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Research Article

Morpho-physiological diversity in indian spring wheat cultivars and identification of promising donor under terminal heat stress

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Abstract

Assessment of genetic diversity and its application for wheat breeding resulted in enhanced and sustainable production and productivity. One hundred and two spring wheat cultivars were evaluated for 19 agro-morphological and physiological traits under late sown conditions for two years. The cluster analysis revealed significant genetic variability among wheat cultivars that shows the excellent opportunity to bring about improvement through hybridizing the selected genotypes present in distant clusters. The data was further subjected to PCA (principal component analysis) and genotype by trait biplot analysis. The first 2 principal components accounted for 33.8% of total variation. Phenological traits and growing degree days had major contribution towards heat tolerance as indicated by principal component analysis. Correlation analysis revealed that grain yield was positively associated with biological yield and harvest index. Promising cultivars were identified for terminal heat tolerance that can be adapted for growing under late sown condition and further used for breeding improved climate resilient wheat cultivars and genomics study.

Keywords: Wheat, terminal heat stress, morpho-physiological diversity memglane stability index, CCI, grain yield, GDD

1. Introduction

Wheat is the second most important crop after rice in India and occupies 30.05 million hectares with a total production of 98.61 million tones and productivity of 3172 kg/ha (IIWBR Annual Report, 2017-18). Global reports indicate a loss of 10-40% in crop production by 21st century because of the impact of climate change (Dutta *et al.,* 2013). Heat and drought are major abiotic stresses that decrease wheat quality and productivity to a considerable extent. Continual heat stress is a problem in about 7 m ha while terminal heat stress is a problem in about 40% of the irrigated wheat growing areas of the world (Joshi *et al.,* 2007). High temperature during grain filling stage in wheat is a major constraint and reduces the number of grain per ear, grain weight and subsequently the harvest index, resulting in reduced grain yields (Bansal *et al.,* 2013). In India, the lower productivity is due to shorter crop duration and period of grain-filling and higher temperatures during crop growth, particularly during grain-filling. Wheat is now grown extensively in countries with tropical and subtropical climates and the importance of high temperature has been recognized by CIMMYT in Mexico which has selection programme for high temperature tolerance (Reynolds *et al.,* 1994). The wheat crop in the northern plains is exposed to higher ambient temperatures at the time of grain filling, which significantly reduces the productivity. The late sown wheat is more affected by high temperature stress leading to reduced yield and quality (Wardlaw and Wringley, 1994). Heat tolerance thus should be essential characteristic of wheat cultivars to be developed. Morphologically similar wheat varieties have showed different degree of tolerance to post anthesis high temperature stress. Hence search for heat stress tolerance in existing cultivars will provide ready to use material in for late sown condition. Further, to boost the breeding programme with novel improved genotypes bestowed with heat tolerance capacity, search for cultivars with greater heat tolerance is warranted. Also analysing diversity based on morpho-physiological traits will help in identification of diverse parents for crossing program and finding better recombinants in terms of terminal heat tolerance. Keeping in view the above points an experiment was conducted to evaluate the wheat cultivars under terminal heat tolerance to identify donor as well as assess genetic diversity .

2. Material and Methods

The experiment was conducted at NBPGR, Farm IARI, New Delhi during 2010-2011 and 2011-2012 with 102 wheat cultivar accessions (Table 1) in augmented block design under natural field conditions along with three checks C306, HDR77 and WR544. The crop was sown on 20th December (late sown) in both the years. Standard agronomic practices were followed for raising the crop. Data were recorded for traits, Days to spike emergence (DSE), Days to anthesis (DA), Days to milk (DM) , Days to dough (DD), Days to physiological maturity(DPM), Growing degree days (GDD), Membrane stability index (MSI), Relative water content (RWC), Chlorophyll content index (CCI), Plant height (PH), Tillers per plant (TP), Grains per spike(GS), Biological yield per plant (BYP), Grain yield per plant (GYP), Harvest index (HI), Spike length(SL), Seed protein(SP), Thousand grain weight (TGW), Protein content (PC). The methods were used for estimation of relative water content (Barrs and Weatherley, 1962) and membrane stability index (Deshmukh *et al.,* 1991). Observations were recorded for different phenophases, chlorophyll concentration index by CCM-200 Plus, membrane stability index, relative water content, canopy temperature by IR thermometer gun (Thermo Fisher Scientific), chlorophyll fluorescence by chlorophyll fluorometer (OS-30 P) made in USA, seed protein content by Infratec 1241 grain analyser, yield and yield attributes under normal and late sown conditions. Physiological observations were taken on fully expanded flag leaves after 15 days of anthesis and yield-related observations were taken at the time of harvest. SPAD chlorophyll metre reading was measured on middle part of flag leaves using portable Minolta SPAD-502 chlorophyll metre (Minolta camera Co. Ltd., Osaka, Japan). MSI

Table 1 List of varieties studied for heat tolerance

S.N.	Acc. ID	Variety	S.N.	Acc. ID	Variety	S.N.	Acc. ID	Variety
1	IC75240	C306 C	37	IC128152	CPAN1676	73	IC252927	RW346
2	IC128184	HDR77 C	38	IC443766	RAJ3765	74	IC75219	SKML1
3	IC296383	WR544 C	39	IC443767	RS31-1	75	IC393885	SONAK
4	IC536265	UP262	40	IC443769	UP115	76	IC145735	SONORA64
5	IC536269	UP368	41	IC443768	Utkalika	77	IC145753	UP2121
6	IC535622	WL1562	42	IC528119	DBW16	78	IC252954	UP2425
7	IC112258	VL401	43	IC116274	AJANTA	79	IC445595	UP2338
8	IC128150	BW11	44	IC144904	AKW381	80	IC75223	WL2265
9	IC128157	DL7843-3	45	IC128155	SANGAM	81	IC113722	MOTIA
10	IC128175	HD2327	46	IC145280	HD1925	82	IC547563	DBW17
11	IC128180	HD2428	47	IC145283	HD1941	83	IC128167	HD2189
12	IC128195	HS240	48	IC145971	HD1949	84	IC443725	HD2285
13	IC128206	J-24	49	IC145286	HD1981	85	IC75242	HD2329
14	IC128210	JU-12	50	IC128209	HD1982	86	IC252612	HD2402
15	IC128213	K7410	51	IC252611	HD2177	87	IC443727	HD2643
16	IC128228	HI385	52	IC128168	HD2204	88	IC252632	HD2687
17	IC128261	VL404	53	IC128169	HD2236	89	IC290174	HD2733
18	IC128266	WH157	54	IC128170	HD2270	90	IC303070	HD2781
19	IC128267	WH283	55	IC128171	HD2278	91	IC443728	HD2824
20	IC128268	WH291	56	IC128172	HD2281	92	IC540910	HD2894
21	IC128270	WH416	57	IC128174	HD2307	93	IC574476	HD2967
22	IC128272	WH410	58	IC128178	HD2385	94	IC574388	HD2987
23	IC128273	WL711	59	IC128181	HD2501	95	IC296299	DBW14
24	IC252742	HW2004	60	IC122726	HUW468	96	IC75191	DL 153-2
25	IC252818	Lal Bahadur	61	IC252732	HUW510	97	IC138631	DL788-2
26	IC252951	UP2338	62	IC128211	K65	98	IC443722	DL803-3
27	IC290230	K9644	63	IC128212	K68	99	IC402064	HD2851
28	IC335540	Kharachia 65	64	IC128235	Narmada-4	100	IC437081	HD2864
29	IC75234	HD2009	65	IC128244	PBW154	101	IC528118	HD2888
30	IC75212	PBW12	66	IC128666	PBW222	102	IC519900	HD2932
31	IC75226	WG377	67	IC420923	PBW226	103	IC309874	PBW343
32	IC144903	AKW1071	68	IC420925	PBW299	104	IC240798	PBW373
33	IC128177	HD2380	69	IC75215	PBW34	105	IC565811	PBW590
34	IC145420	HUW213	70	IC75389	RAJ2184			
35	IC443729	HD2833	71	IC410028	RAJ4037			
36	IC282300	K7903	72	IC252929	RW3016			

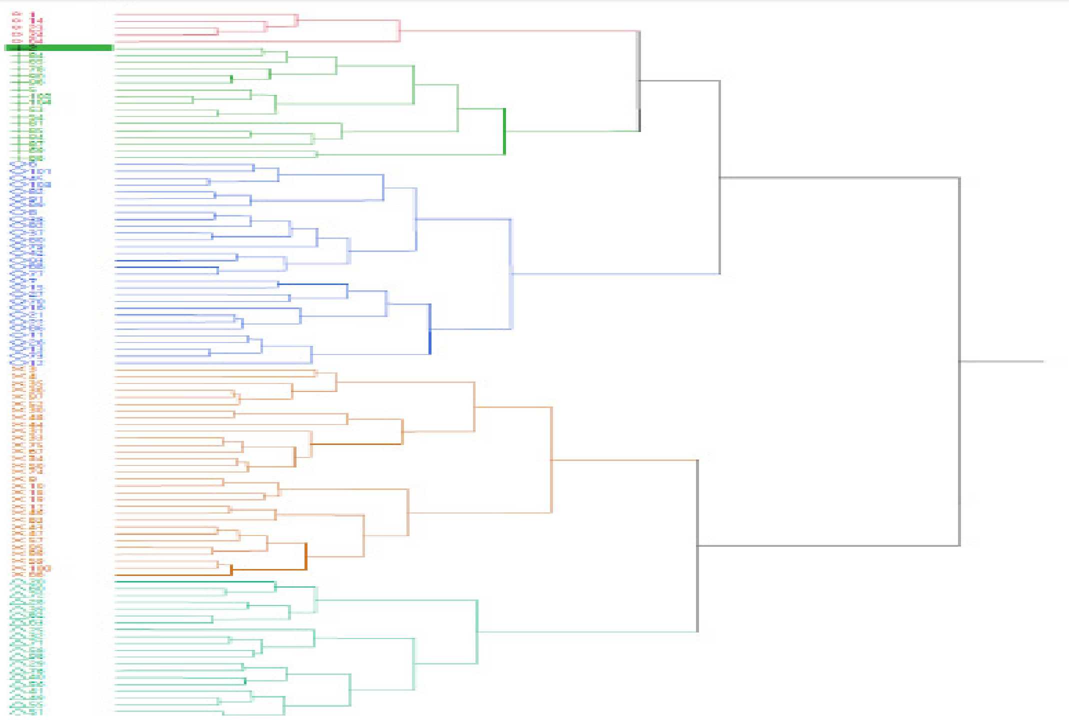

Fig 1. Dendrogram depicting genetic diversity among wheat genotypes under terminal stress condition

was measured using method of Sairam et al. (1997) for which 100 mg leaf material was taken in test tubes containing 10 ml of double distilled water. Initial (at 40°C) and final (at 100°C) conductivity of the solution was recorded on a conductivity bridge (Century, Water soil analysis kit, CMK 751) as C1 and C2. MSI was calculated as MSI = [1 - (C1/C2)] * 100. Statistical analysis for Analysis of Variance, clustering, correlation and Principal Component Analysis were performed by SAS 9.3 and SAS JMP Software.

3. Results & Discussion

Terminal heat stress at grain filling stage alters the physiological mechanism which ultimately affects the yield of wheat crop. It may be due to reduced Pn, membrane stability and total chlorophyll. Several authors (Viswanathan and Khanna-Chopra 2001; Sareen et al. 2012; Sharma et al. 2013) screened the wheat genotypes for heat tolerance based on stress susceptibility indices of yield. In present study, multiple physiological traits were used to screen the wheat genotypes under heat stress condition. Genotypes were highly variable for the traits studied as revealed by mean and range value mentioned in Table 2. Details of promising accessions for various phenophases and other morpho-physiological traits under late sown condition are shown in Table 2 for example, days to spike emergence: IC536265, IC443729, IC144904, IC296383, IC282300 (<77 days); days to milk stage: IC296383(<92days); physiological maturity: IC536265, IC443729, IC144904, IC443722, IC75226(<117 days). Chlorophyll concentration index at post anthesis stage: IC443729 and IC445595(>52.43%); canopy temperature at post anthesis stage: IC128213(69°C); membrane stability index at grain development stage: IC540910 (61%); relative water content at grain development stage: IC443727(79.57 %) IC128213 (69%), number of tillers/plant: IC128235, IC128261(>7); length of spike: IC128206(14.4cm); number of seeds/spike: IC128213(75); biomass/plant IC252632(32.22g); seed yield/plant IC252632(12.8g); 1000 grain wt. IC443727(>43g) and harvest index: IC75215(61.1%) and seed protein content IC128235(7.5%). Donor for multiple traits including grain yield under heat stress identified were: IC252632/HD2687 (GYP and BYP), IC75215/PBW34(GYP, BYP, HI and CCI), IC75240/C306(GYP, BYP, PH,TP and GDD), IC128170/HD2270(GYP,HI and MSI). Almeselmani *et al.,* (2006) recorded significant reduction in wheat chlorophyll concentration index, relative water content and membrane stability index in all genotypes under late and very late sowings at all the stages of plant growth. Kushwaha *et al.* (2006) recorded that late planting in wheat produced significantly less tillers/m^2, spike length and spikelets/ear. This is due to short duration of growth period and increasing temperature

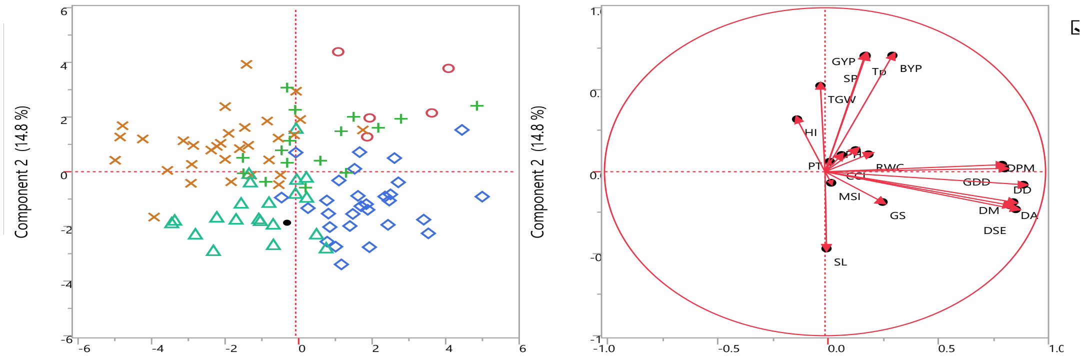

Fig 2. Genotype and trait biplot based on correlation based principal compenent analysis

Table 2: promising accessions for various traits studied under late sown condition; Genotypes in bold are multiple donor for grain yield and other traits

Traits	Mean	Range	Top five accessions under stress condition
DSE	82.75±0.31	75.50-90.50	IC536265, IC443729, IC144904, IC296383, IC282300 (<77.00)
DA	88.75±0.30	82.00-97.00	IC296383, IC282300, IC393885, IC75226, IC443729 (<84.00)
DM	97.82±0.24	91.50-103.00	IC296383, IC128267, IC128268, IC75234, IC75212 (<94.50)
DD	109.84±0.21	105.00-114.50	IC296383, IC443729, IC144904, IC420923, IC128261(<107.00)
DPM	118.95±0.15	114.00-123.50	IC536265, IC443729, IC144904, IC443722, IC75226(<117.00)
GDD	2354.06±3.94	2210.40-2474.98	IC128228, IC75240, IC252742, IC128268, IC128152 (>2410.88)
MSI	53.58±0.39	40.77-61.14	IC540910, IC252632, IC112258, IC75242, IC128170 (>59.91)
RWC	60.42±0.35	51.47-68.54	IC128213, IC128267, IC574388, IC536265, IC112258 (>66.66)
CCI	37.92±0.51	27.35-52.85	IC443729, IC445595, IC75215, IC574388, IC138631(>46.80)
PH (cm)	91.59±1.10	65.00-138.00	IC128235, IC443767, IC128212, IC128211, IC75240 (>113.25)
TP	5.10±0.07	3.50-7.50	IC128235, IC128261, IC128184, IC75240, IC128157 (>6.25)
GS	52.20±0.83	34.00-75.00	IC128213, IC536265, IC290230, IC112258, IC75226 (>68.50)
BYP (g)	17.36±0.33	10.64-32.23	IC252632, IC128235, IC75240, IC75215, IC290230 (>22.98)
GYP (g)	6.71±0.17	3.21-12.82	IC252632, IC75215, IC75240, IC128170, IC303070 (>9.62)
HI (%)	38.43±0.56	24.42-51.35	IC75215, IC113722, IC128170, IC128167, IC145753 (>49.45)
SL (cm)	11.61±0.12	8.40-14.40	IC128206, IC128213, IC252954, IC75389, IC252927 (>13.80)
SP	5.02±0.07	3.50-7.50	IC128235, IC128261, IC128184, IC128266, IC128167 (>6.25)
TGW (g)	30.53±0.44	20.00-43.00	IC443727, IC113722, IC144904, IC574388, IC128175 (>38.25)
PC(%)	13.27±0.07	11.36-14.75	IC443729, IC252927, IC290230, IC113722, IC536265 (>14.27)

Table 3: Correlation study among morpho-physiological traits.

	BYP	CCI	DA	DD	DM	DPM	DSE	GDD	GS	GYP	HI	MSI	PH	PT	RWC	SL	SP	TGW	TP
BYP (g)	1.00																		
CCI	0.11	1.00																	
DA	0.13	0.13	1.00																
DD	0.19	0.07	0.74**	1.00															
DM	0.06	0.18	0.82**	0.74**	1.00														
DPM	0.20	-0.08	0.49**	0.69**	0.50**	1.00													
DSE	0.10	0.05	0.90**	0.77**	0.80**	0.56**	1.00												
GDD	0.18	-0.09	0.52**	0.72**	0.56**	0.93**	0.53**	1.00											
GS	0.23	0.12	0.27	0.19	0.17	0.06	0.25	0.05	1.00										
GYP (g)	0.83**	0.21	0.10	0.13	-0.01	0.06	0.09	0.04	0.22	1.00									
HI (%)	0.10	0.20	-0.04	-0.07	-0.11	-0.18	-0.04	-0.19	0.05	0.63**	1.00								
MSI	0.11	0.01	0.03	0.03	0.02	0.01	0.06	-0.04	0.16	0.18	0.18	1.00							
PH (cm)	0.20	-0.26	-0.08	0.05	0.12	0.21	0.03	0.19	-0.08	0.05	-0.15	0.00	1.00						
PC	0.07	0.18	-0.06	0.04	0.01	-0.05	-0.01	-0.04	0.05	-0.03	-0.14	-0.02	0.16	1.00					
RWC	0.04	0.06	0.10	0.15	0.07	0.17	0.10	0.12	0.06	-0.06	-0.17	0.01	0.01	0.19	1.00				
SL (cm)	-0.11	0.01	0.04	-0.01	0.02	-0.04	0.07	-0.02	0.36*	-0.20	-0.21	0.00	-0.08	-0.04	-0.07	1.00			
SP	0.39*	-0.05	0.01	0.06	0.01	0.17	-0.03	0.15	-0.12	0.21	-0.12	-0.22	0.09	0.10	0.20	-0.18	1.00		
TGW(g)	0.24	0.12	-0.08	-0.03	-0.06	0.06	-0.13	0.10	-0.33*	0.36*	0.31*	-0.05	-0.02	-0.17	0.02	-0.37*	0.08	1.00	
TP	0.40*	0.00	0.01	0.06	0.01	0.16	-0.04	0.15	-0.12	0.21	-0.14	-0.21	0.04	0.10	0.19	-0.17	0.97**	0.10	1.00

DSE: Days to spike emergence, DA: Days to anthesis, DM: Days to milk, DD: Days to dough, DPM: Days to physiological maturity, GDD: Growing degree days, MSI: Membrane stability index, RWC: Relative water content, CCI: Chlorophyll content index, PH: Plant height, TP: Tillers per plant, GS: Grains per spike , BYP: Biological yield per plant, GYP: Grain yield per plant, HI: Harvest index, SL: Spike length, SP: Seed protein, TGW: Thousand grain weight, PC: Protein content

Table 4. Cluster wise mean performance of different morpho-physiological traits in spring wheat cultivars under terminal heat stress

Cluster	Number	DSE	DA	DM	DD	DPM	GDD	MSI	RWC	CCI	PH
1	5	83.90	89.00	99.90	111.85	121.25	2415.09	50.92	62.44	34.37	113.85
2	17	83.71	89.93	97.81	111.00	119.12	2357.01	54.81	59.73	40.45	87.75
3	30	85.87	91.33	100.05	111.53	119.83	2376.31	53.46	60.71	38.34	92.17
4	31	79.47	86.26	95.76	107.77	117.87	2326.84	53.20	61.07	38.52	86.60
5	22	82.02	87.76	97.21	109.05	118.62	2345.52	53.94	59.13	35.25	95.95

Cluster	Number	TP	GS	BYP	GYP	HI	SL	SP	TGW	PC
1	5	6.25	45.30	21.95	7.68	34.44	9.80	6.13	33.55	13.60
2	17	4.96	51.68	20.14	8.99	44.86	11.36	4.89	34.45	13.08
3	30	5.02	57.53	16.54	5.97	36.06	12.15	4.98	28.00	13.55
4	31	5.52	51.45	17.62	6.82	39.05	11.65	5.41	31.44	13.30
5	22	4.40	47.76	15.05	5.53	36.62	11.43	4.35	28.90	12.89

DSE: Days to spike emergence, DA: Days to anthesis, DM: Days to milk, DD: Days to dough, DPM: Days to physiological maturity, GDD: Growing degree days, MSI: Membrane stability index, RWC: Relative water content, CCI: Chlorophyll content index, PH: Plant height, TP: Tillers per plant, GS: Grains per spike , BYP: Biological yield per plant, GYP: Grain yield per plant, HI: Harvest index, SL: Spike length, SP: Seed protein, TGW: Thousand grain weight, PC: Protein content

at grain filling stage. Singh *et al.,* (2011) reported general reduction in the values of all traits under late planting condition as compared to normal planting due to temperature stress.

3.1. Correlation analysis

Correlation analysis was carried out among the 19 morpho-physiological and phenological traits of 102 genotypes under terminal heat stress condition and correlation matrix is given in Table 3. Our correlation analysis showed positive association of grain yield per plant with BYP (0.83), HI (0.63), 1000 grain weight (0.36) which are significant at p (0.01) level. HI is important trait for heat stress tolerance which indicates the partitioning of photosynthates and sink capacity of plants under heat stress conditions (Fischer et al. 2014). The physiological traits like 1000 grain weight, seed yield, biomass, stay green habit, stomatal conductance and canopy temperature deficit has been utilized successfully under breeding programme to screen wheat genotypes for their performance under heat stress conditions (Reynolds et al. 2012; Joshi et al. 2007).

Table 5. Principle component analysis of various morpho-physiological traits in spring wheat under terminal heat stress

	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6
DSE	0.40	-0.14	0.10	0.00	-0.07	-0.02
DA	0.40	-0.11	0.12	0.02	-0.18	-0.08
DM	0.39	-0.12	0.04	-0.03	-0.14	0.06
DD	0.42	-0.05	0.03	-0.05	-0.04	0.04
DPM	0.37	0.03	-0.13	-0.18	0.16	-0.01
GDD	0.38	0.01	-0.13	-0.20	0.13	-0.03
MSI	0.01	-0.04	0.29	0.05	0.30	0.24
RWC	0.09	0.06	-0.18	0.18	-0.18	0.37
CCI	0.03	0.06	0.26	0.20	-0.51	0.21
PH(cm)	0.06	0.08	-0.18	-0.08	0.56	0.34
TP	0.08	0.42	-0.33	0.20	-0.14	-0.22
GS	0.12	-0.11	0.25	0.50	0.17	-0.15
BYP(g)	0.14	0.42	0.16	0.22	0.27	-0.03
GYP(g)	0.09	0.42	0.40	0.09	0.16	-0.04
HI(%)	-0.06	0.19	0.48	-0.14	-0.07	-0.02
SL(cm)	0.00	-0.28	0.01	0.37	0.14	-0.34
SP	0.09	0.42	-0.33	0.19	-0.11	-0.21
TGW(g)	-0.01	0.31	0.14	-0.43	-0.15	0.06
PC	0.01	0.04	-0.12	0.35	-0.07	0.63

3.2. Cluster analysis

These 102 genotypes were divided into five cluster using Ward's method which is based on square euclidean distance for different morpho-physiological traits. Clusters 1, 2, 3,4 and 5 comprised of 5,17,30,31 and 22 genotypes respectively (Fig1). Mean values of different physiological traits under different cluster is given in Table 4. It showed that mean value of different physiological traits is higher in cluster I and II than other clusters. The genotypes of cluster I showed considerably higher mean values of biological yield (21.95g), GDD (18 %), GYP (7.68g), plant height (113.85cm), tillers per plant (6.25), RWC (62.44) than any other cluster mean for respective traits hence seemed comprising of tolerant cultivars.

Table 6. Values representing the relative importance of the physiological traits in grouping of genotypes in different clusters based on communalities of PCA

Physiological traits	Communalities of PCA
DSE	0.89200
DA	0.87369
DM	0.82519
DD	0.77260
DPM	0.91016
GDD	0.91613
MSI	0.16356
RWC	0.17204
CCI	0.38411
PH (cm)	0.37039
TP	0.94659
GS	0.41025
BYP (g)	0.98260
GYP (g)	0.98924
HI (%)	0.96340
SL (cm)	0.31284
SP	0.94553
TGW (g)	0.43083
PC	0.23908

3.3. Principal component analysis

The conservation and exploitation of genetic resources could be made by partitioning the total variance into its components. It also provides opportunity for utilization of appropriate germplasm in crop improvement for particular plant traits. The principal component (PC) analysis divides the total variance into different factors. In this study, out of total 19, six principal components (PCs) were extracted having Eigen value >1. The PC I contributed maximum towards the variability (24.4%) followed by PC II (12.8%) (Table 5). High contribution of first few PCs in total variability based on various plant traits has already been reported in the literature (Ali *et al.*,2011). In our experiment, PC 1 was mainly due to variations in phenological traits and growing degree days. PC2 variation was mainly contributed yield components like BYP, GYP, tillers per plant. Values representing the relative importance of the physiological traits in grouping of genotypes in different clusters based on communalities of PCA are given in Table 6 which revealed the maximum importance of GYP followed by BYP, HI, TP and SP. Principal component 1 and 2 were used to construct trait biplot as well as genotype biplot (Fig 2). The PCA biplot analysis confirmed the amount of variation for the traits among the studied wheat cultivars and it is in congruence with the clustering pattern also. This variability could be utilized in designing a breeding program aimed at improving heat stress tolerance in wheat. The genotypes 1(C-306), 14(JU-12), 23(WL-711), 24(HW-2004) and 64 (Narmada-4) belonging to cluster 1 and first quadrant of PCA are relatively more heat tolerant and can be further used as parental genotypes for developing terminal heat tolerant wheat genotypes in breeding programmes. Overall, it can be concluded that substantial variation in terminal heat stress tolerance among wheat cultivars was found in this study, and several relatively heat stress tolerant wheat genotypes based on morpho-physiological traits were identified.

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