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MULTIVARIATE ANALYSIS FOR TRAIT ALLIANCE OF BREAD WHEAT YIELD UNDER TERMINAL HEAT STRESS CONDITIONS

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ABSTRACT

One hundred twenty five bread wheat recombinant inbred lines were subjected to late heat stress under field conditions during the year 2010-11. The experiment was conducted at CCS Haryana Agricultural University Hisar using randomized complete block design with three replications under late sown conditions. The correlation coefficients revealed that the genotypes having high grain yield also had more number of yield components, namely, number of tillers per plant, number of grains per plant, grain weight per spike, number of grains per spike. These genotypes also had high biomass, harvest index and better in terms of heat stress related parameters,i.e.,canopy temperature depression and membrane thermo stability and earlier in heading and maturity.Path coefficient analysis indicated that the grain weight/spike had the highest direct contribution towards grain yield per plant followed by number of grains/plant, number of tillers/plant,biomass and harvest index. Multiple regression analyses revealed that number of tillers per plant was the most contributing trait fallowed by numbers of grains per plant,grain weight per spike,number of grains per spike,biomass per plant and harvest index. The diversity analysis indicated that the genotypes in one cluster were in general different from another in terms of their response to heat stress.

KEY WORDS
Canopy temperature depression
Cluster analysis
Membrane thermo stability
Multiple linear regression

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INTRODUCTION

Heat stress during post-anthesis growth stages predominantly influences assimilates availability, translocation of photosynthates to the grain and starch synthesis and deposition in the developing grain (Modarresi *et al*.2010). The net result is a lower grain yield because of lower grain weight (Gibson and Paulsen, 1999; Pandey *et al*. 2015). It is difficult to make progress for grain yield and yield components under heat stress conditions as these are highly intricate in nature and entails several tolerance and avoidance mechanisms. A detailed understanding of the genetics and physiology of grain yield and its components under heat stress conditions would improve the efficiency of breeding programs by identifying relevent indices for selecting varieties (Kalyar *et al*.2014).

Among the heat stress related traits, cell membrane is a major physiological parameter which disrupts due to heat stress thus the lipid bilayer membranes become more fluid by either due to denaturation of proteins or an intensification of unsaturated fatty acids (Savchenko *et al*.2002) and measurement of solute leakage from plasma membrane or membrane thermo stability can be used to estimate the heat stress tolerance (Blum, 2001; Dhanda and Munjal, 2006). Canopy temperature depression (CTD) is another trait involved in a number of different physiological and metabolic processes including stomatal conductance, photosynthetic rate, vascular capacity, transpiration rate, stomatal conductance and may be indicative of heat avoidance capability of plant through keeping the canopy cooler during high demand for transpiration, and photo assimilation caused by rapidly filling kernels (i.e. sink strength) and good vascular system. Hence, CTD is positively associated with yield under heat stress (Bahar *et al*. 2011).

Statistical techniques have important role in establishing relationship between the yield and its related components. Though correlation coefficient is an important statistical method in selection for higher yield (Steel and Torrie, 1960), may not provide adequate details about the relationship among different variables (Del Moral *et al*.2003), as much as multivariate statistical methods give. Multiple linear regression, stepwise regression, path analysis, principal component analysis and cluster analysis have been established as useful methods to decipher these relations in crop plants (Saed-Moucheshi *et al*.2013). The multivariate statistical analyses can furnish insights the structure of data and traits' alliance. This study was directed to describe and interpreting the association between wheat grain yield and its related components under terminal heat stress conditions with aiming to provide theoretical foundations guiding wheat breeders for researching the alliance of the main yield components and their influences on wheat plant productivity. Thus the present investigation was initiated to examine the influence of high temperature stress at anthesis and post anthesis stages of plant growth, to identify important traits contributing heat tolerance and promising combination of traits which may be used as the source of heat tolerance in wheat improvement programme.

MATERIALS AND METHODS

Creation of heat stress environments

The experiment was laid out in randomized complete block design with three replications at CCS HAU, Hisar (29°10N′ lat., 75°46′E long., 215 m alt.) during 2010-2011. The plot size was of single row of 2.5 m in length with the spacing of 10 cm × 22.5 cm. In order to create heat stress during anthesis and reproductive stages, the sowing was delayed by about one and half month from normal period of sowing, i.e., in last week of December. Mean weekly maximum and minimum temperatures over the growth period were recorded from Agro-Meteorological Observatory, CCS Haryana Agricultural University, Hisar, India (Fig. 1).

Plant materials

The present investigation was carried out on 125 RILs of bread wheat derived from the cross WH 730 (a thermo-tolerant variety) and WH147 (a thermo-sensitive variety). Observations were recorded as the average of five competitive plants selected randomly from each.

Canopy temperature depression

A hand held infrared thermometer, (model AG-42, Tele temp crop, Fullerton CA) used for instantaneous measurement of canopy minus air temperature as canopy temperature depression. Observations were recorded between 12:00 hrs. to 14:00 hrs.

Membrane thermo stability

For membrane thermo stability, method given by Sullivan (1972), modified later by Ibrahim and Quick (2001) was followed. Membrane thermo stability was measured by the formula given below.

$$MTS \quad 1-\frac{T_1}{T_2} \quad 100$$

T_1 = initial conductance value taken after incubation of leaf discs in a controlled temperature water bath at 49°C for 45 min followed by cooling.

T_2 = final conductance value taken after autoclaved at 0.01 M Pa pressure for 10 min to release all the electrolytes from the leaf discs

Statistical Analysis

Correlation coefficients The phenotypic coefficients of correlation between two characters were determined by using the variance and covariance components as suggested by Al-Jibouri *et al*. (1958).

Multiple linear regressions

Multiple linear regression and partial coefficient of determination (R^2) was estimated for each yield component (Snedecor and Cochran, 1981) in order to assess the relative contribution and to develop the prediction model for grain yield.

Stepwise regression

Stepwise regression was used in order to determine the variable accounting for the majority of total yield variability as suggested by Draper and Smith, 1981.

Principal components analysis

Principal component analysis (PCA) was used to reduce data dimensionality by covariance analysis between variables (Everitt and Dunn, 1992).

Cluster analysis

This analysis was performed using a measure of similarity levels and Euclidean distance (Everitt, 1993).

Path coefficient analysis

Path coefficient analysis was carried out using phenotypic and genotypic correlation values of yield components as suggested by Dewey and Lu (1959).

All statistical analyses were performed using SAS (SAS Institute, 2009) and statistical language R (R Core Team, 2013).

RESULTS

Phenotypic correlation analysis

Results revealed that grain yield per plant exhibited positive significant correlations with number of tillers per plant (0.58**), number of grains per plant (0.78**), grain weight per spike

Table 1: Phenotypic correlation coefficients between various characters in recombinant inbred lines of bread wheat under heat stress conditions

Variables	Grain yield /plant	No. of tillers/ plant	No. of grains/ plant	Grain weight/ spike	No. of grains/ spike	Biomass	Plant height	HI	DTH	DTM	CTD
No. of tillers/plant (X1)	0.58**	1									
No. of grains/plant (X2)	0.78**	0.52**	1								
Grain weight/spike (X3)	0.43**	-0.40**	0.31**	1							
No. of grain/spike (X4)	0.33**	-0.29**	0.58**	0.79**	1						
Biomass (X5)	0.82**	0.65**	0.66**	0.20*	0.15	1					
Plant height (X6)	0.41**	0.25**	0.28**	0.16	0.09	0.41**	1				
HI (X7)	0.48**	0.03	0.37**	0.48**	0.38**	-0.05	0.08	1			
DTH (X8)	-0.23**	-0.07	-0.19*	-0.20*	-0.18*	-0.16	-0.35**	-0.14	1		
DTM (X9)	-0.23**	-0.07	-0.20*	-0.21*	-0.19*	-0.16	-0.33**	-0.14	0.95**	1	
CTD (X10)	0.20*	0.17	0.24**	0.06	0.13	0.19*	-0.05	0.08	0.09	0.05	1
MTS (X11)	0.18*	0.16	0.19*	0.02	0.05	0.13	< 0.01	0.1	-0.18*	-0.20*	0.22*

*,**: Significant at 5% and 1% level of probability, respectively

Table 2: The regression coefficient (b), standard error (SE), t-value and probability of the estimated variables in predicting wheat grain yield by the multiple linear regression analysis

Variables	DF	Parameter estimate	Standard error	t-value	Pr > |t|
X1	1	0.06425	0.06907	0.93	0.3542
X2	1	0.00537	0.00111	4.85	<.0001**
X3	1	1.21177	0.32507	3.73	0.0003**
X4	1	-0.03024	0.00621	-4.87	<.0001**
X5	1	0.21503	0.01799	11.95	<.0001**
X6	1	0.00277	0.00332	0.83	0.4062
X7	1	21.89501	1.73669	12.61	<.0001**
X8	1	0.03633	0.03313	1.10	0.2751
X9	1	-0.04531	0.03304	-1.37	0.1729
X10	1	-0.01486	0.02932	-0.51	0.6134
X11	1	0.00113	0.00449	0.25	0.8019

*, **: Significant at 5% and 1% level of probability, respectively. Y-intercept (a) = "4.53647; SE = 1.52, R^2 = 0.9806, Root MSE =0.36393, Adj R^2 = 0.9788.

Table 3: Relative contribution (partial and model R^2) in predicting wheat grain yield, F-value and probability by the stepwise procedure analysis

Step	Variable entered	Partial R^2	Model R^2	SE of estimates
1	X5	0.6963	0.6963	0.00481
2	X7	0.2791	0.9754	0.75078
3	X9	0.0005	0.9758	0.00891

Table 4: Regression coefficient (b), standard error (SE), F-value and probability (significance) of the accepted variables that can be used to predict wheat grain yield by the stepwise procedure

Variables	Coefficient of regression (B)	Standard error (SE)	F value	Significance
X5	0.28057	0.00481	3397.64	<.0001**
X7	27.70354	0.75078	1361.59	<.0001**
X9	-0.01382	0.00891	2.40	0.1236

*, **: Significant at 5% and 1% level of probability, respectively

Table 5:Eigen value of the correlation matrix for the estimated variables of wheat using the principal component procedure

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11
X1	0.202	0.576	0.076	0.039	0.274	-0.151	-0.047	0.061	0.650	0.043	0.304
X2	0.430	0.152	0.300	0.099	0.114	-0.086	-0.357	-0.373	-0.529	-0.042	0.351
X3	0.295	-0.472	0.153	0.137	-0.144	0.174	0.083	0.523	0.100	-0.003	0.553
X 4	0.323	-0.398	0.266	0.088	-0.190	0.036	-0.350	-0.339	0.486	0.044	-0.382
X5	0.351	0.392	0.107	0.217	-0.205	0.119	-0.130	0.554	-0.196	-0.012	-0.496
X6	0.299	0.131	-0.210	0.410	-0.168	0.390	0.592	-0.387	0.025	-0.014	0.016
X7	0.268	-0.244	0.228	-0.037	0.721	-0.172	0.409	0.085	-0.077	-0.005	-0.292
X8	-0.355	0.079	0.525	0.167	0.030	0.235	0.054	-0.019	0.064	-0.707	-0.002
X9	-0.356	0.078	0.512	0.204	0.045	0.252	0.055	-0.017	-0.040	0.702	0.016
X10	0.124	0.111	0.404	-0.419	-0.503	-0.421	0.447	-0.056	-0.014	0.024	0.011
X11	0.175	0.097	0.041	-0.707	0.100	0.668	-0.034	-0.025	0.011	0.011	-0.005
Eigenvalue	3.366	2.270	1.726	1.147	0.775	0.651	0.627	0.343	0.050	0.029	0.016
Proportion	30.60	20.64	15.700	10.42	7.05	5.92	5.70	3.12	0.45	0.27	0.15
Cumulative (%)	30.60	51.23	66.93	77.35	84.40	90.31	96.01	99.13	99.58	99.85	100

(0.43**),number of grains per spike (0.33**), biomass (0.82**),plant height (0.41**), HI (0.48**), canopy temperature depression (0.20*) and membrane thermo stability (0.18*) whereas, negative significant correlation with days to heading (-0.23**) and days to maturity (-0.23**) (Table 1). Number of tillers per plant showed significant correlations with number of grains per plant (0.78**), biomass (0.65**) and plant height (0.25**),while negative significant correlations with grain weight per spike (-0.40) and grains per spike (-0.29**). Number of grains per plant appeared to an important trait as it showed significant positive associations with all the traits except days to heading and maturity where it was significantly negatively correlated. Grain weight per spike and number of grains per spike were also related to majority of the traits except canopy temperature depression and membrane thermo stability indicating their limited role in heat stress related parameters.Biomass per plant showed significantly correlated with plant height (0.41**) and canopy temperature depression. Plant height showed negative correlations with days to heading (-0.35**) and days to maturity (-0.33**). Harvest index was positively related with the grain yield and majority of its components but non significantly

Table 6: Normal Root-Mean-Square Distance of cluster formation

Number of cluster	Clusters Joined		Frequency	Normal Root-Mean- Square Distance
11	X3	X7	2	0.0107
10	X1	X10	2	0.0174
9	Y	CL10	3	0.0258
8	CL9	CL11	5	0.0529
7	X6	X8	2	0.1200
6	CL7	X11	3	0.1649
5	CL8	X5	6	0.2073
4	CL6	X9	4	0.3066
3	CL5	X4	7	0.3943
2	CL3	CL4	11	0.6766
1	CL2	X2	12	2.1672

Table 7: Path coefficient analysis of grain yield per plant with its component characters in recombinant inbred lines of bread wheat

Variables	Effects via X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	Direct effect	Indirect effect	Genotypic correlation coefficients
X1	0.292	0.299	-0.278	0.155	0.153	0.017	0.000	-0.010	0.008	0.001	0.001	0.292	0.346	0.638**
X2	0.160	0.544	0.155	-0.306	0.140	0.019	0.057	-0.028	0.023	0.001	0.002	0.544	0.223	0.767**
X3	-0.125	0.130	0.647	-0.413	0.023	0.011	0.080	-0.032	0.026	<0.001	<0.001	0.647	-0.2996	0.3474**
X4	-0.084	0.310	0.498	-0.537	0.019	0.006	0.070	-0.027	0.022	0.000	<0.001	-0.537	0.8152	0.2782**
X5	0.210	0.359	0.071	-0.048	0.213	0.031	-0.003	-0.028	0.021	0.001	0.001	0.213	0.615	0.828**
X6	0.075	0.163	0.116	-0.053	0.100	0.066	0.003	-0.048	0.036	-<0.001	0.0001	0.066	0.3919	0.4579**
X7	0.000	0.184	0.311	-0.225	-0.004	0.001	0.167	-0.026	0.019	<0.001	0.001	0.167	0.2613	0.4283**
X8	-0.023	-0.119	-0.161	0.113	-0.044	-0.024	-0.033	0.130	-0.103	<0.001	-0.002	0.130	-0.3956	-0.2656**
X9	-0.023	-0.119	-0.161	0.113	-0.042	-0.022	-0.030	0.126	-0.106	<0.001	-0.002	-0.106	-0.1598	-0.2658**
X10	0.052	0.141	0.038	-0.080	0.044	-0.003	0.013	0.013	-0.006	0.004	0.002	0.004	0.214	0.218**
X11	0.052	0.114	0.012	-0.032	0.034	0.007	0.018	-0.024	0.022	0.001	0.010	0.010	0.204	0.214**

Residual Effect = 0.2030;*, **: Significant at 5% and 1% level of probability, respectively

related with canopy temperature depression and membrane thermo stability. Among the heat stress related parameters, membrane thermo stability seemed to be a reliable parameter as it showed significant negative correlations with days to heading (-0.18*), days to maturity (-0.22*) and significant positive correlation with canopy temperature depression (0.22*).

Multiple linear regression analysis

Table 2 demonstrated regression coefficients and the probability of the estimated variables in predicting wheat genotypes grain yield under terminal heat stress conditions. In view of following equation.

Y= -4.53647 + 0.00537X2 + 1.21177X3 - 0.03024X4 + 0.21503X5 + 21.89501X7.

The equation explained almost 98% of the total variation within the grain yield components and the remaining 2 % assigned to residual effects. The t-test showed that number of tillers per plant (X1), numbers of grains per plant (X2), grain weight per spike (X3), number of grains per spike (X4), biomass (X5) and HI (X7) significantly contributed the grain yield. These results showed the importance of the mentioned variables in breeding programs of wheat under terminal heat stress conditions.

Stepwise multiple linear regression analysis

Data presented in Table 3 shows the partial and cumulative R^2 and the probability for the accepted limiting three variables in grain yield prediction. These variables are biomass (69.63%), harvest index (27.91 %), days to maturity (0.05%). According to the results 97.59% of the total variation in grain yield could be attributed to these aforementioned three variables. Regression coefficients for the accepted variables are shown in Table 4. The equation for prediction of grain yield was obtained as given below:

Y-intercept (a) = "6.22311 + 0.28057X5 + 27.70354X7 - 0.01382X9

Principal component analysis

Results of the principal component analysis (Table 3 and Fig. 2) revealed that an increase in the number of components was associated with a decrease in eigen values. This trend reached its maximum at five factors. Accordingly, the principal components analysis had grouped the estimated wheat variables into six main components which all together accounted for 84.4% of the total variation of grain yield under terminal heat stress conditions. The first principal component which accounted for 30.6% of total variation in grain yield and mainly contributed by the characters numbers of grains/ plant (0.430), numbers of grain/spike (0.323) and biomass (0.351). The second principal component made 20.64% contribution towards total variation in grain yield and represented mainly by the characters, namely, numbers of tillers/plant (0.576) and biomass (0.392). The contribution of third principal component was 15.70% and represented mainly by numbers of grains/plant (0.300), days to heading (0.525), days to maturity (0.512) and canopy temperature depression (0.404). The fourth principal component accounted 10.42% towards total variation and mainly responsible for plant height (0.390). The contribution of fifth principal component was 7.05 and it was mainly responsible for harvest index (0.721).

Cluster analysis

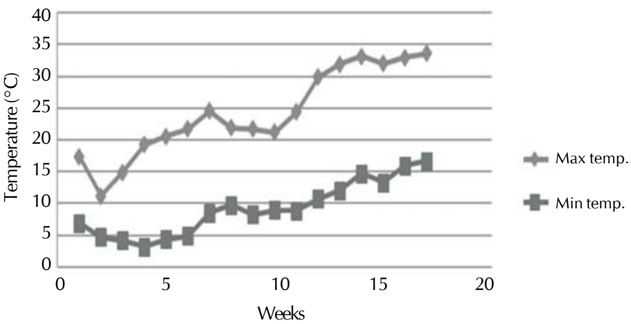

Figure 1: Weekly maximum and minimum temperatures observed during the growth period of December 2010–April 2011

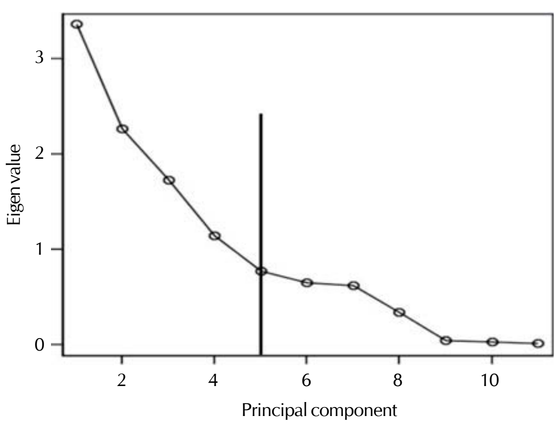

Figure 2: Scree plot showing eigen values in response to the number of components for the estimated variables of wheat under terminal heat stress conditions

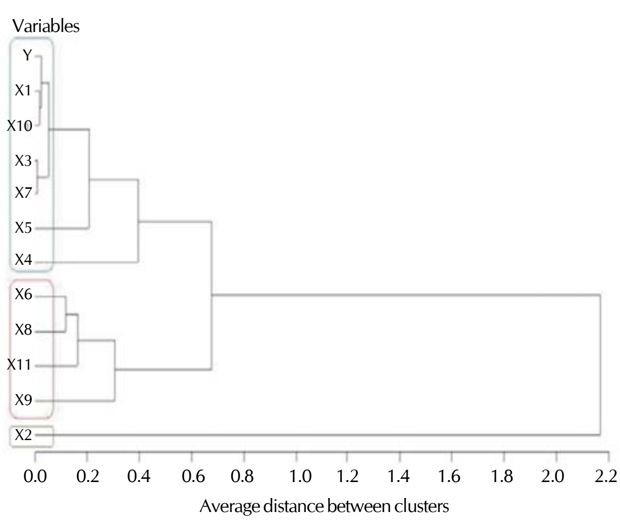

Figure 3: Similarity levels of the estimated eleven wheat variables using the hierarchical cluster analysis under terminal heat stress condition

Using hierarchical cluster analysis the distance of each variable in relation to other variables is calculated. Groups were then formed by the process of agglomeration division. As shown in Table 6 the similarity level increased as the number of clusters increased. Below is the formation process of the clusters. Traits X3 and X7 form the initial cluster (CL11) with normal RMS (Root Mean Square) Distance of 0.0107. The next cluster formed with X1 and X10 (CL10) having a Normal RMS Distance of 0.0174, Y and CL10 have formed another cluster (CL 9) with Normal RMS Distance of 0.0258. CL 9 is joined by CL11 to form CL8 with a distance of 0.0529.The traits X6 and X8 form the cluster (CL7) with Normal RMS Distance of 0.1200. CL7 and X11 are united at a distance of 0.1649 to form CL6. The clusters CL8 and X5 are joined to at a distance of 0.2073 to form CL5. Next clusters CL6 and X9 are joined at a distance of 0.3066 to form CL4. Similarly, clusters CL5 and X4 are joined to form CL3 at a distance of 0.3943 which is again joined with CL4 at a distance of 0.6766 to form CL2. CL2 and X2 are joined at a distance of 2.1672 to form the final cluster CL1.

The distance levels of the estimated 12 wheat characters using the hierarchical cluster analysis could be agglomerated into 3 clusters. Cluster 1 could be numbers of grains/plant (X2), while Cluster 2 includes DTM (X9), MTS (X11), days to heading (X8) and plant height (X6). Cluster 3 includes numbers of grain/spike (X4), biomass (X5), HI (X7), grain weight/spike (X3), CTD (X10), MTS (X11) and grain yield/plant (Y). Data reflected the tendency of each grouped variables in one cluster to relate closely to each other. Hence, the study results proved that numbers of grain/spike (X4), biomass (X5), HI (X7), grain weight/spike (X3), CTD (X10) and numbers of tillers/plant (X1) were more closely related to grain yield.

Path analysis

The correlation coefficients were partitioned into direct and indirect effects (Table 7).The residual effect (0.2030) was considerably low indicating a high contribution of independent traits to the dependent trait grain yield. The direct effects revealed that grain weight/spike (0.647) had the highest direct contribution towards grain yield per plant followed by number of grains/plant (0.544), number of tillers/plant (0.292) and biomass. The highest indirect effects on grain yield were observed with number of grain/spike (0.815), biomass (0.615), plant height (0.392), number of tillers/plant (0.346), HI (0.2613) number of grains/plant (0.223), CTD (0.214) and MTS (0.204). On the other hand, days to heading and days to maturity recorded negative direct effect (-0.149) on grain yield. This indicated that the grain weight per spike was the most important trait followed by number of grains per spike, number of tillers per plant and biomass for selection grain yield in the present set of material. However the characters, namely, number of grains per spike and biomass were also of considerable importance in terms of their high indirect effects towards grain yield.

DISCUSSION

Grain yield, a complex trait, is governed by many quantitative trait loci dispersed throughout the genome (Zhang *et al.*2010) and with low heritability (Blum, 1988), and is highly influenced by environmental fluctuations (Zhang *et al.*2013), and therefore, grain yield itself cannot be used as the sole criterion for selection (Dhanda and Munjal, 2006). Hence, breeding programmes should combine specific plant factors which would buffer yield against severe reduction under heat stress

(Dhanda and Munjal, 2012). Positive associations of biomass (Riaz-Ud-Din *et al.* 2010) and HI (Sud and Bhagwat, 2010; Riaz-Ud-Din *et al.*2010) with grain yield under heat stress environment indicative of efficient utilization of photosynthates. The significant correlations of grain yield with number of tillers per plant, number of grains per plant, grain weight per spike, number of grains per spike under heat stress conditions suggested contribution of component traits under heat stress (Ferrante *et al.*2013; Slafera *et al.*2014). The positive association of plant height with grain yield revealed that the taller plants,in general, more biomass. The results are in agreement with Jat *et al.* (2015). Days to heading and days to maturity indicated that the genotypes earlier in heading and maturity were better for grain yield and its components. These may be due to the escape from heat stress by accelerating their life cycle. These results are in line with those reported by Mondal *et al.* (2013); Zarei *et al.* (2013). Canopy temperature depression played an important role because of its positive correlations with numbers of grains per plant, numbers of grains per plant and biomass (Bahar *et al.*2011). Membrane thermo stability also appeared to very important trait as the genotypes having high membrane thermo stability were also had high grain yield, number of grains per plant, high canopy temperature depression and earlier in days to heading and maturity. Ram *et al.* (2014) also found positive association between membrane thermo stability and grain yield. High temperature during day time particularly day temperatures above thresh hold level may have deleterious effects on plant physiological process (Trivedi, 2015). Therefore, the characters, namely membrane thermo stability, canopy temperature depression and earlier days to heading may help the plant to face heat stress (Satbhai *et al.* 2014).

Correlation coefficient analysis revealed that the genotypes having higher grain yield under heat stress environment were not only having higher number of yield component traits but also high membrane thermo stability, high canopy temperature depression and earlier in days to heading and maturity. Membrane thermo stability may be measured through the estimation of electrolyte leakage from the leaves of the plant when exposed to high temperature. Thus genotypes with low leakage of electrolyte may contribute heat tolerance. Canopy temperature depression also helps the plant to protect itself from heat stress by making its canopy cooler during high evaporative demand (Waiker and Arun 2015). In addition the high grain yield also contributed by the traits like earlier heading and earlier maturity under present study. Identification of suitable recombinant lines with high temperature tolerance may play is an important role in improvement of wheat productivity particularly under heat stressed areas. Furthermore, the multiple statistical procedures which have been used in this study showed that simple correlation and path analysis cannot distinguish crucial variables affecting grain yield, the conclusive assertion cannot be done on the basis of these methods as such. Using multiple statistical analysis of wheat yield under stressed conditions, Leilah and Khateeb (2005) showed that weight of grains/spike, harvest index and biomass were the crucial yield attributing parameters. It seems that use multivariable statistical methods like, stepwise regression together with cluster analysis and principal component analyses are stronger statistical methods to be used in breeding programs for screening important traits.

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Abbreviations: MTS = Membrane thermo stability, CTD = Canopy temperature depression, DTM = Days to maturity, DTH = Days to heading, HI = Harvest index