerant genotypes (Fernandez, 1992).

Short stature genotypes had low yield in both stress and non-stress conditions possibly due to less development of root system and less availability of stored reserves to growing grains. It is proven that genotypes with *Rht-B1b* and *Rht-D1b* genes had lower yield under either condition than genotypes lacking these two genes (Butler *et al.,* 2005; Borrell *et al.,* 1993). In the present study, local check Pakistan 2013 was with reduced height and took less days to mature among all other studied local checks and thus was able to maintain high grain yield, high grains per spike and this high tiller number. The short stature of Pakistan 2013 might affected other yield components under optimum conditions, thus reducing their rank for yield under non stress conditions (Dodig *et al.,* 2012).

The PCA analysis showed that area, GY, FLW, SL, STI and Biomass were more influential during stress and contributed maximum to variation explained by first two components. All these traits must be selected together to maximize yield under drought stress conditions. Furthermore, selection of genotypes based on yield components could enhance selection of alleles of genes favoring yield under stressful environments, accumulation of these genes in wheat accessions may result in increased survival rate at the expense of grain yield (Passioura, 2012). In non-stress treatment many yield components have positive loading indicating importance of these traits in selection and simultaneous incorporation in breeding program.

Accumulation of osmolytes for example proline, sugar and various antioxidant enzyme is first and strong response to stress and is proven in various studies by (Rampino *et al.,* 2006; Vendruscolo *et al.,* 2007; Bowne *et al.,* 2012). Most genotypes vary in their response to stress and so is the accumulation of proline and other osmolytes, usually tolerant genotypes accumulate more proline than others. Increase concentration of proline in wheat genotypes play a vital role in osmotic adjustment and thus enhance their tolerance to stress (Nio *et al.,* 2011; Lum *et al.,* 2014). Role of proline in osmotic adjustment and enhancing tolerance of genotypes to drought stress is reported in various other crops including; sugar beet (*Beta vulgaris*) (Gzik, 1996) and alfalfa (*Medicago sativa*) (Irigoyen *et al.,* 1992). Under non stress conditions, proline content had significant but weak association with grain yield suggesting its role in osmo-protection, but it cannot be used as indicator for selection under drought stress. In a similar study (Tardieu, 2005) reported non-significant weak correlation between grain yield and proline content. So, these finding suggest that proline content cannot be used as selection criteria but due to its positive correlation with yield under stress it is an important trait for enhancing grain yield under adverse conditions (Zahedi *et al.,* 2016). In the present study proline content was found positively associated with grain yield under drought stress is in accordance with previous finding suggesting its major role in drought tolerance. So, in conclusion, proline is accumulated in response to stress

in various plants and it had a key role in mitigating stress, but when it is measured at a single point it may not serve as good indicator for selection of genotypes based on their yield. Its positive association with grain yield in some studies proved its role in maintenance of grain yield under stressful conditions, but further studies are still required to find out rate of proline accumulation among genotypes with change in stress severity and growth stage of plant. This can be achieved by comparing proline accumulation in a set of stress tolerant and susceptible genotypes. The results from the present study showed that the germplasm had useful diversity for drought tolerance.

Conclusion

This study was aimed at evaluating drought tolerant genotypes from CIMMYT bread wheat nurseries using agro-traits and proline content. Results discovered that proline content of genotypes was increased under drought stress and showed a weak correlation with yield and yield components. Besides, this study identified 15 high yielding varieties and recommended for further research. These can be incorporated in local breeding program for developing drought tolerant varieties to meet our needs.

Acknowledgements

The principal author is thankful to Dr Jalal ud Din from NARC, Islamabad for providing Land for field experiments.

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(Received for publication 22 April 2019)