

## **Population structure, molecular and physiological characterisation of elite wheat varieties used as parents in drought and heat stress breeding in India**

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### **Abstract**

**In the present study 24 modern cultivars of wheat were subjected to genetic and physiological characterisation in order to ascertain their use as parents in drought and heat tolerance breeding. The physiological traits, canopy temperature and chlorophyll content of flag leaves were found to be widely distributed. Population structure analysis grouped them into seven clusters with optimum k value=7. AMOVA and PCoA (Principal Coordinate Analysis) revealed higher level of polymorphism among the resistant and susceptible groups. The study revealed that the varieties used in breeding program are highly divergent pure breeding lines with unique pattern of alleles.**

**Key words:** Drought, genetic diversity, heat, physiological traits, wheat

Remarkable progress in wheat production has been achieved by India during the past four decades (Nagarajan 2005) and is currently the second largest wheat producer in the world with annual production of 95.85 million tonnes (FAOSTAT). There have been contradictory claims on diversity of varieties bred through modern plant breeding techniques with the common perception of reduction in genetic diversity seen as a curse of modern plant breeding (Vallve 1993; Clunies-Ross 1995). Such genetic erosion could have serious impacts in the response of plant varieties to pest and diseases as well as the changing climate scenario (Clunies-Ross 1995). Breeding for drought and terminal heat tolerance is an important aspect of Indian wheat improvement program and is largely dependent on molecular marker assisted selection

strategies. Chlorophyll content and canopy temperature (CT) were identified as new and cheap method for indirect selection in wheat for improving drought tolerance (Talebi 2005). The previous reports of genetic structure of Indian wheat varieties mainly concentrated on pre-and post green revolution era cultivars and landraces (Jain and Yadav 2009; Mir et al. 2012). Considering the fact that there is a major lacuna in available information regarding molecular genetic diversity of elite breeding lines and parents used in abiotic stress breeding the present study was carried out to study the physiological parameters CT and chlorophyll content associated with drought screening among the selected elite varieties. The study also focussed to investigate the structure and genetic divergence among parents used for MARS (Marker Assisted Recurrent Selection) and MABC (Marker Assisted Back Crossing) program.

Twenty four wheat varieties which are being utilized as parents in drought breeding program at IARI, New Delhi were used for study. Details are given in Table 1. Physiological data were measured on canopy temperature and chlorophyll contents at vegetative and reproductive stages of plant growth. Data collection was done on the basis of 10 plants per plot. A set of 194 microsatellite markers was selected covering all the seven chromosomes for genotyping on the basis of their chromosomal location. The selected SSR markers covered all the three genomes. Primer sequences were obtained from the Graingenes website.

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**Table 1.** List of varieties used in the study, their parentage and susceptibility or resistance nature to heat and drought

Name	Pedigree	Nature of variety
HI 1500	HW 2002*2/Strempalli/ PNC 5	R
HUW 510	HD 2278/HUW 234//DL 230-16	S
DBW 42	OASIS/SKAU Z//4*BCN/3/ 2*PASTOR	S
GW366	DL 802-3/GW 232	S
Baviacora	BO BWHITE/NA COZARI-76// V EERY/ 3/ BLUE JAY/COCORA QUE -75	R
DBW 43	BA BAX/LR42// BABAX*2/3/VIVITSI	S
HD 2733	A TT ILA/ 3/T UI/CARC// CHEN/CHTO/4/ATTILA	S
Bacanora	JUP/BJY//URES-81	R
PBW 550	WH 594/ RAJ 3856/ W 485	S
Kundan	Tonari 71/NP 890	R
HD 2888	C 306/ <i>T. sphaerococcum</i> /HW 2004	R
WH 730	CPAN 2092/Improved Lok-1	S
NW 2036	BO W/ CROW/ BUC/ PV N	S
Raj 3765	HD 2402/ VL 639	S
NI 5439	RE MP 80/3*NP 710	R
C 306	RGN /CSK3// 2*C591/3/C217/ N14/ /C281	R
Halna	HD 1982/ K 816	S
PBW 175	HD 2160/ WG 1025	R
GW 322	PBW 173/ GW 196	S
GW 326	PBW 175/LOK-1	S
HD 3016	PBW 652*/PASTOR	R
HD 2987	HI 1011/HD 2348//MENDOS// IWP72/DL 153-2	R
HD 2967	ALD/ CO C//URE SH/ HD 2160 M/ HD 2278	R
HW 2004	C 306*7//T R 380-14#7/3 AG 14	R

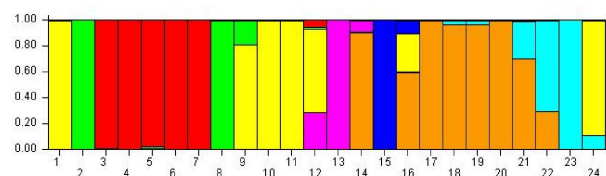
R = Resistant; S = Susceptible

(<http://wheat.pw.usda.gov/GG2/index.shtml>).

Principal component analysis of physiological data was done with the help of software package GENSTAT version 15 (VSN International Ltd, UK). The software STRUCTURE ver.2.3.3 was used for study of population genetic structure among wheat varieties. Analysis of Molecular Variance (AMOVA) was carried out to evaluate the population differentiation among the 7 assumed sub populations using the software Arlequin version 3.5.1.2. The Principal Coordinate Analysis (PCoA) was conducted to evaluate the allelic compositions of the susceptible and resistant parents with the help of software GENALEX version 6.

The varieties Baviacora and Bacanora were found to have low CT values while Raj 3765 and Halna were found to have high CT values. Chlorophyll content among flag leaves was highest in HW 2004 followed by HD 2987 and C 306. In the present study when the variates CT, chlorophyll content and grain yield were considered together the first principal component (PC-1) captured 58.39% variation while the second component (PC-2) captured 29.04% variation. PC-1 was mainly contributed by CT and chlorophyll while grain yield was reflected in PC-2.

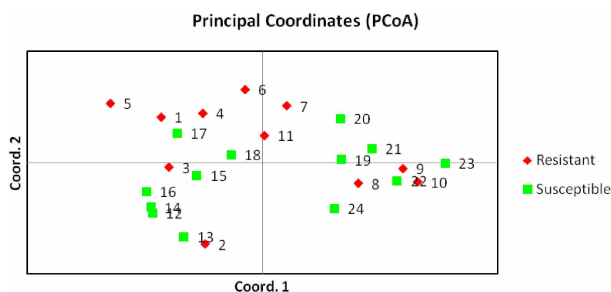
Of the 194 SSR markers used for genotyping of 24 wheat varieties, 162 were finally selected based on amplification profile and low amount of missing data points. Total number of alleles detected was 660 with a mean value of 4.07 alleles per locus. The largest number of alleles (11) was detected by *barc119*. The PIC value of SSR markers varied between 0.11 to 0.87 depending upon number of alleles detected and the allelic frequency and the mean PIC value was 0.58. STRUCTURE analysis helped to group genetically similar parents together in the same cluster. The optimum k value was calculated as 7 and hence the number of subpopulations were also limited as 7 (Fig. 1). The proportion of individuals in different clusters varied from 0.047 in cluster 3 to 0.269 in cluster 7. The average distance between individuals (expected heterozygosities) in the same cluster varied between 0.0421 in cluster 5 to 0.4906 in cluster 4. The molecular variance among the seven identified clusters was calculated using AMOVA. There was considerable admixture in clusters 4, 5, 6 and 7. So these clusters were grouped together and the other three with lesser admixture were kept in the second group. AMOVA revealed that maximum variation was among individuals within clusters (83.73%) and also among the clusters (15.63%). The  $F_{ST}$  value was found to be significant (0.15732\*\*\*). AMOVA results are given in



**Fig. 1.** Population structure of 24 wheat varieties grouped into 7 sub populations at k=7. The varieties are arranged in the following order: HI 1500, HUW 510, DBW 42, GW 366, Baviacora, DBW 43, HD 2733, Bacanora, PBW 550, Kundan, HD 2888, WH 730, NW 2036, Raj 3765, NI 5439, C 306, Halna, PBW 175, GW 322, GW 326, HD 3016, HD 2987, HD 2967 and HW 2004

Table 2. When these clusters were grouped together the variance attributed to grouping was only 0.63% and the F-statistics were not significant. Both the inbreeding co-efficients  $F_{IS}$  and  $F_{IT}$  were found to be 1.0000\*\*\*. The distance between different clusters was calculated as maximum pair wise  $F_{ST}$ . Maximum  $F_{ST}$  value of 0.52941 was noted between clusters 5 and 3, 6 and 3 as well as 2 and 3.

The results of PCoA revealed higher polymorphism between the resistant and susceptible group of parents (Fig. 2). The first axis of PCoA explained 23.92% of variation and the second axis



**Fig. 2. PCoA between resistant and susceptible parents. The varieties are spread all over the 4 axes indicating their wide diversity**

**Table 2.** Analysis of Molecular Variance (AMOVA) in elite wheat varieties among the 7 groups identified through STRUCTURE

Source of variation	Sum of squares	Variance components	Percentage of variation	F Statistics
Among groups	52.908	0.10740	0.63542	FCT=0.00635
Among populations within groups	221.244	2.64212	15.63244	FSC= 0.15732***
Among individuals within populations	476.029	14.15200	83.73214	
Within individuals	0.000	0.00000	0.00000	
Total	750.180	16.90152		

explained 18.48% variation. The first three axes cumulatively explained 58.73% of variation. The Nei's genetic distance between these two populations was estimated to be 0.114. The allelic pattern across the two populations also differed considerably. Number of private alleles for susceptible population was 0.803 and for resistant population was 0.742. The Nei's genetic distance between susceptible and resistant population was 0.114.

Intensive plant breeding often manifests itself in reduced genetic diversity and genetic erosion in modern cultivars. This often leads to increased vulnerability to biotic and abiotic stresses of crop varieties (Vallve 1993). Therefore it is essential to estimate the genetic diversity among the different cultivars used as parents in the breeding program in order to gain information about the genetic variability and allelic composition. The present study used SSR markers for investigating population structure and genetic diversity of wheat varieties used as parents in breeding for drought and heat tolerance. Simultaneously the physiological traits like CT and chlorophyll content contributing towards resistance to drought and heat stress was also studied. These markers will be further employed in MARS and MABB for drought and heat tolerance. The physiological traits, CT and chlorophyll content of flag leaves were found

to be widely distributed with none being too bad or too good. The study revealed that the varieties used in breeding program are highly divergent pure breeding lines with unique pattern of alleles.

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