Original Research Article

Study of Genetic Diversity of Recombinant Inbred Lines in Bread Wheat (Triticum aestivum L.) for Heat Tolerance

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A B S T R A C T

As a cool season crop, wheat (Triticum aestivum L.) has maximum growing temperature of 15°C during the reproductive stage. With increase in global climate change, heat stress is becoming an increasingly severe constraint on production of wheat. An experiment was conducted on 32 bread wheat genotypes to evaluate the genetic diversity for yield and yield contributing parameters under heat stress conditions. The genotypes were congregated into six clusters namely cluster I having 25 genotypes, followed by cluster v (3) and cluster II, III, IV and VI each having one genotype each. The maximum inter cluster distance was observed between genotype of the cluster II and VI (96.6). Among all characters number of days to fifty per cent flowering (40%), relative water content (16.44) and flag leaf area (15.52) contributed maximum to divergence.

Keywords
- Heat stress
- Divergence
- Canopy temperature
- Relative water content

Accepted: 10 January 2021
Available Online: 10 February 2021

Introduction

Bread wheat (Triticum aestivum L.) is a self-pollinating annual plant, belonging to the family poaceae. It is a hexaploid (AABBDD) with chromosome number of 42 (2n=6x=42) and a genome size of 16 GB. Globally, wheat production stands at 768.49 million metric tonnes with an area of 220.24 million hectares and productivity of 3.49 metric tonnes per hectare (USDA 2020/21). Heat stress is an international issue related to adverse effect on agriculture that causes morpho-physiological, anatomical and biochemical changes resulted in heavy losses in crop production (Wahid et al., 2007).

Moisture stress at post-anthesis, decreases duration of maturation, grain filling, grain yield, mean grain weight, grain number and 1000-grain weight (Kaur and Behl, 2010). Heat stresses during grain filling results in
decrease in starch contents reducing the grain quality as well as grain weight (Sial et al., 2005). However, the average global temperature is reported to be increasing at a rate of 0.18°C every decade (Hansen et al., 2012). Heat stress results in deterioration of cellular structure and results in damage to various plant metabolic pathways, mainly those concerning to membrane thermostability, photosynthesis and starch synthesis (Larkindale et al., 2002). Denaturation of proteins caused by heat stress disrupts water, ion, and organic solute movement across membrane, resulting in increased cell membrane permeability, and in turn, inhibition of cellular function (Cossani, 2002). Thylakoid membranes and PS II are considered the most heat-labile cell components (Ristic et al., 2007). Under high temperature thylakoid membranes causes swelling, increased leakiness, physical separation of the chlorophyll light harvesting complex II from the PSII core complex, and damage of PSII-mediated electron transfer (Ristic et al., 2008). Starch synthesis is highly sensitive to high temperature stress due to the susceptibility of the soluble starch synthase in developing wheat kernels (Keeling et al., 1994).

Genetic diversity is essential for adaptability and survival of wheat species against abiotic and biotic stress (Fu and Somers 2009). Diversity originates either due to geographical separation or due to genetic barriers and it plays crucial role in plant breeding. Genetic divergence analysis decides the level of diversity present among selected genotypes (Mondal. 2003). Study on the nature and degree of genetic diversity assists the plant breeder in selecting the diverse parents for purposeful hybridization (Samsuddin et al., 1985). The $D^2$ statistics (Mahalanobis. 1936) is one of the most scientific biometrical techniques to determine genetic divergence present in a population. Selection of parents based on genetic divergence has been successfully exploited in several crops (Jain et al., 1981). Present experiment was conducted to identify genetically diverse genotypes among RILs as donors with desirable traits for hybridization particularly for heat stress with high yield and its contributing traits.

**Materials and Methods**

**Plant material and field trials**

The experiment was planted during Rabi 2017-2018 and Rabi 2018-19 at the Experimentation Centre of Department of Genetics and Plant Breeding, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Science and Technology of Jammu, Chatha which is situated at an altitude of 332 m above sea level with 32° 39′ degree N latitude and 74° 58′ E longitude with an annual rainfall of 1000 mm and, represents sub-tropical conditions.

The experimental material consist of 450 recombinant inbred lines population derived from three crosses (LOK-1 × HUW-468, LOK 1 × HUW 234 and Raj-4014 × PBN-51 ) which were grown during Rabi 2017-2018 in an augmented design with two checks namely PBN 51 (heat tolerant) and HUW 234 (heat susceptible) line. After every 20 lines, a total of 450 RILs were further evaluated for various morpho-physiological characters. Based on the mean data of 450 lines, 10 RILs from each of the three crosses were selected exhibiting better morpho-physiological character. Total of 30 RIL along with two check namely PBN 51 and HUW 234 were evaluated during Rabi 2018-2019 in two rows of five meter using RBD design in three replication for morphological and physiological traits to select elite RILs for heat tolerance and grain yield. The meteorological data for experimental period were obtained from Meteorological Station, SKUAST-J Chatha

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Jammu. There were 77 days with temperature above 25°C. These days coincided with flowering and grain filling stages which are most critical stages that determine yield. There were several temperature peaks before and during flowering.

Data were recorded on five randomly selected competitive plants from each plot on thirteen quantitative characters namely Plant yield (g), and yield attributing trait such as number of days to flowering, number of days to maturity, plant height (cm), number of tillers per plant, number of grains per ear, thousand grain weight (g), flag leaf area (cm²), chlorophyll content (SPAD unit), relative water content (%), relative water loss (%), canopy temperature (°C) and canopy temperature depression (°C).

The analysis of recorded data was done for genetic diversity by using Mahalanobis $D^2$ statistic (Mahalanobis. 1956) and clustering of the genotypes was done according to Tocher’s method (Rao. 1952). The analysis was done using window stat software.

**Genetic divergence analysis**

Based on Mahalanobis $D^2$ analysis thirty two genotypes were grouped into six clusters depicted in table 1. Verma et al., (2014) referred to cluster analysis as the art of finding group. The aim of clustering is to find groups that are very diverse from each other, and whose members are very analogous to each other.

The grouping pattern of the genotypes suggested no parallelism between genetic divergence and geographical distribution of genotypes. (Singh et al., 2012), (Lokhande et al., 1987) and (Mohanty and Prusti, 2002) described that genotype diversity was independent of geographical region. In present investigation Cluster 1 had maximum genotypes (25), followed by cluster fifth (1). Second, third, fourth and sixth cluster had one genotype only. On the basis of dissimilarity coefficient at 50% from the dendogram. (Hailegiorgis et al., 2011) grouped the 49 bread wheat genotypes into 22 different clusters with cluster 9 and 13 deserve for their direct use as parents in hybridization programs for the development of high yielding varieties. (Singh et al., 2011) clustered 60 genotypes of bread wheat into six clusters.

To get more heterotic and largest number of desirable transgressive segregants, selection of parents for hybridization should be evaluated on basis of genetic diversity rather than geographic diversity.

**Intra and inter cluster distances**

The intra and inter cluster distance values between six clusters is presented in table 2. The magnitude of intra cluster distances measure the extent of genetic diversity between the cultivars of same cluster.

Inter cluster distance is a method to determine of genetic distance between two clusters. The genotype of the cluster II and VI exhibited maximum divergence (96.06) followed by the genotypes of cluster III and VI (79.73), V and VI (71.26), IV and VI (61.08), I and VI (60.23), II and IV (58.53), III and IV (54.61). The average inter cluster distance between the genotypes of cluster I (21.82) was maximum followed by clusters V (12.62).

Highest inter-cluster distance than intra-cluster distance, shows wide genetic diversity among the genotypes. More the distance between the clusters wider the genetic diversity would be present between the genotypes. Highly divergent genotypes would produce a broad range of segregation in the successive generations facilitating further selection of desirable genotypes. (Singh et al., 2012)
Table 1 Distribution pattern of 32 genotypes of wheat in various clusters (I-VI) for various heat related traits.

| Clusters No. | No. of Genotype | Name of Genotypes |
|--------------|-----------------|-------------------|
| I            | 25              | Cross 1 RIL 1,2,3,4,5,6,7,8,10. Cross 2 RIL 11, 12, 13,14,16,17,18,19,20. Cross 3 RIL 21, 22, 26, 27, 29. Check HUW 234 |
| II           | 1               | Cross 3 RIL 15    |
| III          | 1               | Cross 2 RIL 15    |
| IV           | 1               | Check PBN 51      |
| V            | 3               | Cross 1 RIL 9, Cross 3 RIL 24,28 |
| VI           | 1               | Cross 3 RIL 30    |

Table 2 Inter and intra (diagonal) cluster average distance of $D^2$ among different clusters in wheat to study genetic divergence among various RILs

| Clusters No. | Cluster I | Cluster II | Cluster III | Cluster IV | Cluster V | Cluster VI |
|--------------|-----------|------------|-------------|------------|-----------|------------|
| Cluster I    | 21.82     | 33.45      | 30.93       | 34.43      | 37.67     | 60.23      |
| Cluster II   | 0.00      | 8.94       | 58.53       | 23.21      | 96.06     |            |
| Cluster III  | 0.00      | 0.00       | 54.61       | 22.29      | 79.73     |            |
| Cluster IV   | 0.00      | 0.00       | 45.51       | 61.08      | 45.51     |            |
| Cluster V    |           |            | 12.62       | 71.26      | 0.00      |            |
| Cluster VI   |           |            |             |            |           | 0.00       |
**Table 3** Cluster mean values of six clusters for various characters of 32 wheat genotype.

| Character/Cluster                        | I     | II    | III   | IV    | V     | VI    |
|-----------------------------------------|-------|-------|-------|-------|-------|-------|
| Number of days to fifty percent flowering | 97.90 | 103.67| 101.45| 102.00| 103.33| 78.67 |
| Number of days to maturity              | 120.13| 105.33| 122.33| 117.67| 108.67| 128.67|
| Plant height (cm)                       | 86.20 | 72.54 | 69.51 | 116.78| 82.35 | 76.36 |
| Number of tillers per plant             | 9.07  | 8.56  | 9.22  | 10.60 | 8.19  | 8.33  |
| Number of grains per ear                | 41.07 | 41.89 | 39.78 | 37.09 | 38.55 | 37.78 |
| Thousand grain weight (g)               | 30.36 | 36.71 | 29.34 | 33.67 | 30.32 | 33.29 |
| Flag leaf area (cm²)                    | 32.91 | 31.76 | 31.15 | 31.43 | 22.04 | 25.33 |
| Chlorophyll content                     | 38.66 | 38.88 | 41.44 | 41.78 | 38.31 | 38.47 |
| Relative water content (%)              | 57.55 | 54.48 | 53.18 | 59.59 | 55.87 | 50.88 |
| Relative water loss (%)                 | 75.90 | 74.10 | 76.40 | 67.52 | 73.84 | 74.74 |
| Canopy temperature (⁰C)                 | 21.54 | 26.87 | 21.03 | 20.65 | 21.01 | 21.51 |
| Canopy temperature depression (⁰C)      | 7.64  | 7.27  | 7.30  | 6.22  | 7.50  | 7.14  |
| Grain yield ¹                          | 16.21 | 16.77 | 16.42 | 20.16 | 16.80 | 18.08 |

**Table 4** Percent contribution towards variability of each character for 32 wheat genotype for various heat related traits

| S.No | Character                                           | Contribution % |
|------|-----------------------------------------------------|----------------|
| 1    | Number of days to fifty percent flowering           | 40.32          |
| 2    | Number of days to maturity                          | 7.46           |
| 3    | Plant height (cm)                                   | 2.02           |
| 4    | Number of tillers per plant                         | 3.63           |
| 5    | Number of grains per ear                            | 1.01           |
| 6    | Thousand Grain weight (g)                           | 2.02           |
| 7    | Flag leaf area (cm²)                                | 15.52          |
| 8    | Chlorophyll content (SPAD Unit)                     | 6.00           |
| 9    | Relative water content (%)                          | 16.94          |
| 10   | Relative water loss (%)                             | 2.22           |
| 11   | Canopy temperature (⁰C)                             | 1.41           |
| 12   | Canopy temperature depression (⁰C)                  | 2.02           |
| 13   | Grain yield ¹ (g)                                   | 4.48           |

**Cluster mean values**

Mean values of seven clusters of 32 genotypes is presented in Table 3. On the basis of cluster mean, flag leaf area and canopy temperature depression showed maximum values in cluster I. Cluster II recorded maximum mean value for thousand grain weight, number of grains per ear and minimum values for number of days to maturity. Cluster IV recorded high mean value for tillers per plant, relative water content and grain yield per plant whereas it
recorded minimum mean value for relative water loss and canopy temperature. Cluster VI recorded minimum mean value for days to fifty per cent flowering. On the basis of divergence and cluster mean it may be concluded that maximum heterosis and good recombinant would be possible from crosses between genotypes of clusters I, II, IV and VI cluster.

**Percent contribution towards total divergence**

The characters contributing maximum to the $D^2$ values are to be given greater emphasis for deciding the cluster for the purpose of further selection. (Singh et al., 2012) Under the present investigation the maximum contribution towards the total divergence as given in table 4 was exhibited by days to flowering (40.32%), followed by relative water content (16.44%), flag leaf area (15.52%), days to maturity (7.46%), chlorophyll content (6%), grain yield per plant (4.48%), tillers per plant (3.63%), relative water loss (2.22%), plant height (2.02%). Therefore, these characters should be given importance during hybridization and selection in the segregating population for improvement of yield and its component traits.

Under present investigation 32 genotypes were grouped into six clusters. The genotype of the cluster II and VI exhibited maximum divergence. The higher inter cluster distance indicated the presence of wide diversity among the genotypes included among these clusters. Hybrid produced from the selected genotypes from these clusters showing high inter cluster distance may produce appropriate transgressive segregation of high magnitude of heterosis. Cluster mean values indicated that genotypes like Cross 3 RIL 15 from cluster IV, PBN-51 from cluster IV, Cross 3 RIL 30 from cluster VI could be used as potential donors for hybridization program to produce desirable recombinant genotypes with high grain yield suitable for heat stress conditions. The trait namely days to flowering, relative water content, flag leaf area, days to maturity, chlorophyll content and grain yield per plant contributed more than 70% towards divergence. Hence, these characters should be given importance during hybridization and selection in process for improvement of yield and its component traits.

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