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# **RESEARCH PAPER**

# Characterization of wheat genotypes for stay green and physiological traits by principal component analysis under drought condition

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**Abstract :** An experiment was conducted to examine the magnitude of genetic diversity and characters contributing to genetic diversity among 35 core elite wheat germplasm from INDIA and CIMMYT under water deficit condition. Principal components (PC) analysis showed that three components explained 67.73 per cent of the total variation among traits. The first PC contribute 38.8 per cent, second PC contribute 17.17 per cent and third PC contribute 11.66 per cent of total variation between traits. The first PC was more related to LSR, DSI, SCMR, RWC, ear weight per plant, harvest index and grain yield. The second PC was more related to plant height, LSR, tillers per plant, biological yield, thousand kernel weight and RWC. Therefore, selection based on first component is helpful for a good hybridization breeding program. Genetic divergence was carried out and grouped genotypes into six genetically distinct clusters. Cluster II genotypes *viz.*, CHIRYA7, HW2041 and PBW502 shows superiority for functional stay green trait by exhibiting low cluster mean for leaf and DSI, and high cluster mean for SCMR, photosynthetic rate, RWC, tillers per plant, ear weight, 1000 kernel weight, biological yield, harvest Index, grain yield per plant and in contrast Cluster IV genotypes are non-stay green and drought susceptible by exhibiting high cluster mean for LSR and DSI. A three dimensional (3D Plot) depicts maximum genetic divergence between HW2041 and CBW38 and CHIRYA7 and HW2033. Stay green trait and all yield attributing traits except plant height can be improved by intermating HW2041 with CBW38 and CHIRYA7 with HW2033 genotypes which result in a highly heterotic hybrid for these traits under water deficit stress in wheat.

Key Words : Principal component analysis, Genetic diversity, Leaf senescence rate, Wheat, Drought

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INTRODUCTION	per cent of the world's cereal area, and over 220 million
Wheat (Triticum antium I) is a starle feed for	ha, often under abiotic stress which consequently lowers
wheat ( <i>Triticum destivum</i> L.) is a staple food for	its yield (Cossani and Reynolds, 2012). Wheat yields have
more than 35 per cent of the world population with 30	been reported to reduce by 50-90 per cent of their

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Traits that may be associated with post flowering drought tolerance include improved rooting depth (Sharp et al., 2004), stay-green (Rajcan and Tollenaar, 1999 and Borrell et al., 2000), longer seed filling duration, increased seed filling rate and increased individual seed weight (Harris et al., 2007). Drought leads to premature leaf senescence leads to decrease in leaf area duration and decrease in crop yield. Senescence rate in wheat is particularly sensitive to water and heat stress and like many other traits in crops, genetic variation for this trait has been reported in wheat (Falqueto et al., 2009; Srivalli and Khanna-Chopra, 2009). Photosynthesis, the most important process influencing crop production, inhibited by drought stress and high photosynthetic rate (Pn) is one of the most important breeding strategies for crop improvement. Morphological characters, such as root length, tiller, number of spike per m<sup>-2</sup>, grain per spike number, fertile tillers per plant, 1000 grain weight, peduncle length, spike weight, stem weight, awn length, grain weight per spike, grain yield, biomass, harvest index, plant height, and main spike length etc., affect the wheat tolerance to the water shortage in the soil (Plaut et al., 2004).

Modern cultivars in wheat and other crops are often genetically similar, with a rather narrow genetic base. The presence of genetic diversity and genetic relationships among genotypes is a prerequisite and paramount important for successful wheat breeding programme under drought condition because artificial crossing among dissimilar parents allows a large segregation and combination of different favourable alleles as emphasized by Bered et al. (2002). A number of methods are available for analysis of genetic diversity in germplasm accessions, breeding lines and populations. Multivariate analysis methods are also useful tool to access stability and can be used to identify groups with desirable traits for breeding. As a multivariate statistical technique, the principal components analysis (PCA) has the ability to transform a number of possibly correlated variables into as maller number of variables and explained the variation among genotypes. This approach is very helpful in deciding which agronomic traits of crop contributing most to yield, subsequently, these agronomic traits should be emphasized in the breeding program. There are substantial differences between the groups, but the individuals within a single group are similar (Einstein, 1996). Keeping these in mind, the present study was undertaken with the aim of examining the magnitude of genetic diversity and characters contributing to genetic diversity among wheat genotypes under drought condition.

# MATERIAL AND METHODS

A pot culture experiment was conducted during Rabi season of 2012-13 and on thirty five wheat germplasm with recommended package of practices for wheat. Plants were subjected to water deficit stress for 8 days after anthesis (DAA) by withholding irrigation (RWC 65-70%), while in irrigated plants RWC ranged from 80-85 per cent. The response of plants in terms of growth and physiological traits were studied in upper most fully expanded flag leaf at 50 per cent anthesis stage. Leaves were categorized into green and yellow/dead leaves, and the rate of photosynthesis (Pn) was measured using portable infrared gas analyser (IRGA), LI-6400XT Model (Li-COR Ltd., Lincoln, Nebraska, USA) by operating the IRGA in the closed mode between 10.00-11.00 a.m. when relative humidity, temperature, photosynthetic photon flux density and CO<sub>2</sub> conc. ranged from 50-60 per cent, 30 to 35°C, 1200 µmol m<sup>-2</sup>s<sup>-1</sup> and 350 to 360 µmol mol<sup>-1</sup>, respectively. Fifteen flag leaves per treatment were selected at random for Pn measurement and expressed in µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>1</sup>.Soil and plant analyser development (SPAD) values were measured in the middle part of flag leaves using portable Minolta SPAD-502 chlorophyll meter (Minolta camera Co. Ltd., Osaka, Japan) after flowering at the end of stress period for 8 days. The average readings of 10 leaves per pot was recorded and used in analysis. Measurements were carried out twelve times between flowering and the end of senescence on three flag leaves for each genotype. Phenotyping for leaf senescence was done visually and senescence score was estimated, dividing the percentage of estimate area that is dead by time duration in days as per Lu et al. (2011). A senescence scoring of 10 indicated essentially no leaf death, 5-6 indicated approximately 50 per cent mature leaf area dead, while 0 indicate 100 per cent leaf senescence. 10 = no leaf dead area; 9 = 10 per cent dead area; 8 = 20 per cent dead area; 7 = 30 per cent dead area; 6 = 40 per cent dead area; 5 = 50 per cent dead area; 4 = 60 per cent dead area; 3 = 70 per cent dead area; 2 = 80 per cebt dead area; 1 = 90 per cent dead area and 0 = 100 per cent dead area. The twelve dates of assessments were expressed as  $(\Sigma t_1 -$   $\Sigma t_{12}$ ) and the corresponding senescence score  $(S_1 - S_{12})$ . The average senescence  $(S_a)$  was calculated after 20 days from 50 per cent anthesis as  $(S_i +_{20} - S_i) / (\Sigma t_i +_{20} - \Sigma t_i)$ . The plants were harvested separately from control and water stressed pots. Measurements on grain yield per plant were recorded as economic yield. The whole plant dry weight was measured as biological yield. The drought susceptibility index was calculated using the formulae (Fischer and Maurer, 1978) given by :

 $\mathbf{S} = (\mathbf{1} \cdot \mathbf{Y} / \mathbf{Y} \mathbf{p}) \ / \ \mathbf{D}$ 

where Y is yield under stress, Yp is yield without stress and X and Xp represent average yield over all varieties under stress and non-stress condition, respectively.

#### **D** = Stress intensity = 1-X/Xp

X is mean Y of all genotypes, Xp is mean Yp of all genotypes. The S was used to characterize the relative drought stress tolerance of the various species (S $\leq$ 0.50 high drought tolerant, S $\geq$ 0.50 $\leq$ 1.00 moderately stress tolerant and S>1.00 Susceptible). Simple statistics and numerical taxonomic techniques were analyzed using the procedure of cluster and principal component analysis with the help of computer software INDOSTAT software version 9.2. Cluster analysis was conducted on the basis of average distance of k-means and the accessions in each cluster were then analyzed for basic statistics.

### **RESULTS AND DISCUSSION**

The average data were analyzed by using principal

component analysis. Principial component analysis is a form of multivariate analysis utilized in present study, reflects the importance of the largest contributor to the total variation at each axis of differentiation. The eigen values are often used to determine how many factors to retain. The sum of the eigen values is usually equal to the number of variables (Sharma, 1998). Three principal components showed more than one eigen value. The variations were identified for twelve different characters in 35 core elite wheat germplasm from India and CIMMYT. Most of the variation accounted by the first three canonical vectors represented the primary, secondary and tertiary axes of differentiation. In the principal component analysis, out of six, three principal component exhibited more than one eigen value and showed 67.73 per cent of variability. Hence, this three were given due importance for further explanation (Table 1). The first principal component vector explained 38.88 per cent of the variation. The second principal component vector explained about 17.17 per cent of the variation. The third principal component contributed about 11.11 per cent to the total variation. It was concluded that maximum variation was present in first PC. So selection of genotypes from this PC will be useful.

In the present study, differentiation of the genotypes into different clusters was because of relatively high contribution of few characters rather than small contribution from each character. The positive and negative loading show the presence of positive and negative correlation trends between the components and

cent of variation	(s) for twelve morpho-	physiological traits in 3	5 genotypes of wheat	with eigen values and	cumulative per
	1 Vector	2 Vector	3 Vector	4 Vector	5 Vector
Leaf senescence rate	0.306	0.375	0.289	0.057	0.244
SCMR	-0.368	-0.136	0.010	-0.219	0.031
Photosynthetic rate	-0.255	0.017	0.633	-0.208	0.114
RWC	-0.341	-0.310	-0.243	-0.001	-0.313
Plant height	-0.117	0.461	0.095	0.411	-0.375
Tillers/ plant	-0.204	0.359	0.187	-0.407	-0.446
Ear weight/ Per plant	-0.353	0.108	-0.110	-0.250	0.031
1000 kernel weight	-0.240	-0.400	0.366	0.150	0.343
Biological yield	-0.059	0.348	-0.385	-0.451	0.501
Harvest index	-0.291	0.262	-0.023	0.410	0.326
Grain yield/ plant	-0.364	0.177	0.140	0.073	0.063
DSI	0.362	-0.098	0.313	-0.330	-0.098
Eigene value (Root)	4.666	2.061	1.400	1.128	0.719
% var. exp.	38.887	17.176	11.667	9.398	5.993
Cum. var. exp.	38.887	56.064	67.731	77.129	83.122

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the variables. Therefore, the above mentioned characters which load high positively or negatively contributed more to the diversity and they were the ones that most differentiated the clusters. Accordingly, from Table 1 the first principal component (PC-I) had high positive component loading from LSR, DSI, whereas SCMR, grain yield per plant, relative water content, ear weight per plant and harvest index exhibited high negative loading. In the second principal component (PC-II) high positive component loading was observed from plant height, LSR, tillers per plant, biological yield followed by high negative loading from thousand kernel weight and RWC. The major contributing characters for the diversity in the third principal component (PC-III) were photosynthetic rate, thousand kernel weight and DSI exhibited high value for positive loadings and biological yield showed the high negative values. From the three PCs it was clear that among all the twelve variables, DSI, LSR, photosynthetic rate, plant height, tillers per plant, biological yield, grain yield had high value which is agreement with Chahal and Gosal (2002) who showed that characters with largest absolute value closer to unity within the first principal component influence the clustering more than those with lower absolute value closer to zero. From this study, it was concluded that a good hybridization breeding program can be initiated by the selection of genotypes from the PC1 as it contributed maximum toward diversity with maximum eigene value. Table 2 shows that the 35 wheat genotypes were grouped into six clusters using K-means non-hirerichical clustering. Ranjbar et al. (2007); Escobar-hernandez et al. (2005); Sapra and Lal (2003); Maqbool et al. (2010) and Ahmadizadeh et al. (2011) also used principal component was used by Rajbar et al. (2007); Escobar-Hernandez et al. (2005); Sapra and Lal (2003); Maqbool et al. (2010) and Ahmadizadeh et al. (2011) for grouping of wheat germplasm. From these scores given to genotypes on the basis of first and second PC, breeders can select genotypes with highest score having desirable characters for further breeding programmes. Cluster VI comprised 12 genotypes followed by cluster I and V comprising 8 genotypes, whereas cluster II comprises 3 genotypes and lastly cluster III and IV comprises 6, respectively. K-cluster mean of principal component analysis for all the 12 characters presented in Table 3. The individual cluster reflected superiority for various traits depicted by cluster mean value in Table 3.

Cluster II genotypes shows functional stay green nature exhibiting minimum cluster mean for leaf senescence rate and DSI and maximum cluster mean for SCMR, photosynthetic rate, RWC, tillers per plant, ear weight per plant, 1000 kernel weight, biological yield, harvest Index, grain yield per plant. Cluster III genotypes are drought sensitive and non-stay green nature exhibiting maximum cluster mean for LSR, DSI and minimum cluster mean for SCMR, Photosynthetic rate, RWC, ear weight per plant, thousand kernel weight, harvest index and grain yield which is in agreement with Ahmad *et al.* 

Table 2 : Clustering of 35wheat germplasm into different clusters based on K-mean cluster analysis							
Group K	n	Cluster members					
1.	8	PBW373 HW2042 HW2055 HW2008 HW2027 HW4024 HW2060 HW4010					
2.	3	CHIRYA7 HW2041 PBW502					
3.	2	CBW38 HW2033					
4.	2	HW4050 HW2063					
5.	8	CHIRYA1 LOK64 UP2696 HW4060, HW4203 HW4009 SHANGHAI HW2051					
6.	12	HW2020 HW2085 HW2061 PBW555 HD2894 CHIRYA3 GW322 HW4007 HW4030 HW2080 WL711 HD2789					

Table 3 : K- Cluster mean twelve morpho-physiological characters of 35 wheat germplasm												
	LSR	SCMR	PnRate	RWC	PH	TPP	EWP	TKW	BYP	HI	GYP	DSI
1 Cluster	0.279	45.092	6.720	54.946	86.646	9.668	10.893	30.112	24.503	28.693	7.428	0.809
2 Cluster	0.189	51.156	16.050	61.635	79.833	10.370	15.605	40.652	26.560	32.003	8.253	0.658
3 Cluster	0.483	37.467	2.033	45.910	79.500	9.822	2.265	32.869	12.803	16.873	1.824	1.211
4 Cluster	0.200	42.500	8.060	57.236	63.167	5.625	6.737	39.997	11.700	19.552	2.500	1.275
5 Cluster	0.375	43.004	5.867	50.364	77.208	7.879	5.928	27.801	15.619	19.812	2.615	1.153
6 Cluster	0.318	44.642	6.721	52.886	81.361	7.501	7.244	27.621	17.870	25.611	4.315	1.046

LSR= Leaf Senescence Rate, SCMR= SPAD Chlorophyll Meter Reading, RWC= Relative Water Content, DSI= Drought Susceptibility Index, PHT= Plant Height, TPP= Tillers per plant, EW= Ear weight, TKW= Thousand Kernel Weight, BY=Biological yield, HI=Harvest Index,

GY= Grain Yield, Pn rate= Photosynthetic rate

CHARACTERIZATION OF WHEAT GENOTYPES FOR STAY GREEN & PHYSIOLOGICAL TRAITS BY PRINCIPAL COMPONENT ANALYSIS

Table	Table 4 : Mean performance of 35 wheat germplasm for twelve different agro-morphological traits												
Sr. No.	Character	LSR	SCMR	Pn rate	RWC	PHT	TPP	EW	TKW	BY	HI	GY	DSI
1.	PBW373	0.2167	45.6667	4.0833	58.2487	79.3333	7.1333	9.8000	23.5807	19.4667	32.2000	6.2667	0.7160
2.	HW2042	0.3167	49.3333	15.4333	51.9030	92.8333	9.3333	9.3867	33.9857	26.3667	25.7453	6.8000	0.9790
3.	HW2055	0.3167	47.0000	3.2500	51.1810	86.3333	9.5333	12.3333	22.8240	28.4443	15.4917	5.0000	0.7360
4.	HW2020	0.2000	43.0000	4.6500	57.6113	76.8333	11.4167	14.1100	23.6287	25.3890	21.5840	2.2833	1.0957
5.	HW2085	0.1833	45.6667	5.0833	59.1350	81.6667	5.7223	6.7733	27.3500	18.7777	29.2473	5.3167	0.8940
6.	HW2061	0.2167	46.3333	10.3100	52.2333	78.3333	6.7443	7.7367	36.2627	19.3433	35.1087	6.7333	0.9890
7.	HW2008	0.2667	42.0000	3.1167	53.6337	95.0000	7.0810	10.4800	35.5130	21.8633	42.1853	9.0667	0.6070
8.	HW2041	0.2167	50.8667	16.2200	63.5083	91.5000	11.8667	16.9667	52.5480	25.1033	28.9733	7.3333	0.5883
9.	HW2027	0.3167	40.3333	6.9933	54.7727	91.0000	10.1943	6.8100	25.6523	19.2557	35.9947	6.8667	0.9160
10.	HW4024	0.2667	43.6667	9.3483	57.4693	85.3333	7.6500	11.2200	48.9410	23.1443	29.0887	9.9500	0.8110
11.	PBW555	0.2167	45.3333	5.6267	56.0380	76.6667	6.8447	9.4633	23.7307	17.3777	27.4800	4.6667	1.0490
12.	HW2060	0.2167	47.7000	5.2667	58.7707	75.3333	6.7300	12.0767	31.2753	17.7603	30.5257	8.3333	0.7880
13.	HD2894	0.2667	41.6333	5.3667	52.6657	89.6667	7.4113	4.9933	34.5933	14.6193	28.2420	4.1333	0.7743
14.	CHIRYA7	0.1333	54.2667	20.3333	62.0633	66.6667	11.0000	15.6333	63.0243	32.2667	26.5500	8.3597	0.6950
15.	CBW-38	0.4833	37.4000	1.7300	46.5367	71.0000	11.0057	3.1000	14.9030	17.0167	10.8233	1.8477	1.2520
16.	HW4010	0.3167	45.0333	6.2667	53.5870	88.0000	19.6867	15.0367	19.1273	39.7210	18.3143	7.1400	0.9220
17.	PBW502	0.2167	48.3333	11.5967	59.3320	81.3333	8.2443	14.2150	30.3843	22.3113	40.4843	9.0667	0.6920
18.	Chirya-3	0.2667	42.6667	6.0367	52.5423	66.6667	5.7500	8.1667	31.3203	14.7787	31.5803	4.6833	1.0560
19.	Chirya-1	0.2667	41.0000	2.8733	55.4493	74.3333	7.7500	6.4850	27.2413	13.9000	19.3627	2.6667	1.0620
20.	HW4050	0.2167	45.0000	4.2867	55.4950	65.0000	5.8500	4.5600	45.1133	10.7667	26.6880	2.8667	1.1810
21.	GW-322	0.4167	39.0667	4.3800	50.6517	73.6667	5.5000	7.8667	31.5660	13.0220	31.3620	3.9333	1.0363
22.	LOK-64	0.2667	46.3333	2.6933	52.4170	65.1667	7.1467	7.0100	34.0840	17.3550	22.7740	3.9000	1.0480
23.	UP-2696	0.4167	38.8667	7.9033	49.3080	65.3333	7.3333	5.0000	31.8833	14.5333	17.2800	2.4000	1.1850
24.	HW4007	0.3833	47.0000	13.8167	53.0163	92.6667	8.6300	3.1963	22.6887	22.9543	12.5567	2.9000	1.1820
25.	HW4060	0.4167	38.8333	5.4767	48.7940	88.3333	9.4667	5.1533	35.0333	11.3153	26.8587	3.0333	1.1850
26.	HW4203	0.2667	46.6667	9.2333	51.6477	88.6667	8.4443	6.7533	25.1183	14.7557	14.4843	2.1533	1.1400
27.	HW2063	0.1833	40.0000	11.8333	58.9777	61.3333	5.4000	8.9133	34.8807	12.6340	12.4157	2.1333	1.3693
28.	HW4009	0.4167	41.0000	4.4733	49.7000	81.5000	4.0777	3.4000	21.4967	22.0110	28.9653	2.4667	1.1220
29.	HW4030	0.4333	46.6667	3.1667	52.1037	87.3333	6.3333	6.2200	21.5827	15.1110	28.4623	4.2667	1.0540
30.	HW2080	0.4500	44.3333	5.9000	49.5157	82.1667	8.6167	6.2967	24.6023	15.4453	11.6847	4.6667	1.1533
31.	WL711	0.4667	47.0000	7.4800	47.5617	77.0000	7.1500	5.7667	34.7763	13.3000	34.8490	4.6000	1.1193
32.	Shanghai1	0.4833	46.3333	9.4867	46.3547	72.6667	11.6110	3.4067	23.9363	14.4180	16.8610	2.3000	1.2333
33.	HW2033	0.4833	37.5333	2.3367	45.2827	88.0000	8.6390	1.4300	14.8347	8.5890	22.9233	1.8000	1.1690
34.	HD2789	0.3167	47.0000	8.8300	51.5613	93.6667	9.8890	6.3333	19.3530	24.3227	15.1787	3.6000	1.1477
35.	HW2051	0.4667	45.0000	4.7933	49.2430	81.6667	7.2000	10.2133	23.6133	16.6667	11.9077	2.0000	1.2490

Table 5 : Diverse parents for traits based on genetic distance, K-cluster mean and mean performance of different characters

Sr. No.	Characters	Cluster	Suitable parents
1.	LSR (Fast and slow senescing)	II andIII	CHIRYA7, HW2041/ CBW38 and HW2033
2.	SCMR	II and III	CHIRYA7, HW2041/ CBW38 and HW2033
3.	Photosynthetic rate	II andIII	CHIRYA7, HW2041/ CBW38 and HW2033
4.	RWC	II andIII	CHIRYA7, HW2041/ CBW38 and HW2033
5.	Plant height cm	IV and I	HW4050 HW2063/ HW2008 and HW2027
6.	Tillers/ plant	II and IV	CHIRYA7, HW2041 and PBW502/ HW4050, HW2063
7.	Ear weight/ plant	II andIII	CHIRYA7, HW2041, PBW502/ CBW38 and HW2033
8.	1000 kernel weight	II andIII	CHIRYA7, HW2041, PBW502/ CBW38 and HW2033
9.	Biological yield / plant	II and IV	CHIRYA7, HW2041, PBW502/ HW4050, HW2063,
10.	Harvest index / plant	II andIII	CHIRYA7, HW2041, PBW502/ CBW38 and HW2033
11.	Grain yield / plant	II andIII	CHIRYA7, HW2041, PBW502/ CBW38 and HW2033
12.	DSI	II and IV	CHIRYA7, HW2041, PBW502/ HW4050 and HW2063

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(2014) who showed that Cluster-II exhibited the maximum mean value for spike length, average values for plant height and 1000 grain weight was higher in cluster-III. Cluster IV exhibit low cluster mean for plant height, tillers per plant, biological yield per plant, and high DSI. A three dimensional (3D Plot) configuration of 35 wheat genotypes has been presented in Fig. 1. The figure depicts spatial distance between genotypes as a measure for genetic divergence between them. Biplot had been used by many researchers in comparing different genotypes. Kaya et al. (2002); Dadbakhsh et al. (2011); Abdolshahi et al. (2010) were able to reveal that bread wheat genotypes with larger PCA1 and lower PCA2 scores gave high yields (stable genotypes) and genotypes with lower PCA1 and larger PCA2 scores had low yields (unstable genotypes). In the present study for the improvement of wheat genotypes, diverse parents were selected based on genetic distance, K-cluster mean (Table 3) and mean performance (Table 4) for different characters as presented in Table 4. CHIRYA7 and HW2041 show superiority for functional stay green traits such as low LSR, high SCMR, photosynthetic rate, RWC (Table 5). CHIRYA7, HW2041 and PBW502 for ear weight, 1000 kernel weight, tillers per plant, biological yield per plant, harvest index per plant, grain yield per plant and low DSI. HW4050 and HW2063 were selected for plant height. As genetic divergence was found maximum between HW2041 and CBW38 and CHIRYA7 and HW2033 (Fig. 1) (yield attributing traits) traits such as ear weight per plant, 1000 kernel weight, tillers per plant, biological yield, harvest index per plant, grain yield, DSI, SCMR, photosynthetic rate and RWC



Fig. 1: Three dimensional representation of Indian wheat genotypes showing divergence based on principal component analysis

can be improved by internating of these genotypes which result in a maximum expression of heterosis for these functional stay green traits under water deficit condition.

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