Plant Archives Vol. 25, No. 1, 2025 pp. 476-482 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.no.1.071

CLUSTERING AND GENETIC DIVERGENCE ANALYSIS AMONG BREAD WHEAT GENOTYPES UNDER HEAT STRESS CONDITIONS

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Orchid id : 0009-0006-6862-325763

(Date of Receiving-11-12-2024; Date of Acceptance-13-02-2025)

ABSTRACT

A set of 205 bread wheat genotypes including 5 checks were evaluated in augmented design for genetic divergence in the Research area of Wheat and Barley Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar during *Rabi* 2022-23 and *Rabi* 2023-24 under late sowing conditions for exposing the genotypes to high temperatures. Observations were recorded for 23 different morpho-physiological and quality traits. The statistical analysis for genetic divergence was done using Mahalanobis D^2 statistics and clustering of genotypes was done using Ward's method. The genetic diversity analysis revealed the formation of eight clusters suggested the presence of wide genetic diversity among the 205 genotypes studied. Cluster VI had maximum number of genotypes *i.e.*, 37, while cluster-II had only 11 genotypes. Highest and lowest average intra-cluster distance was exhibited by cluster III and VIII respectively. Further, genotypes of clusters II and VIII exhibited maximum inter-cluster distance whereas lowest inter-cluster distance was observed between clusters I and VI. Hence, crossing of genotypes from cluster II with that from cluster VIII would produce desirable recombinants in segregating generations with improvement in traits to enhance the yield. This study provided valuable insights into the extent of genetic diversity present in the evaluated materials, offering a foundation for developing superior genotypes with enhanced yield potential and improved physiological resilience to heat stress conditions.

Key words : Cluster analysis, D^2 statistic, Genetic diversity, Heat stress, Wheat.

Introduction

Wheat (*Triticum aestivum* L.), a cereal grass of Poaceae family, is the world's largest staple cereal crop that holds immense global importance after rice for human consumption and livestock feed (Braun *et al.*, 2010). Wheat is commonly known as the "King of Cereals" due to its remarkable adaptability to diverse agroclimatic conditions, high nutritional value, high productivity and the prominent position it holds in the food grain trade (Bhanu *et al.*, 2018; Mitra *et al.*, 2024). It serves as a primary source of food and energy, offering a wide range of end-use products such as chapati, bread, biscuits and pasta, while also providing valuable fodder for animals.

However, wheat productivity is increasingly threatened by several biotic and abiotic stresses and among these stresses, rising global temperature poses a major challenge to wheat cultivation (Fernie *et al.*, 2022; Anonymous, 2022). In India, high-temperature stress (>30°C) at the time of grain filling stage is a major constraint in increasing productivity of wheat in tropical and sub-tropical countries (Rane and Nagarajan, 2004). Heat stress disrupts vital physiological and biochemical processes in wheat, impairing metabolic functions at all developmental stages and during the post-anthesis stage, it adversely affects the availability and translocation of photosynthates to developing kernels, as well as starch synthesis and deposition, ultimately reducing grain yield, grain weight and quality (Al-Ashkar *et al.*, 2020; Fernie *et al.*, 2022). An increase in temperature from 15–20 °C

(day/night) to 40–15 °C on the third day after anthesis has been reported to reduce wheat yield by up to 23%. This highlights the need to identify wheat genotypes capable of thriving under adverse environmental conditions (Belete *et al*., 2021; Ramya *et al*., 2017). Genetic diversity analysis is a powerful approach to identify superior donor genotypes for heat tolerance, providing a valuable resource for wheat improvement and breeding programs aimed at mitigating the impacts of heat stress in wheat.

Precise knowledge of the nature and extent of genetic divergence is crucial for plant breeders in selecting genetically diverse parents for targeted hybridization (Arunachalam, 1981). The genetic improvement of any crop largely relies on the availability and utilization of genetic diversity (Joshi and Dhawan, 1966). Various statistical methods, including D^2-statistics and hierarchical Euclidean cluster analysis, have been developed to assess genetic diversity. These methods quantify genetic divergence based on the similarity or dissimilarity of genotypes, considering the combined effects of multiple economically important traits. Cluster analysis, in particular, is a reliable approach for determining family relationships and evaluating the genetic distance among genotypes. Understanding genetic diversity for grain yield is critical to achieving diverse plant breeding objectives, such as enhancing yield, ensuring wide adaptability and improving desirable quality traits (Lal *et al*., 2009).

Hybridization followed by selection is a fundamental approach in wheat breeding. Parents' choice is the first step in plant breeding program through hybridization. In order to obtain transgressive segregants, genetic distance between parents is necessary (Joshi *et al*., 2004). Estimation of genetic distance is one of appropriate tools for parental selection in wheat hybridization programs. Appropriate selection of the parents is essential to be used in crossing nurseries to enhance the genetic recombination for potential yield increase (Jaiswal *et al.*, 2019). Therefore, it is essential to evaluate bread wheat genotypes based on morpho-physiological and quality traits across different sowing environments to identify stable genotypes with superior yield and heat tolerance.

Materials and Methods

The present experiment was conducted in Research area of Wheat and Barley Section, Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar during *Rabi* 2022-23 and *Rabi* 2023-24 under late sowing conditions for exposing genotypes to high temperatures. The experimental material comprising of 205 bread wheat genotypes, including five checks (DBW 222, HD 3086, WH 1105, HD 3059 and WH 1124) was evaluated in augmented design involving eight blocks, each containing 25 test genotypes and 5 checks. Each plot comprised of one genotype with three rows of 2 m length. Further, row -to-row and plant-to-plant distance were kept at 20 cm and 10 cm, respectively. Standard agronomic practices recommended for wheat cultivation were followed to ensure healthy crop growth. For data collection, five representative plants were randomly selected and tagged in each plot to record observations for morpho-physiological and quality traits. Pooled mean values across the two seasons were used for genetic divergence analysis. Mahalanobis' D^2 statistics (Mahalanobis, 1936) were employed for estimating genetic divergence, and clustering of genotypes was performed using Ward's Minimum Variance Method (Ward, 1963).

Results and Discussion

The success of any breeding program relies heavily on the extent of variability present within the breeding population. During selection, useful variations might remain untapped if not identified by the breeder. Therefore, assessment of the variability is crucial for the identifying genotypes capable of generating further variability. In the present investigation, experimental material exhibited a high degree of variation for all the studied traits. These findings are consistent with the results reported by Ajayi *et al.* (2022), Pandey *et al.* (2022) and Singh *et al.* (2021).

Genetic diversity analysis

Crossing diverse genotypes is a highly effective method in order to generate variation in crops. Grouping of genotypes facilitates the identification and selection of suitable diverse parents for crossing program. To achieve this, cluster analysis is useful to classify genotypes into clusters, ensuring that genotypes within the same cluster share similar traits, while clusters remain distinct from each other. In this study, Ward's D^2 cluster analysis, based on D^2 values and Bayesian Information Criterion (BIC), was used to assess genetic divergence across morpho-physiological and quality traits, grouping 205 bread wheat genotypes into eight clusters (Fig. 1).

Cluster analysis

A total of 205 bread wheat genotypes were categorized into eight clusters based on the degree of genetic divergence among the genotypes (Table 1, Figs. 3 and 4). Highest number of genotypes belonged to cluster VI (37) followed by cluster V (36), cluster III (35), cluster I (29), cluster VIII (21), cluster IV (19),

Table 1 : Distribution of 205 bread wheat genotypes into different clusters using Ward's method.

Cluster	No. of genotypes	Genotype
I	**29**	DBW 222, HD 3086, DBW 14, DBW 16, HS 277, HD 1941, HD 1981, HD 1982, HD 2009, HD 2135, HD 2177, HD 2189, HD 2204, HD 2270, HD 2285, HD 2307, HD 2643, HD 2687, HD 2824, HP 1102, HS 207, HW 657, K 7903, K 9423, NP 715, NW 1014, NW 2036, PBN 142, WG 377
II	**11**	WH 1124, HD 3059, HD 2402, HW 2045, HW 517, JWS 17, K 65, K 7410, PBW 65, UP 115, UP 2425
III	**35**	WH 1105, HD 2733, HI 977, HPW 184, HPW 89, HS 1097-17, HYB 65, K 88, KSML 3, MACS 2496, MLKS 11, NP 771, NP 799, NP 809, NP 836, PBW 138, PBW 226, PBW 343, PBW 373, PBW 396, PBW 443, PV 18, Raj 2184, K 9351, Raj 3077, Raj 821, TAWA 267, UP 215, UP 2338, UP 368, VL 616, VL 738, VL 832, WH 1025, WL 2265
IV	**19**	C 306, HI 1500, HI 1612, HW 2004, HY 12, HY 5, K 816, NARMADA 112, NP 111, NP 114, NP 165, NP 4, NP 761, NP 818, NP 839, NP 890, PBW 175, SONORA 64, UTKALIKA
V	**36**	HD 2236, HD 2327, HD 2501, HI 1418, WL 410, HP 1744, HP 1761, HS 1138-6-4, J 405, K 8020, K 9533, KRL 19, KRLI 4, LOK 1, NIAW 301, NIAW 34, NP 101, NW 1067, PBN 51, PBW 120, PBW 154, PBW 509, PBW 54, Raj 4037, SKW 196, SONALIKA, UP 1109, UP 2565, UP 262, VL 421, VL 802, VL 804, WH 157, WH 542, WH 771, WL 1562
VI	**37**	HD 2278, HD 2281, HD 2380, HD 2985, HD 2781, HD 2833, HD 2851, HD 2864, HDR 77, HI 1454, HS 86, HP 1731, HS 295, HS 365, HS 420, HUW 12, HUW 37, HUW 55, J 1-7, K 9162, KSHIPRA, NARMADA 4, NP 120, NP 710, NP 770, PBW 12, Raj 1972, RSP 561, UP 2003, UP 2121, UP 2382, VIDISHA (DL 788-2), VL 401, VL 404, WG 357, WH 147, WL 711
VII	**17**	HI 784, HPW 147, HS 375, HUW 206, HUW 213, HW 741, J 24, K 53, K 78, K 8434, K 8962, K 9006, K 9644, NP 792, NP 832, Raj 1482, VL 829
VIII	**21**	HP 1493, K 8027, MANDAKINI, NI 345, NI 5439, NP 12, NP 52, NP 718, NP 721, NP 745, NP 823, NP 824, NP 825, PBW 1ZN, Raj 1114, RIDLEY, RS 31-1, S 331, SAFEDLERMA, SHARBATI SONORA, WH 283

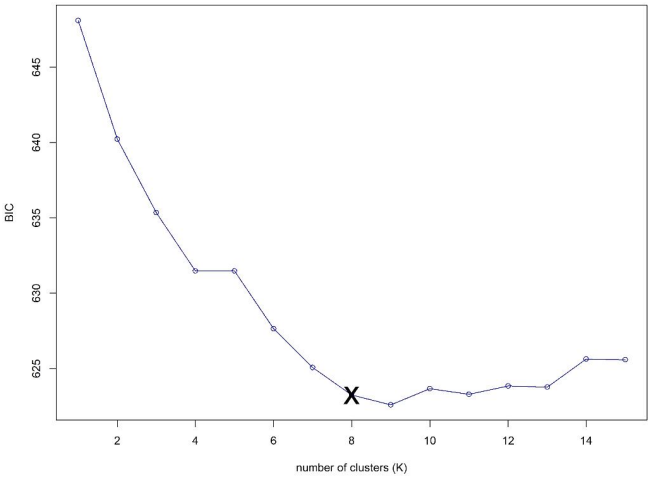

Fig. 1 : Detection of number of clusters based on BIC for grouping of bread wheat genotypes.

cluster 17) whereas lowest number of genotypes belonged to cluster II (11). Divergence analysis revealed that genotypes related by their place of origin have shown a tendency to group in the same cluster to some extent, which may be due to dependence upon the directional selection pressure.

Average intra and inter cluster distances

The average intra and inter-cluster distances (Table 2) were calculated to determine the genetic relationship among the genotypes within a cluster and between different clusters. The highest average intra-cluster was obsereved by cluster III (807.64) followed by cluster IV (649.30), cluster VII (589.04), cluster VI (577.74), cluster V (564.47), cluster II (506.18), cluster I (476.21) and cluster VIII (454.39). Higher intra-cluster distances indicated greater genetic diversity among the genotypes within these clusters compared to clusters with lower intra-cluster distances. Heatmap showing dissimilarity index on the basis of Euclidean distances among 205 bread wheat genotypes is depicted in Fig. 2.

Inter-cluster distance is the main criterion for selecting the genotypes using D^2 statistics (Khare *et al*., 2015). In this study, it was observed that the genotypes of clusters II and VIII exhibited maximum divergence (1019.04), followed by the genotypes of clusters II and VI (997.66), II and IV (960.53), II and V (946.54), II and VII (931.05) while the lowest inter-cluster distance was observed between clusters I and VI (541.23). Higher inter-cluster distances indicated greater genetic diversity among the

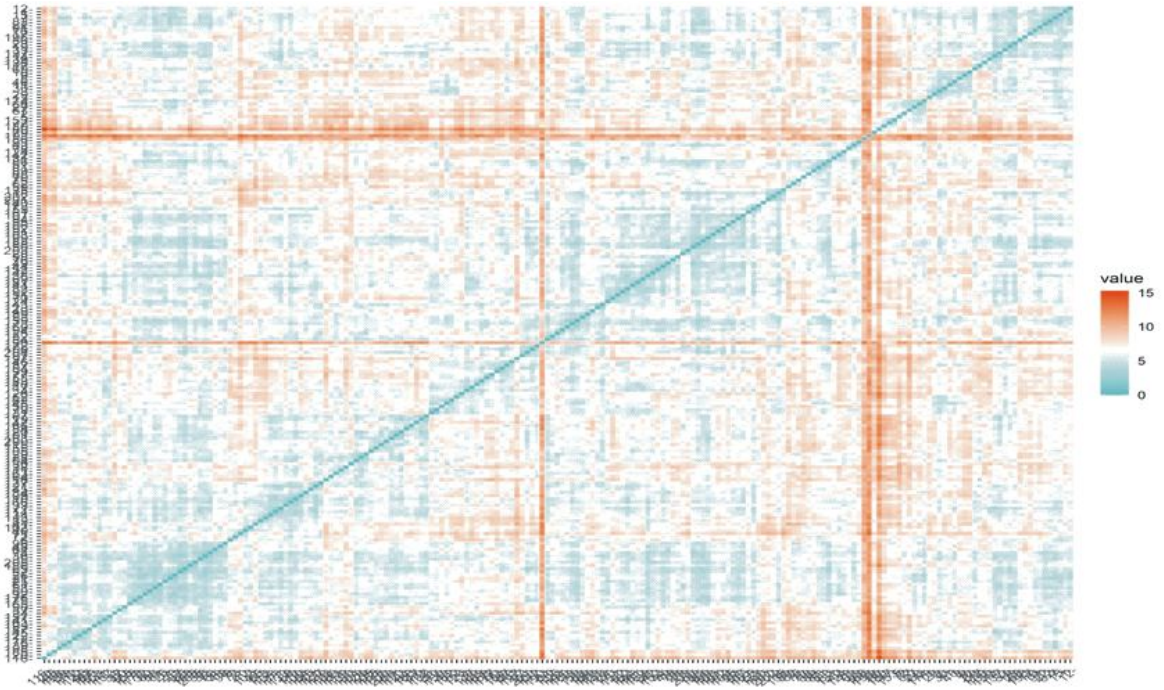

Fig. 2 : Heatmap showing dissimilarity index on the basis of Euclidean distances among 205 bread wheat genotypes.

Table 2 : Average inter-cluster and intra-cluster (diagonal) distances among different clusters for 205 bread wheat genotypes.

Cluster	I	II	III	IV	V	VI	VII	VIII
I	**476.21**							
II	930.10	**506.18**						
III	825.45	915.62	**807.64**					
IV	618.94	960.53	856.09	**649.30**				
V	548.44	946.54	841.60	734.72	**564.47**			
VI	541.23	997.66	887.46	677.02	612.66	**577.74**		
VII	589.25	931.05	825.98	820.27	604.61	650.21	**589.04**	
VIII	561.70	1019.04	910.99	700.37	635.23	599.44	674.07	**454.39**

genotypes within these clusters. Notably, genotypes in clusters II and VIII exhibited significant divergence, highlighting their potential for producing a higher number of superior hybrids, recombinants, and transgressive segregants. The observed genetic divergence is influenced by several factors such as exchange of breeding material, genetic drift, natural selection, artificial selection, and geographical diversity.

In the similar way, Yasin *et al.* (2024) grouped 40 bread wheat lines into ten clusters to assess intra and inter-cluster distances by using D^2 analysis. Cluster X exhibited the highest intra-cluster distance, while appreciable inter-cluster distances were observed between Cluster VI and Cluster X followed by Cluster IX and Cluster X. Grain yield per plant contributed the most to total divergence followed by spike length, effective tillers per plant, 1000-grain weight and plant height. Santosh and Jaiswal (2024) screen 32 diverse bread wheat genotypes and grouped into six clusters. Cluster II contained the highest number of genotypes (11), while Cluster VI had only one genotype. Cluster V showed the highest intra-cluster distance, whereas Cluster VI had the lowest. The greatest inter-cluster distance was observed between Clusters III and VI, while the smallest was between Clusters IV and I. Chauhan *et al.* (2023b) divided 40 wheat genotypes into six clusters and cluster III found to have the highest number of genotypes followed by clusters V, IV, II, I and VI. Cluster VI had the highest intra-cluster distance (2.621) followed by clusters V (2.323) and IV. Clusters I and VI had the largest inter-cluster distance followed by clusters I and V and clusters I and IV. Furthermore, similar results were also reported by Singh *et al.* (2022), Majid and Dar (2020), Jaiswal *et al.* (2019), Kumar *et al.* (2019), Chauhan *et al.* (2023), Rani *et al.* (2023) and Chaudhary *et al.* (2022).

Table 3 : Cluster mean values of 23 morpho-physiological and quality traits for eight clusters of 205 bread wheat genotypes.

S. no.	Traits	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7	Cluster 8
1	DH	80.45	78.73	83.91	79.45	81.06	79.26	82.09	82.14
2	DA	83.88	82.05	87.44	82.63	84.17	82.62	85.29	85.71
3	DPM	120.84	122.94	120.94	119.91	121.08	119.82	122.85	122.55
4	PH	88.02	94.42	93.65	115.93	90.01	93.62	100.48	111.71
5	NET/M	98.44	97.74	92.27	97.79	89.44	89.85	90.73	87.36
6	SL	8.86	9.71	9.22	8.93	9.56	9.29	10.45	8.69
7	PL	34.76	36.84	34.62	40.69	34.58	33.16	36.78	33.36
8	NS/S	17.12	17.24	17.24	16.14	17.43	16.68	18.91	16.70
9	GFD	36.97	40.89	33.50	37.27	36.92	37.20	37.56	36.83
10	SW	2.47	2.81	2.42	2.41	2.62	2.38	2.70	2.38
11	NG/S	39.31	40.51	39.31	33.80	41.02	34.84	38.80	34.07
12	BY/P	1247.38	1797.91	1348.65	1269.35	1257.31	1219.99	1364.94	1172.51
13	GY/P	450.33	598.82	469.63	436.26	494.71	417.65	477.50	392.38
14	HI	36.43	33.42	35.07	34.86	39.61	34.33	35.30	33.52
15	TGW	37.32	40.22	38.05	39.42	39.46	36.12	35.13	35.43
16	CF	0.66	0.68	0.67	0.61	0.65	0.66	0.65	0.65
17	CC	39.38	39.25	37.55	35.42	38.80	36.29	37.69	32.81
18	CTD-I	5.74	7.41	8.22	7.57	7.63	7.64	7.44	7.42
19	CTD-II	4.51	5.23	6.23	5.68	5.89	6.03	5.68	5.62
20	CP	11.26	11.05	10.45	10.67	10.28	11.19	11.39	10.27
21	GC	28.21	30.27	26.28	27.92	25.13	28.65	28.29	24.90
22	HW	75.41	75.21	72.99	76.07	74.95	72.96	75.97	74.97
23	SC	703.38	704.86	705.89	706.87	707.88	706.47	699.56	715.07

DH: Days to 50% heading; **DA**: Days to anthesis; **DPM**: Days to physiological maturity; **PH**: Plant height (cm); **NET/M**: Number of effective tillers/meter; **SL**: Spike length (cm); **PL**: Peduncle length (cm); **NS/S**: Number of spikelets/spike; **GFD**: Grain filling duration (days); **SW**: Spike weight (g); **NG/S**: Number of grains/spike; **BY/P**: Biological yield/plot (g); **GY/P**: Grain yield /plot (g); **HI**: Harvest index (%); **TGW**: 1000-grain weight (g); **CF**: Chlorophyll Fluorescence (Fv/Fm); **CC**: Chlorophyll content (SPAD value); **CTD-I**: Canopy Temperature Depression at anthesis (°C); **CTD-II**: Canopy Temperature Depression at 15 days after anthesis (°C); **CP**: Crude Protein (%); **GC**: Gluten content (%); **HW**: Hectolitre weight (kg/hl); **SC**: Starch (mg/g).

Cluster means of different cluster for various traits

The cluster mean for each trait is presented in Table 3 indicating considerable differences among the clusters. Cluster I exhibited maximum cluster mean values for number of effective tillers/meter (98.44) and chlorophyll content (39.38) while minimum cluster mean values for plant height (88.02), canopy temperature depression at anthesis (5.74) and canopy temperature depression at 15 days after anthesis (4.51). Cluster II exhibited maximum cluster mean values for days to physiological maturity (122.94), grain filling duration (40.89), spike weight (2.81), biological yield/ plot (1797.91), grain yield/plot (598.82), 1000-grain weight (40.22), chlorophyll fluorescence (0.68) and gluten content (30.27) while minimum cluster mean values for days to 50% heading (78.73), days to anthesis (82.05) and harvest index (33.42). Cluster III exhibited maximum cluster mean values for days to 50% heading (83.91), days to anthesis (87.44), canopy temperature depression at anthesis (8.22) and canopy temperature depression at 15 days after anthesis (6.23) while minimum cluster mean value for grain filling duration (33.50). Cluster IV exhibited maximum cluster mean values for plant height (115.93), peduncle length (40.69) and hectolitre weight (76.07) while minimum cluster mean values for number of spikelets per spike (16.14), number of grains per spike (33.80) and chlorophyll fluorescence (0.61). Cluster V exhibited maximum cluster mean values for number of grains per spike (41.02) and harvest index (39.61). Cluster VI exhibited minimum cluster mean values for days to physiological maturity (119.82), peduncle length (33.16), spike weight (2.38) and hectolitre weight (72.96). Cluster VII exhibited maximum cluster mean values for spike length (10.45), number of spikelets/ spike (18.91) and crude protein (11.39) while minimum cluster mean values for 1000-grain weight (35.13) and starch content (699.56). Cluster VIII exhibited maximum

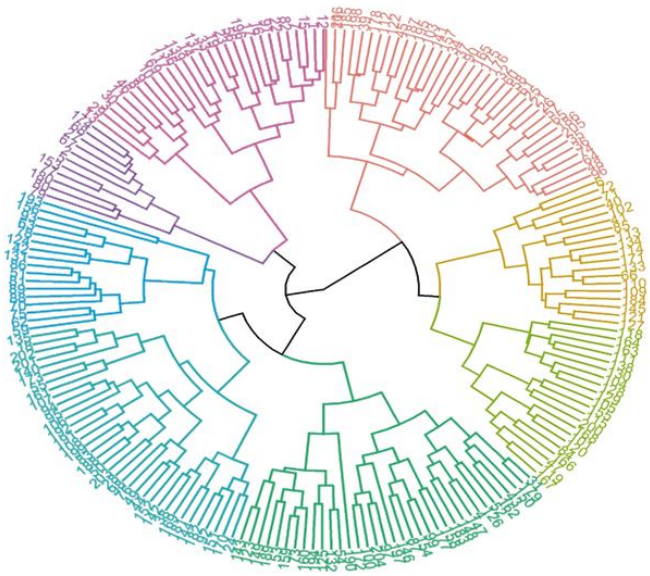

Fig. 3 : Circular dendrogram representing 205 bread wheat genotypes into 8 clusters.

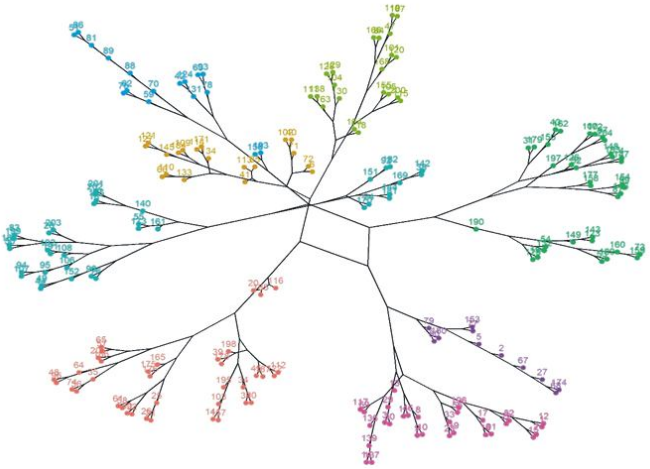

Fig. 4 : Phylogenetic dendrogram representing 205 bread wheat genotypes into 8 clusters.

cluster mean value for starch content (715.07) while minimum cluster mean values for number of effective tillers/meter (87.36), spike length (8.69), spike weight (2.38), biological yield/plot (1172.51), grain yield per plot (392.38), chlorophyll content (32.81), crude protein (10.27) and gluten content (24.90). Clusters exhibiting complementary trait performances serve as valuable sources for selecting parents in transgressive breeding programs.

Similarly, Singh *et al*. (2024) observed that cluster means of 13 characters were highest in clusters IV and V and lowest in clusters III and II. Yadav *et al.* (2023) reported that cluster VII exhibited the highest mean for traits like tillers per plant, spike length, spike weight and grains per spike. Majid and Dar (2020) found that cluster V and cluster VIII exhibited the maximum cluster mean values for the maximum number of traits. Phougat *et al*. (2017) grouped 44 bread wheat genotypes into five clusters and found highest mean values for maximum traits in cluster I. Afzalifar *et al.* (2022) grouped 297 wheat cultivars in three clusters and found cluster I had highest mean values for maximum traits. Similar findings were observed by Singh *et al.* (2022), Chauhan *et al.* (2023), Rani *et al.* (2023) and Chaudhary *et al.* (2022).

Conclusion

Cluster analysis revealed that all the genotype were grouped in eight clusters. Inter and intra-cluster distances provided index of genetic diversity between and within clusters. Larger the distance between the clusters better the chances of getting transgressive segregants. Highest average intra-cluster distance was exhibited by cluster III. Further, genotypes of clusters II and VIII exhibited maximum inter-cluster distance. Hence, crossing of genotypes from cluster II with genotypes from cluster VIII would produce desirable recombinants in segregating generations with improvement in traits to enhance the yield. Selection of genotypes from multiple clusters based on genetic distances and cluster means is an optimal approach. Our findings indicated that the experimental material possessed physiological traits associated with heat tolerance in bread wheat, along with sufficient genetic variability and diversity to enhance yield potential. Hybridization process can be optimized and made more efficient by selecting genotypes of interest from diverse clusters and developing a targeted breeding program around them. This study provides valuable insights into the extent of genetic diversity present in the evaluated materials, offering a foundation for developing superior genotypes with enhanced yield potential and improved physiological resilience to heat stress conditions.

Acknowledgement

The authors are very thankful to the Head of the Department, Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar (Haryana, India) for providing the experimental material, research facility and necessary support to carry out this research work.

Conflict of interest

The authors declare no conflicts of interest.

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