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Research Article

Genetic diversity analysis of wheat under timely, late and very late sowing conditions

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Abstract
Heat stress is a major constraint in wheat (*Triticum aestivum* L.) production, especially when sowing is delayed. The present study assessed the genetic divergence among 30 spring wheat genotypes under three sowing times namely, timely sown , late sown and very late sown, which corresponds to increasing terminal heat stress. Mahalanobis D^2 analysis was used to group genotypes into different clusters and quantify divergence at each sowing time. The analysis revealed the formation of 10 clusters under timely sowing, 11 clusters under late sowing and 7 clusters under very late sowing. Cluster composition varied with sowing time, with many genotypes forming unique singleton clusters under optimal conditions, whereas under severe heat stress most genotypes grouped together, indicating a convergence of performance under stress. The highest inter-cluster distance was observed under late and very late sowing suggesting greater genetic divergence in heat-stressed environments. Cluster mean analysis showed significant differences for agronomic and quality traits. Under late and very late sowing, Cluster V in late, Cluster II in very late sowing exhibited superior mean grain yield per plant along with higher tiller number and grains per spike, reflecting better heat tolerance. In contrast, clusters comprising heat-susceptible genotypes had much lower yields and poor yield components. Trait contribution to divergence also shifted with sowing time: under timely sowing, grain *Fe* content was the single largest contributor to total divergence (33.6%), whereas under late and very late sowing, grain Zn content contributed most (20.2% and 29.4%, respectively), followed by grain yield and grain weight. The results indicate substantial genetic diversity among the wheat genotypes for traits associated with heat stress adaptation. Heat-tolerant genotypes were distinct in cluster analysis, possessing traits like early maturity, higher tillering, and better grain-filling under stress. The diverse genotypes can be harnessed in breeding programs to improve wheat terminal heat stress tolerance.

Keywords: Wheat, Heat stress, Divergence, yield, Nutritional traits

INTRODUCTION

Bread wheat is one of the world's most important cereal crops, serving as a staple food for a large proportion of the global population. In India, wheat is second only to rice in total consumption and is cultivated in over 30 million hectares, producing about 107 million tonnes annually (Ministry of Agriculture and Farmers Welfare, 2023). However, rising temperatures and climate change pose significant challenges to sustaining wheat productivity. Global average temperatures are projected to increase by 1.5–5 °C by the end of this century, leading to more frequent heat waves and higher incidence of terminal heat stress in many wheat-growing regions (IPCC, 2014). Wheat is highly sensitive to high temperature stress, particularly during the reproductive (anthesis) and grain-filling stages. Temperatures above ~30 °C during grain filling can cause yield reductions exceeding 20%, and even brief heat episodes can induce pollen sterility and shrivelled grain (Asseng *et al*., 2015; Wahid *et al*., 2007). Field studies have shown that delayed sowing, which exposes wheat to higher temperatures during grain filling, can drastically reduce yield. For instance, sowing just 3–4 weeks late

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(mid-December vs. mid-November) has been reported to reduce yield by around 50% due to terminal heat stress (Rashid *et al*., 2004). In North Indian conditions, timely sowing (November) allows the crop to mature before the hottest pre-summer period, whereas late-sown crops experience supra-optimal temperatures during grain development, resulting in accelerated senescence, reduced grain weight and yield loss (Singh and Dwivedi, 2015). High temperature stress also impairs grain quality parameters such as test weight, protein content and micronutrient (zinc and iron) accumulation in the grain (Wahid *et al*., 2007).

Genetic improvement for heat tolerance is a priority in wheat breeding to mitigate the impacts of global warming on crop production. The existence of sufficient genetic variability for heat stress adaptation is a prerequisite for breeding thermo-tolerant wheat varieties (Joshi and Dhawan, 1966). Wheat genotypes differ markedly in their phenological development, morphological traits and physiological responses to high temperatures. For example, some genotypes escape terminal heat by maturing earlier or have traits that confer resilience (such as sustained kernel growth rate or heat-protective proteins), whereas others are extremely susceptible (Wahid *et al*., 2007). Evaluating the extent of genetic diversity among the available germplasm under heat stress can help identify parents likely to produce superior recombinants. D^2 statistics (Mahalanobis, 1936) is a powerful multivariate technique for quantifying genetic divergence based on multiple traits simultaneously. It has been widely used in wheat and other crops to group genotypes into clusters such that genotypes within a cluster have small trait divergences and those in different clusters have large divergences. The D^2 clustering approach helps plant breeders select genetically diverse parents for hybridization between divergent parents are expected to generate high heterosis and broad variability in progeny (Arunachalam, 1981, Baranwal, 2013). In wheat, numerous studies have employed D^2 analysis to assess diversity for yield and stress-adaptive traits. For instance, Arya *et al*. (2017) grouped 49 bread wheat genotypes into eight clusters and noted considerable diversity for yield components, while Dhanda and Munjal (2017) evaluated genetic divergence under heat stress conditions and identified divergent heat-tolerant lines. However, most such studies have focused on a single environment or average performance. Given that genotype performance can vary with the environment (G×E interaction), it is insightful to evaluate genetic divergence under contrasting sowing conditions (normal vs. late planting). This can reveal whether the same groups of genotypes remain distinct across environments and identify genotypes that consistently show heat tolerance.

The present investigation was undertaken to quantify genetic divergence among 30 diverse wheat genotypes under timely, late and very late sowing conditions, corresponding to increasing levels of terminal heat stress. The specific objectives were to (1) group the genotypes into clusters under each sowing time and determine the inter- and intra-cluster distances, (2) compare cluster mean traits to understand the distinguishing characteristics of clusters (particularly those associated with heat tolerance) and (3) estimate the contribution of different traits to overall divergence in each sowing environment. By focusing on Mahalanobis D^2 results, we aim to identify genetically divergent and heat-tolerant genotypes that could be valuable in breeding programs for developing heat-resilient wheat varieties.

MATERIALS AND METHODS

Plant Materials and Experimental Design: Thirty (**Table 1**) spring wheat (*Triticum aestivum* L.) genotypes were selected for this study. These comprised a mix of released cultivars and advanced breeding lines obtained from diverse sources. The genotypes were evaluated in the *Rabi* 2022–23 growing season under three sowing dates: S_1 – timely sowing on 15th November 2022 (optimal conditions), S_2 – late sowing on 15th December 2022 (moderate terminal heat stress), and S_3 – very late sowing on 15th January 2023 (severe terminal heat stress). The trials were conducted at the experimental research farm at PGI MPKV Rahuri, in a randomized complete block design with two replications for each sowing time. Each plot consisted of a 20-row bed, 2 meters in length, with 20 cm row spacing, covering an area of approximately 8.0 m². Standard crop management and agronomic practices (irrigation, fertilization, weed control) were followed uniformly across all sowing dates to ensure that temperature timing was the primary differing factor.

Climatic Conditions: The field experiment was conducted during the *Rabi* season of 2022–23 at MPKV, Rahuri, under typical dryland agro-climatic conditions. The growing period, spanning from November to May, began with relatively cool and dry weather that gradually transitioned to warmer conditions as the season progressed. During this period, the average maximum temperatures ranged from 29°C in November to 38°C in April and May, while the minimum temperatures increased from 19°C to 26°C, respectively. In January and February, corresponding to the tillering and flowering stages, the crop experienced optimal temperatures, with maximums of around 29–32°C and minimums of 18–20°C, which were favourable for both vegetative and reproductive development. Notably, in early sown plots (November sowing), the grain-filling stage occurred prior to the rise in temperature, helping the crop avoid exposure to terminal heat stress. In contrast, later sown treatments entered reproductive and grain-filling stages during March and April, when temperatures reached 35–38°C, increasing the likelihood of heat-induced stress. All sowings were carried out at the same location under uniform agronomic practices, with each date considered a separate treatment.

Table 1. List of genotypes and their pedigree

S. No.	Genotype	Pedigree
1.	Trimbak	SERI 82 /3/ MRS/ JUP//HORK ‘S’
2.	HI 1605	BOW/VEE/5/ND/VG9144//KAL/BB/3/YACO /4/ CHIL/6/C ASKOR/3/CROC_1/AE.SQ(224)// OPATA /7/PASTOR/MILAN/KAUZ/3/BAV92
3.	Phule Samadhan	NIAW 34 X PBW 435
4.	PDKV Sardar	3 SSN-DF-99-186-Sel
5.	RAJ 3765	HD 2402/VL639
6.	MACS6222	HD 2189*2//MACS 2496
7.	MACS2496	SERI”S”
8.	MACS2478	CS/Th.sc//3*PVN/3MIRLO/BUC/4/MILAN/5/TILHI
9.	HI1633	GW 322/PBW 498
10.	DBW107	TUKURU/INQLAB
11.	NIAW34	CNO 79/PRL “S”
12.	PBS02	-
13.	UP3088	-
14.	NEQ-2021-1	-
15.	DBW71	PRINIA/UP2425
16.	QLD124	-
17.	INDB2121	-
18.	PH132	-
19.	NIAW3170	SKOLL / ROLF 07
20.	HD2932	KAUZ/STAR//HD 2643
21.	RWP1174	-
22.	CG2117	-
23.	IC336999	BPS-2462
24.	IC416119	PAU –539
25.	PBW771	-
26.	IC28584	-
27.	8499246	ROLF07*2/SHORTENED SR26 TRANSLOCATION/ /SUP152/ KENYA SUNBIRD/3/ SWSR22T.B./KACHU//2*KACHU
28.	8485982	KACHU/SAUAL/3/TACUPETO F2001/BRAMBLING// KIRITATI/ 4/KACHU*2/3/ ND643//2*PRL/2*PASTOR
29.	IC0401999	DL 788-2/RAJ 3717
30.	IC445348	-

Observations Recorded: Data were collected on a total of 14 quantitative traits related to phenology, yield and grain quality. The phenological traits were days to first flowering (heading) and days to physiological maturity, recorded on a plot basis as the number of days from sowing to when ~50% of plants reached each stage. The morphological and yield-contributing traits (measured from five representative plants or on a plot yield sample) included plant height, number of productive tillers per plant, spike length (cm), number of spikelets per spike, number of grains per spike, 1000-grain weight (g) and grain yield per plant (g). Near-Infrared Reflectance Spectroscopy (NIRS) was employed to estimate grain protein, zinc, iron, and total carotenoid content (expressed as β-carotene equivalents), utilizing pre-calibrated models derived from standard wet chemistry techniques. This approach enables rapid and non-destructive analysis of grain composition and is widely recognized in cereal quality assessment (Delwiche, 1995; Williams and Norris, 1987). Gluten strength, indicative of bread-making quality, was assessed through the SDS sedimentation test, following the modified procedure described by Axford *et al*. (1978). The total carotenoid content of the grain was determined spectrophotometrically at 450 nm and expressed as β-carotene equivalents (ppm) following standard protocols (Abdel-Aal *et al.,* 2007; Hentschel *et al.,* 2002). Because NIRS calibration was based on β-carotene standards, the values represent total carotenoids rather than true β-carotene concentration.

Data Analysis: For each sowing environment (S_1 , S_2, S_3), the multi-trait data of 30 genotypes were subjected to Mahalanobis' D² statistic to assess genetic divergence (Mahalanobis, 1936). Prior to analysis, trait data were mean-centered and standardized (to mean zero and unit variance) to eliminate scale differences. The pooled within-group variance-covariance matrix was used to compute D² distances between all possible genotype pairs. A total of *N(N–1)/2* pairwise distances were obtained for *N=30* genotypes (thus 435 pairwise D² values per environment). To group the genotypes into clusters based on these distances, Tocher's method of non-hierarchical clustering was applied, as described by Rao (1952). The cluster membership for each sowing time was computed separately. The intra-cluster distance was calculated as the √D² within each cluster (for clusters with more than one genotype), and inter-cluster distances were similarly derived for all pairs of clusters using the cluster centroid values.

Cluster Means and Trait Contributions: For each sowing time, the mean values of all traits were calculated for each cluster to facilitate interpretation of cluster characteristics. Key cluster means (e.g., for grain yield, tiller number, grains/spike, 1000-grain weight, and quality traits) were compared to identify clusters excelling in specific traits. Additionally, the proportional contribution of each trait to the total divergence was estimated by the method proposed by Singh and Chaudhary (1985). In this method, for each environment the number of times a given trait had the highest contribution to the D² distance among all trait components for a particular genotype pair is tallied (i.e., count of "times ranked first" for each trait). The percentage contribution of that trait is then calculated as: *(Number of times trait was first / Total number of genotype pairs) × 100*. This provides an intuitive measure of which traits most frequently dominated the genetic divergence between genotypes. Traits with 0% contribution never constituted the largest divergence for any pair, indicating relatively low differentiation in those traits. All analyses were performed using Indostat statistical software, and results were tabulated in detail for each sowing condition.

Significance Testing: Wilks' lambda (λ) criterion was used to test overall multivariate differences among the genotypes for each sowing time prior to clustering. The λ statistic was highly significant ($P<0.01$) in all three sowing environments, confirming that the 30 genotypes differed significantly when considering the combined set of traits. Univariate analysis of variance was also conducted for each trait (not presented in detail here), and the traits showing little to no genotypic variance in a given environment (e.g. days to maturity in S_1) correspondingly had negligible contributions in the divergence analysis.

RESULTS AND DISCUSSION

Clustering patterns across sowing times: The Mahalanobis D² analysis grouped the 30 wheat genotypes into 10 clusters under timely sowing (S_1), 11 clusters under late sowing (S_2), and seven clusters under very late sowing (S_3) (**Fig.1a,1b,1c. and Table 2**). Under optimal (S_1) conditions, clusters were relatively small e.g. Cluster I had 12 genotypes, Cluster II had 7, Cluster V had 4, and seven clusters were monogenotypic. In contrast, terminal heat stress (S_2) caused most genotypes to converge: 20 genotypes fell into Cluster I, five into Cluster II, and the remaining five clusters were singletons. This shift in clustering patterns indicates that heat stress tended to homogenize performance (making genotypes behave similarly poor under stress) while only a few lines retained distinct, tolerant profiles. Notably, clusters did not track pedigree or origin, confirming that divergence was driven by trait differences rather than geographic source.

Inter-and intra-cluster distances: In S_1, only Clusters I, II and V had multiple genotypes, with intra-cluster √D² of 9–13 (largest in Cluster V, √D² ≈12.8) (**Table 3**). Even the smallest inter-cluster distance (8–11) exceeded all intra-distances, confirming well-separated clusters. In S_2, two clusters had multiple members (Cluster I with 17 genotypes, √D² ≈10.7; Cluster IV with 4 genotypes, ≈9.95) and all others were singleton (intra-distance=0). The maximum inter-cluster D² in S_2 was 26.41 (between Clusters XI and X), higher than in S_1, reflecting that some genotypes were extremely divergent under moderate heat. Under severe stress (S_3), Clusters I (20 genotypes, √D² ≈10.5) and II (5 genotypes, ≈10.6) had the only non-zero intra-cluster distances, while all other clusters were singletons. The largest inter-cluster distance in S_3 was 25.56 (between Clusters VII and III). Overall, the largest inter-cluster separations occurred under late/very late sowing. Such large D² values (many >20) imply great genetic divergence. Hybridisation of genotypes from these divergent clusters is expected to produce high heterosis and broad transgressive variation. For example, Cluster VII (a singleton outlier under heat) was highly divergent from multiple other clusters, making its genotype a candidate for hybridization with high-yield clusters to combine complementary traits.

Cluster trait means and heat tolerance: S_1 (Timely sowing): Cluster means (**Table 4a**) reveal that under optimal conditions, genotypes varied moderately. Clusters I and II, the largest groups, flowered and matured in mid-range (~63–65 days to flowering, ~123–125 days to maturity). Cluster II was the highest-yielding (8.43 g/plant), driven by outstanding spike fertility (19.8 spikelets and 53.4 grains per spike). Cluster X, by contrast, contained a very tall genotype (104 cm) with the heaviest grains (1000-grain weight 53.46 g), yielding 8.00 g despite only moderate grain number. Low-yield clusters (e.g. III, IX: ~5.5 g) had weak yield components (fewer grains/spike). In quality traits, clusters diverged: Cluster X had the highest gluten strength (sedimentation 40.5 mL) and among the highest Zn (46–47 ppm), while Cluster VII

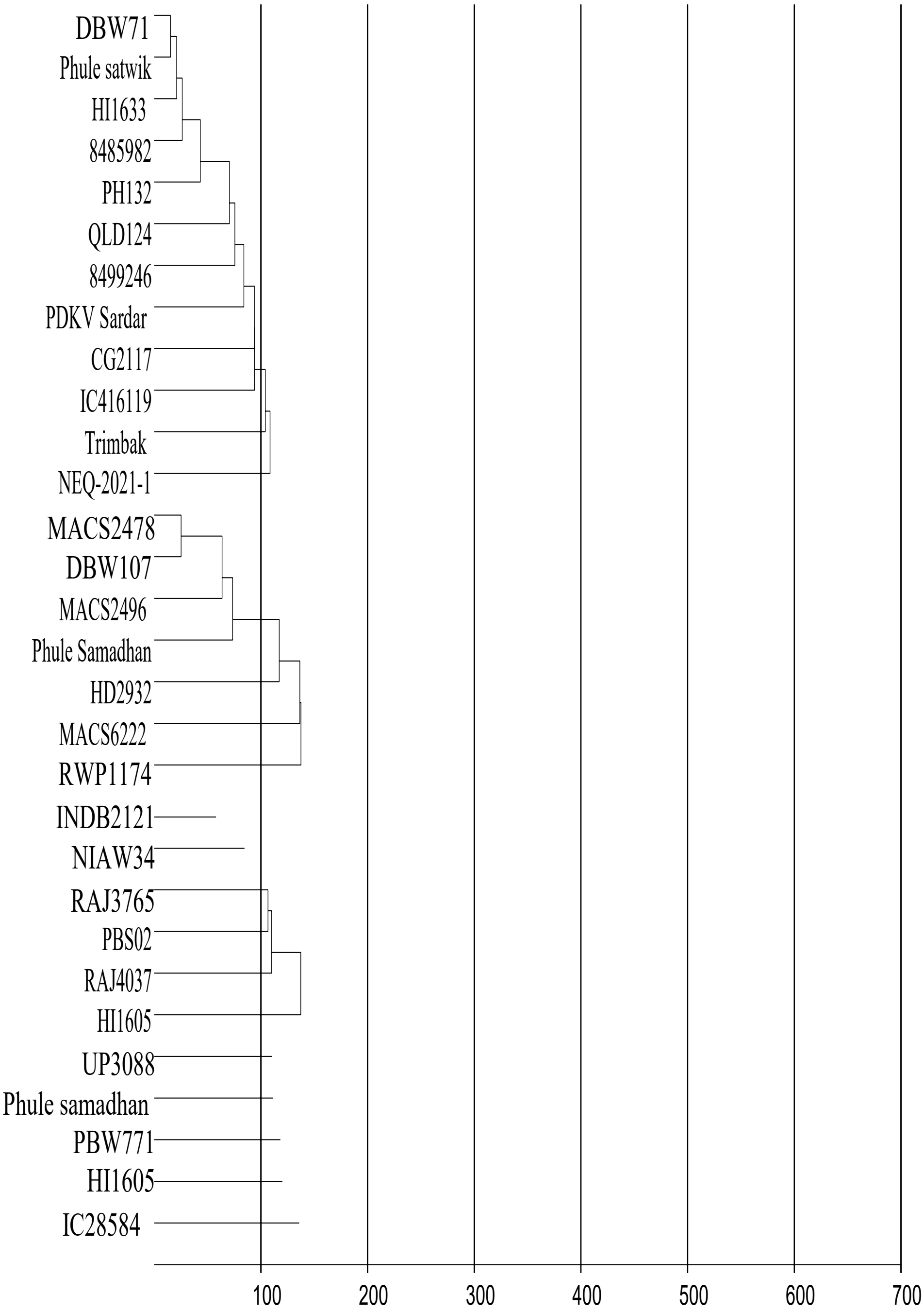

Fig.1a. Clustering of wheat genotypes sown during 15th November 2022 by using Tocher method

(a singleton) had the highest protein (12.91%). Thus, under S_1 some clusters excelled in yield (Cluster II), others in grain weight or nutritional quality (Clusters X, V, VII).

S_2 (Late sowing): Heat stress was observed to reduce overall means. Flowering and maturity occurred ~5–7 days earlier (mean 58–63 days to flower, 118–123 days to maturity). Clusters VIII and XI flowered earliest (~58–60 days). Grain yield per plant in S2 ranged 4.50–7.75 g: Cluster V was highest (7.75 g) and Cluster II was second (7.00 g). These clusters (each small groups) maintained superior spike fertility under heat (Cluster V: 20.0 spikelets, 56.5 grains per spike; Cluster II: 17.5 and 55.3 grains). In contrast, the large Cluster I (17 genotypes) averaged 5.63 g, reflecting typical susceptibility, and

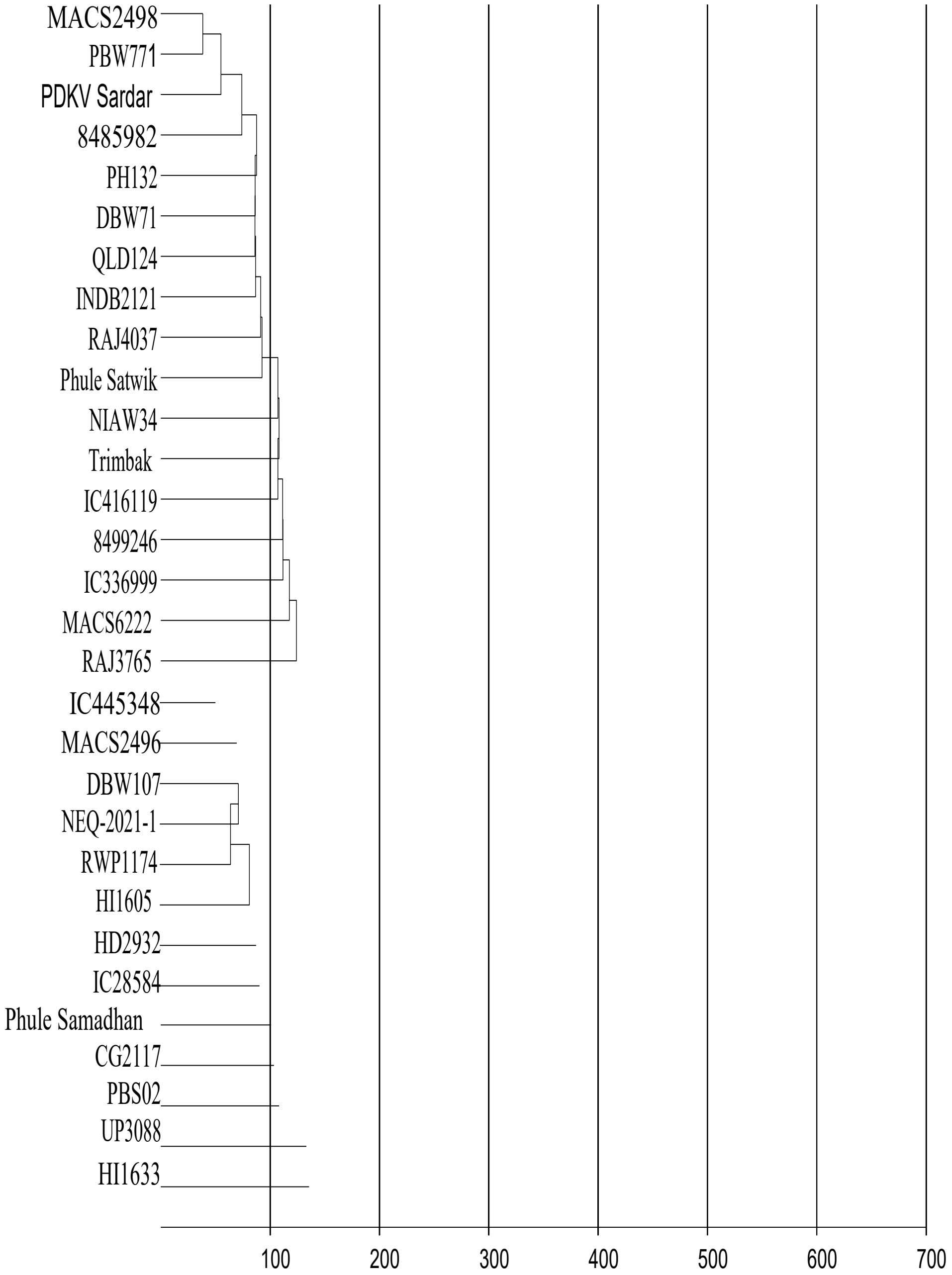

Fig.1b. Clustering of wheat genotypes sown during 15th December 2022 by using Tocher method

Cluster VII was lowest (4.50 g). Plant height tended to decrease, and tiller number was slightly reduced; interestingly, Cluster III produced 6.5 tillers (highest) helping it achieve a decent yield. In quality traits, grain protein was slightly higher (clusters V and IX at ~12.9%), and many clusters showed increased sedimentation. In particular, Cluster XI (singleton) had an exceptionally high sedimentation (55.5 mL) and the highest Zn (49.3 ppm) under stress, although its yield was low. Clusters V and II maintained moderate Zn (~40 ppm) with >12% protein. Overall, Cluster V (S_2) combined the best yield with good quality (high spike fertility, moderate protein, good sedimentation, decent Zn/Fe) (**Table 4b**).

S_3 (Very late sowing): Severe stress further shortened phenology and depressed yield. Cluster V genotypes flowered and matured earliest (54.5 and 114.5 days), demonstrating an escape strategy. Grain yield plummeted to 3.75–5.53 g: Cluster II now had the highest mean (5.53 g), with Clusters V (5.26 g) and VII (5.00 g) close behind. These yields were ~30–35% below their S2 values, underscoring the penalty of additional heat delay.

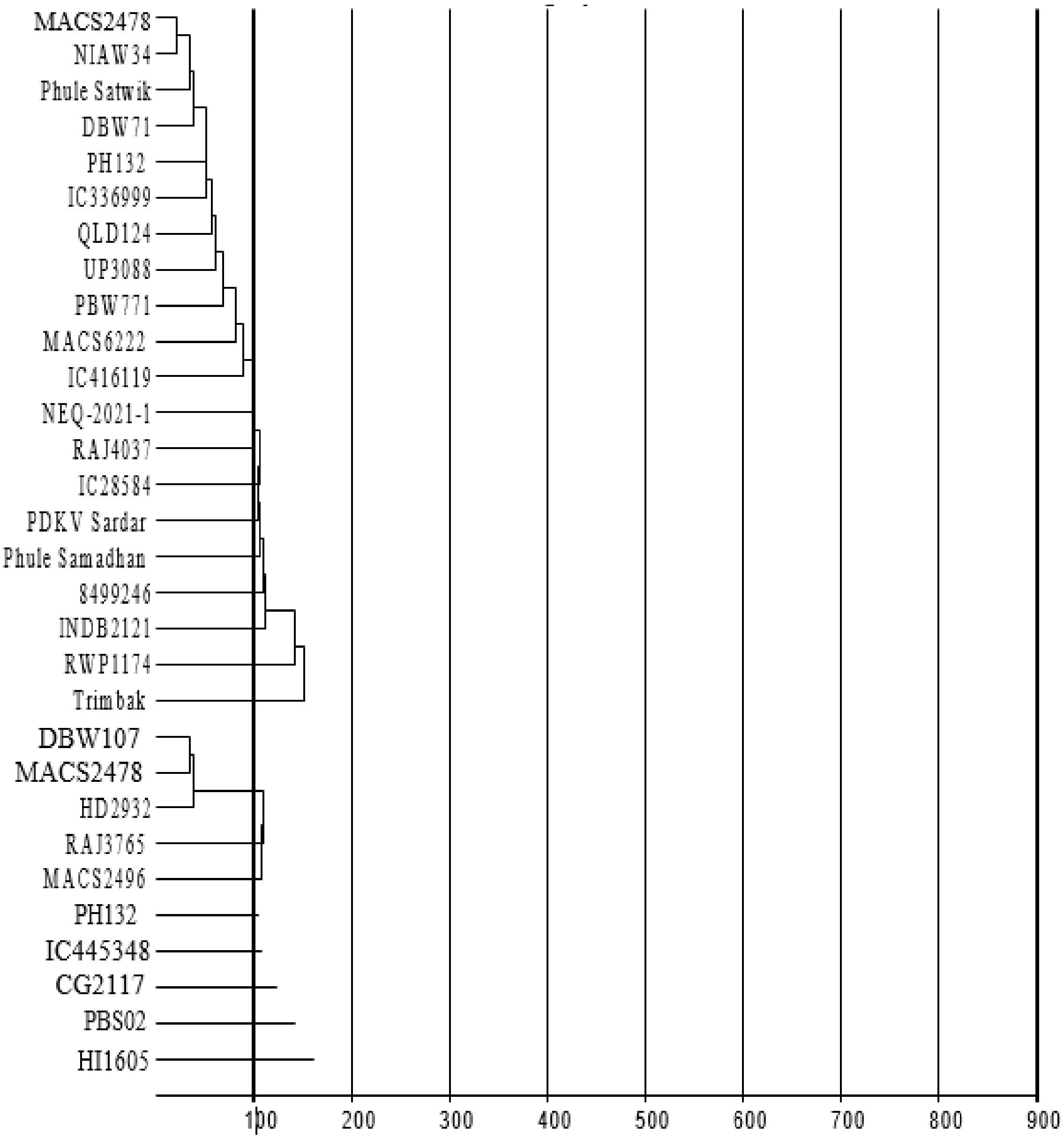

Fig.1c. Clustering of wheat genotypes sown during 15th January 2023 by using Tocher method.

The large Cluster I (bulk of genotypes) averaged only 4.18 g, reflecting widespread susceptibility. The tolerant clusters (II, V, VII) shared traits of earliness, higher tiller numbers, and greater grains per spike, enabling them to outyield others under stress. For example, Cluster VII had the most grains per spike (39.9) and Cluster II was close behind (38.3). Grain size became more variable: Cluster VI (singleton) had the largest grains (1000-weight 35.39 g) and the highest Zn (54.2 ppm), but very low yield due to poor tillering (3.7 tillers). In contrast, Cluster II produced smaller grains (32.36 g) but compensated with more spikes and grains. Clusters V, II and VII (the top-yielders) all showed this strategy of sacrificing grain weight for spike productivity. Overall, the cluster means show that heat-tolerant genotypes (e.g. those in Cluster V of S_2 and Cluster II of S_3) consistently combined earlier phenology, higher tillering and spike fertility, and maintained yield under stress. Susceptible genotypes (e.g. most in Cluster I, S_3) were characterized by late maturity and drastic tiller/spike abortion under heat (**Table 4c**).

*Trait contributions to divergence:*Under S_1, grain iron (Fe) content was by far the largest contributor (33.6%), followed by Zn (13.6%) and protein (13.0%); grain yield was only fourth (10.3%) (**Table 5**). In other words,

Table 2. Distribution of wheat genotypes into different clusters based on D^2 values across three sowing dates (November 15th, December 15th and January 15th, 2022)

Sowing time	Cluster No	Number of genotypes	Name of genotypes
Nov. 15th	I	12	DBW71, Phule Satwik, HI1633, 848598, PH132, QLD124, 8499246, PDKV Sardar, CG2117, IC416119, Trimbak, NEQ-2021-1
	II	7	MACS 2478, DBW107, MACS2496, IC445348, HD2932, MACS6222, RWP1174
	III	1	INDB2121
	IV	1	NIAW34
	V	4	RAJ 3765, PBS02, RAJ4037, IC336999
	VI	1	UP3088
	VII	1	Phule Samadhan
	VIII	1	PBW771
	IX	1	HI1605
	X	1	IC28584
Dec 15th	I	17	MACS2478, PBW771, PDKV Sardar, 8485982, PH132, DBW71, QLD124, INDB2121, Phule Satwik, NIAW34,Trimbak, IC416119, 8499246, IC336999, MACS6222, RAJ3765,RAJ4037
	II	1	IC445348
	III	1	MACS2478
	IV	4	DBW107, NEQ-2021-1, RWP1174, HI1605
	V	1	HD2932
	VI	1	IC28584
	VII	1	Phule Samadhan
	VIII	1	CG2117
	IX	1	PBS02
	X	1	UP3088
	XI	1	HI1633
Jan 15th	I	20	MACS2478, NIAW34, Phule Satwik, DBW71, HI1633, IC336999, QLD124, UP3088, PBW771, MACS6222,IC416119, NEQ-2021-1, RAJ4037, IC28584, PDKV Sardar, Phule Samadhan, 8499246, INDB2121, RWP1174, Trimbak
	II	5	DBW107, 8485982, HD2932, RAJ3765, MACS2478
	III	1	PH132
	IV	1	IC445378
	V	1	CG2117
	VI	1	PBS02
	VII	1	HI1605

Table 3a. Average intra (bold) and inter cluster distance (D^2) values for ten clusters for thirty wheat genotypes sown during November 2022

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X
I	**9.74**	20.47	12.97	11.88	19.28	17.71	13.87	14.41	18.27	14.29
II		**11.71**	19.97	19.93	14.80	14.36	21.07	15.79	14.08	22.10
III			**0.00**	12.67	14.43	16.28	15.10	11.29	21.58	19.45
IV				**0.00**	18.96	18.36	16.82	10.88	20.59	20.87
V					**12.82**	15.48	19.81	14.89	19.27	22.70
VI						**0.00**	20.48	14.77	13.60	18.00
VII							**0.00**	16.24	19.45	18.35
VIII								**0.00**	17.31	20.92
IX									**0.00**	18.94
X										**0.00**

Diagonal: Intra cluster and Above diagonal: Inter cluster D^2 values

Table 3b. Average intra (bold) and inter cluster distance (D^2) values for eleven clusters in thirty wheat genotypes sown during 15th December 2022

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	**10.71**	13.20	12.58	13.33	14.49	12.55	13.99	14.11	14.36	15.96	16.12
II		**0.00**	9.96	13.44	11.96	12.95	18.84	17.28	18.37	13.89	19.69
III			**0.00**	17.52	15.63	12.82	19.04	17.83	12.64	14.55	18.40
IV				**9.95**	14.02	13.18	15.15	14.75	20.33	17.64	18.00
V					**0.00**	12.10	15.14	18.29	18.95	14.60	22.36
VI						**0.00**	15.66	10.28	17.51	13.34	17.74
VII							**0.00**	15.39	19.17	19.25	19.02
VIII								**0.00**	19.83	18.53	16.35
IX									**0.00**	21.56	17.72
X										**0.00**	26.41
XI											**0.00**

Diagonal: Intra cluster and Above diagonal: Inter cluster D^2 values

Table 3c. Average intra (bold) and inter cluster distance (D^2) values for seven clusters in thirty wheat genotypes sown during 15th January 2023

Clusters	I	II	III	IV	V	VI	VII
I	**10.52**	13.81	13.44	14.83	14.00	15.37	17.46
II		**10.64**	17.10	16.00	17.58	14.50	21.27
III			**0.00**	20.66	21.19	16.20	25.56
IV				**0.00**	14.30	15.51	16.12
V					**0.00**	20.64	14.45
VI						**0.00**	23.94
VII							**0.00**

Diagonal: Intra cluster and Above diagonal: Inter cluster D^2 value

under optimal conditions differences in micronutrient and quality traits (especially Fe) dominated the genetic distances, reflecting the presence of high-Zn/Fe lines that stood apart. Under moderate heat (S_2), the pattern shifted: grain Zn rose to 20.2% contribution, yield to 14.7%, sedimentation (quality) to 14.0%, and grains per spike to 13.8%. In S_2, phenological traits (flowering, maturity) contributed negligibly, as all genotypes flowered early. In severe heat (S_3), Zn content dominated (29.4%), followed by 1000-grain weight (13.8%), Total carotenoids (11.7%) and Fe (9.4%). Importantly, grain yield's share fell to only ~5.3% in S_3, likely because nearly all yields collapsed to low values under extreme stress. Overall, these trends show that divergence drivers shifted from quality/micronutrient traits in normal conditions to yield components and Zn under stress. Grain Zn content was consistently a major differentiator in all environments, peaking under stress. Thus, divergence analysis highlights that under late sowing, maintenance of spike fertility and kernel weight become critical, whereas under normal conditions breeders might focus more on nutritional quality.

The wide genetic divergence observed among the genotypes indicates strong scope for improving heat tolerance, yield, and grain quality through strategic hybridization. Large inter-cluster distances suggest that crosses between highly divergent clusters such as high-yielding, heat-tolerant lines from Cluster V (late sowing) and nutrient-dense, quality-rich lines from Cluster XI (late sowing) can produce heterotic and transgressive segregants combining heat resilience and nutritional traits.

Clusters that consistently maintained higher yields under late and very late sowing (e.g., Cluster V in S_2 and Cluster II in S_3) should be prioritized as donor sources for terminal heat tolerance. Clusters rich in grain quality (Cluster XI, S_2; Cluster VI, S_3) provide complementary traits such as higher Zn, Fe, and gluten strength and can be incorporated into high-yielding backgrounds.

Trait-wise, productive tillers, grains per spike, and thousand-grain weight were the most reliable indicators of heat tolerance, while zinc content remained a key

Table 4a. Mean values of 10 clusters for 12 characters in 30 wheat genotypes sown on 15th November 2022

Cluster Number	Days to First flowering	Days to maturity	Plant height (cm)	Number of Productie tillers	Spike length (cm)	Number of spikelets per spike	Number of grains per spike	Test weight (g)	Protein (%)	Sedimentation value (ml)	Zn (ppm)	Fe (ppm)	Total carotenoids (β-carotene equivalents, ppm)	Seed yield per plant (g)
I	63.04	123.08	88.34	3.71	8.90	17.08	45.14	44.41	11.80	37.25	41.99	37.74	11.55	6.14
II	64.71	124.57	82.81	6.45	10.76	19.79	53.37	42.45	11.85	34.61	43.35	37.66	11.29	8.43
III	67.00	124.00	82.50	3.80	8.03	15.00	39.60	40.92	12.33	33.50	47.21	37.14	12.40	5.50
IV	66.00	122.00	82.00	3.20	8.48	17.50	44.50	44.30	11.56	31.50	46.54	39.02	9.50	6.25
V	64.00	123.00	81.65	5.49	9.84	18.00	46.74	42.31	12.35	36.85	47.54	34.42	13.25	7.81
VI	66.00	126.00	97.20	4.80	8.00	16.50	44.10	45.43	11.73	26.80	40.70	31.85	9.29	7.25
VII	64.00	127.00	75.20	4.50	8.57	18.00	47.00	44.77	12.91	42.00	37.40	38.86	11.29	6.00
VIII	65.00	129.50	78.50	4.50	9.55	14.50	45.50	46.20	11.61	37.00	43.49	38.34	11.22	6.50
IX	68.00	124.00	84.10	4.90	8.48	18.00	49.20	37.88	11.23	34.50	32.40	35.37	12.72	5.50
X	63.00	123.00	103.80	5.45	11.09	17.00	50.10	53.46	11.89	40.50	40.54	37.90	10.59	8.00

Table 4b. Mean values of 11 clusters for 12 characters in 30 wheat genotypes sown on 15th December 2022

Cluster Number	Days to First flowering	Days to maturity	Plant height (cm)	Number of Productie tillers	Spike length (cm)	Number of spikelets per spike	Number of grains per spike	Test weight (g)	Protein (%)	Sedimentation value (ml)	Zn (ppm)	Fe (ppm)	Total carotenoids (β-carotene equivalents, ppm)	Seed yield per plant (g)
I	61.00	121.03	78.05	4.08	8.38	15.85	40.67	40.06	12.32	41.81	46.66	39.63	10.21	5.63
II	61.00	119.00	84.60	4.75	10.15	17.50	55.30	35.70	11.87	39.70	47.88	40.98	10.89	7.00
III	61.00	121.00	81.05	6.50	10.26	16.50	46.90	36.08	12.05	40.50	52.21	41.63	10.46	6.50
IV	61.13	122.63	79.02	3.97	9.03	17.38	46.20	40.29	11.78	42.40	41.11	38.48	10.44	6.00
V	63.00	119.00	77.60	4.20	8.04	20.00	56.50	40.29	12.86	42.30	44.20	39.71	8.76	7.75
VI	60.00	120.00	89.40	5.34	9.09	17.00	46.60	44.66	12.18	43.20	44.66	39.21	8.88	6.25
VII	60.00	121.00	72.60	4.40	8.71	15.50	40.80	42.40	13.04	44.50	37.82	41.07	9.67	4.50
VIII	58.00	118.00	95.00	3.86	8.26	15.50	34.10	47.82	11.71	40.90	39.74	41.81	10.13	4.75
IX	62.00	123.00	73.40	4.90	5.75	15.50	38.20	39.29	12.89	46.85	52.67	39.32	12.56	5.00
X	63.00	122.50	93.30	5.00	9.80	17.50	44.40	36.65	12.60	33.05	45.27	36.89	8.62	6.00
XI	60.00	121.50	75.50	3.80	8.30	15.50	38.80	37.72	12.33	55.55	49.29	41.27	9.72	5.50

Table 4c. Mean values of seven clusters for 12 characters in 30 wheat genotypes sown on 15th January 2022

Cluster Number	Days to First flowering	Days to maturity	Plant height (cm)	Number of Productie tillers	Spike length (cm)	Number of spikelets per spike	Number of grains per spike	Test weight (g)	Protein (%)	Sedimen-tation value (ml)	Zn (ppm)	Fe (ppm)	Total carotenoids (β-carotene equivalents, ppm)	Seed yield per plant (g)
I	58.58	119.00	68.39	4.01	7.39	13.35	31.82	34.62	12.67	50.20	47.78	41.15	8.43	4.18
II	59.90	118.20	63.80	5.06	8.01	14.75	38.30	32.36	12.73	50.54	48.74	42.56	8.53	5.53
III	54.50	121.00	63.90	3.40	8.10	12.50	32.30	41.30	12.78	43.00	50.76	40.82	6.78	4.00
IV	59.50	116.00	71.70	4.25	5.90	11.50	36.80	24.68	12.31	43.60	49.23	42.47	9.56	3.75
V	54.50	114.50	69.80	5.39	6.25	14.50	33.20	33.30	12.22	48.25	41.90	43.24	9.10	5.26
VI	60.00	119.50	59.10	3.70	5.65	15.50	33.70	35.39	13.29	56.00	54.22	40.15	12.01	3.75
VII	60.00	119.50	78.50	4.30	8.38	14.50	39.90	31.15	12.26	52.00	43.01	36.72	12.04	5.00

Table 5. Percent contribution of 14 characters for divergence in S_1,S_2 and S_3

S. No.	Source	Contribution (%) during S_1	Contribution (%) during S_2	Contribution (%) during S_3
1	Days to first flowering	0.00	0.00	2.07
2	Days to maturity	0.00	0.92	0.00
3	Plant height	8.74	9.66	5.75
4	Number of productive tillers per plant	7.13	2.53	6.44
5	Spike length	0.00	1.15	6.21
6	Spikelets per spike	0.46	0.00	2.53
7	Grains per spike	1.15	13.76	0.92
8	Test weight	0.46	4.83	13.79
9	Protein	13.01	7.13	3.45
10	Sedimentation value	9.43	14.02	2.99
11	Zn	13.56	20.23	29.43
12	Fe	33.56	5.52	9.43
13	Total carotenoids (β-carotene equivalents)	2.07	5.52	11.72
14	Grain yield per plant	10.34	14.71	5.29

divergent trait even under stress. These findings support environment-specific selection: focusing on yield components under heat stress and on nutritional traits under normal conditions. Overall, the identified divergent clusters and their superior genotypes offer a practical roadmap for developing climate-resilient, high-yielding, and micronutrient-rich wheat varieties.

This study demonstrated substantial genetic divergence among 30 wheat genotypes evaluated under varying levels of terminal heat stress. Mahalanobis D² analysis formed 10, 11, and 7 clusters under timely, late, and very late sowing, respectively, indicating that heat stress reduces the distinctiveness among genotypes while highlighting a few stable heat-tolerant groups. Genotypes in Cluster V (late sowing) and Cluster II (very late sowing) consistently showed superior heat tolerance through early maturity, higher tillering, and greater spike fertility, along with desirable grain zinc and iron content. These genotypes represent strong donors for improving heat resilience and nutritional quality. Trait contribution analysis showed that nutritional traits such as zinc and iron dominated divergence under normal conditions, whereas yield components particularly grains per spike and test weight were more influential under heat stress. The results highlight the feasibility of simultaneously breeding for heat tolerance and micronutrient enrichment. Based on cluster means, specific genotypes like *HI 1605, RAJ 3765, MACS 6222, HD 2932,* and *PBW 771* (identified in diverse superior clusters) can be harnessed to develop breeding populations. The study also underscored that genetic divergence and geographic origin were unrelated, implying breeders should look across different origins for novel diversity.

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