

Genetic Diversity of Bread Wheat (*Triticum aestivum* L.) Using the Multivariate Analysis in Normal Irrigation and Drought Stress Conditions

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SKS and AKS designed and executed the experiment and wrote the first draft of the manuscript. Authors PJ, AK and Banshidhar performed the statistical analysis, managed the analyses of the study and managed the literature searches. Authors MKS and AT provided technical support in interpretation of results and prepared the final draft. All authors read and approved the final draft of the manuscript.

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ABSTRACT

The present investigation was carried out in the fields located at wheat breeding section, Dr Rajendra Prasad Central Agricultural University, Pusa, Samastipur and Bihar. The experimental materials of the study comprised of 39 diverse bread wheat (*Triticum aestivum* L.) genotypes. The genotypes were grown under two environments viz., drought stress (rainfed) and irrigated (well-watered). The experiment in each environment was laid out in Randomized Block Design (RBD) with three replications. Five plants were selected randomly from each plot for recording observations on traits viz., days to fifty per cent flowering, days to maturity, plant height, spike length, flag leaf area, relative water content (RWC), chlorophyll content, canopy temperature, number of tillers per plant, number of grains per spike, 1000 grain weight, harvest index and grain

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yield per plant. Germination percentage and seed vigour index (SVI) were recorded in laboratory condition. The 39 genotypes of bread wheat were grouped into seven clusters using Tocher method in both drought stress and irrigated conditions. The genotypes in cluster III and cluster VI, due to maximum inter-cluster distance between them, exhibited a high degree of genetic diversity and thus may be utilized under inter varietal hybridization programme (transgressive breeding) for getting high yielding recombinants in drought stress condition. Similar inter varietal crosses may be attempted between genotypes in cluster III and IV and cluster III and V in a drought environment. The maximum inter-cluster distance was observed between cluster IV and VI followed by cluster III and IV and cluster VI and VII in irrigated condition indicating the chances of getting high yielding recombinants would be better if the crosses are made among the genotypes of these groups. Cluster VI was suitable for grain yield, number of tillers per plant, number of grains per spike, harvest index and chlorophyll content in both environment (drought and irrigated). Therefore, the selection of parents from this cluster for these traits would be effective. Under stress, the genotypes viz., GW 2007-80, WR 1872, NAIW-1607, NAIW-1342 and WR 1743 were found promising as indicated by low drought susceptibility index.

Keywords: Genetic divergence; bread wheat; multivariate analysis.

1. INTRODUCTION

Wheat (*Triticum aestivum*), one of the largest cereal crops of the world, is the first most important source of staple food. It is the only crop to have produced more than 500 million tons in a single year and to contribute more calories and more protein to the world's diet than any other food crop. Wheat production in the Mediterranean region is often limited by sub-optimal moisture conditions. Most of the countries of the world are facing the problem of drought [1,2].

Drought continues to be an important challenge to agricultural researchers and plant breeders (Nezhadahmadi *et al.*, 2013) [3]. It is assumed that by the year 2025, around 1.8 billion people will face absolute water shortage and 65 per cent of the world's population will live under water-stressed environments. Drought limits agricultural production by preventing the crop plants from expressing their full genetic potential. Estimates of yield losses due to drought range from 15 to 60 per cent which depends on geographical region and length of crop season [4]. Insufficient water is the primary limitation of wheat production worldwide [5]. The performance of wheat under drought is correlated with yield potential and thus this has become a priority area of research [6,7]. Wheat is widely grown as a rainfed crop in semi-arid areas, where large fluctuations occur in the amount and frequency of rainfall events. The development of resistant cultivars, however, has been limited by low heritability for drought resistance and lack of effective selection strategies [8].

Drought tolerance is a typical quantitative trait and breeding programmes to increase yield under reduced water supply conditions are a rather challenging task. A physiological approach can complement empirical breeding to enhance the rate of yield improvement. Nowadays, the effort is focused on improving crop genotypes for drought-prone areas. Understanding of the mechanisms behind drought tolerance, which can lead to the restitution of physiological function and hardening of plants under drought stress is essential for the achievement of such as a goal [9].

For a successful breeding program, the presence of genetic diversity and variability play a vital role. Genetic diversity is essential to meet the diversified goals of plant breeding such as breeding for increasing yield, wider adaptation, desirable quality, pest and disease resistance. Genetic divergence analysis estimates the extent of diversity existed among selected genotypes. Precise information on the nature and degree of genetic diversity helps the plant breeder in choosing the diverse parents for purposeful hybridization [10]. Various quantitative criteria have been proposed for the selection of genotypes based on their yield performance in stress and non-stress environments. Based on these indicators genotypes are compared in irrigated and rainfed conditions or different levels of irrigations [11,12]. Since published works of bread wheat on genetic diversity for drought stress are scanty, this experiment aimed to identify genetically divergent bread wheat parents with desirable traits for drought tolerant [3].

2. MATERIALS AND METHODS

The present investigation was carried out in the fields located at wheat breeding section, Rajendra Agricultural University (RAU), Pusa, Samastipur, Bihar during *rabi* 2011-2012. The experimental site is located at 25.98°N latitude and 85.67°E longitudes and has an altitude of 52.0 m above mean sea level. The experimental materials of the study comprised of 39 diverse bread wheat (*Triticum aestivum* L.) genotypes. The genotypes were grown under two environments viz., drought stress (rainfed) and irrigated (well-watered). The experiment in each environment was laid out in Randomized Block Design (RBD) with three replications. In each replication, each genotype was grown in a plot of 6 rows of 3-meter length each with a spacing of 22.5 cm between rows in both sets of experiment i.e. drought stress and irrigated conditions. After sowing of experiments, three irrigations were applied to the irrigated experiment at crown root initiation stage (CRI stage), late jointing stage and milking stage during the growing season. Whereas the drought stress experiment entirely depended on natural precipitation and no surface irrigation was applied. However, it received 58.7 mm of rainfall from December 2011 to April 2012, out of that about 49 mm rainfall was received during 20-25 days of sowing. Normal agronomic practices like fertilizer application and weed control were applied to both experiments. Five plants were selected randomly from each plot for recording observations on traits viz., days to fifty per cent flowering, days to maturity, plant height, spike length, flag leaf area, relative water content (RWC), chlorophyll content, canopy temperature, number of tillers per plant, number of grains per spike, 1000 grain weight, harvest index and grain yield per plant. Germination percentage and seed vigour index (SVI) were recorded in laboratory condition. Flag leaf length and width of five randomly selected plants were taken by measuring scale and flag leaf area was calculated by the following formula [13].

Flag leaf area (cm²) = flag leaf length (cm) x flag leaf width (cm) x correction factor

Where,

Correction factor = 0.74

Relative water content was calculated by the formula given by Barr and Weatherley [14].

$$RWC = \frac{F.W. - D.W.}{T.W. - D.W.} \times 100$$

Where

F.W. = Fresh weight of flag leaf

D.W. = Dry weight

T.W. = Turgid weight

Seed Vigour Index (SVI) was calculated from the following formula [15]

Seed Vigour Index = Germination % x Seedling dry weight

Drought Susceptibility Index (DSI) was determined by the following formula [16]. The formula is

$$DSI = \frac{1 - Y_r/Y_i}{1 - X_r/X_i}$$

Where,

Y_r = Yield under drought stress condition

Y_i = Yield under irrigated condition

X_r = Mean yield of all cultivars under drought stress condition

X_i = Mean yield of all cultivars under irrigated condition

The data were analyzed using WINDOSTAT version 8.6 software developed by INDOSTAT, Hyderabad for computation of analysis of variance, and genetic divergence.

3. RESULTS AND DISCUSSION

In the present investigation, 39 diverse genotypes of bread wheat were studied to assess their drought tolerance in terms of traits implicated in drought, yield and yield-related traits. The analysis of variance or estimates of MSS indicated that there was highly significant variation among the genotypes for all the traits studied under both the environments. Thus, it is implied that there was reasonably sufficient variability in the material used for their study, which provides ample scope for selecting superior and desirable genotypes by the plant breeder for further improvement for drought tolerance.

Selection of suitable parents for utilization in crop improvement programme is an important but rather difficult task for plant breeders. Genetic diversity is considered to be important for realizing heterotic response in F₁ and a broad spectrum of variability in segregating generations. Diversity analysis helps in assessing the nature of diversity to identified

genetically diverse genotypes for their use in breeding programmes. In the present investigation, 39 genotypes were grouped into seven clusters by Tocher method based on D^2 statistics in both water stress and irrigated conditions and tabulated in Tables 1 and 2, respectively. In drought condition, Cluster II comprises highest number of genotypes (13) followed by cluster VI (11) and cluster III (8), whereas the cluster IV, V and VII were solitary (monogenotypic), comprising single genotype each, namely WR-2099, VW 624 and WR-1743, respectively. Similar studied based on D^2 statistic was also performed by Abarshahr, et al. [17], Dashti, et al. [18], Bousba, et al. [19] and Rajshree and Singh [2]. All the genotypes that were taken for genetic divergence study under irrigated condition differed significantly about the characters studied and displayed marked divergence. Among the seven clusters, cluster II was the largest, comprising of 10 genotypes followed by cluster III with 9 genotypes, cluster IV with 7 genotypes, cluster V with 6 genotypes and cluster I with 5 genotypes. The cluster V and VII were monogenotypic, comprising single genotypes each, namely KO 583 and WR 1743, respectively. The clustering pattern showed that genotypes of different geographical areas were clubbed in one group and also the genotypes of the same geographical area were grouped into the same cluster as well as in different cluster indicating that there was no formal relationship between geographical diversity and genetic diversity. The genetic drift and selection in a different environment could cause greater diversity than geographical distance (Patel and Patel, 2012) [20].

Underwater stress condition, the highest intracluster distance was observed in cluster VI followed by cluster III and cluster II indicating differences in genotypes within the cluster (Table 4). The least intracluster distance was found in cluster I indicating that close resemblance between the genotypes presented in this cluster. The genotypes in cluster III and cluster VI, due to maximum inter-cluster distance between them, exhibited a high degree of genetic diversity and thus may be utilized under inter varietal hybridization programme (transgressive breeding) for getting high yielding recombinants for the rainfed condition. Similar inter varietal crosses may be attempted between genotypes in cluster III and IV and cluster III and V. The lowest inter-cluster distance was observed between cluster IV and V followed by cluster II and IV and cluster II and V implying these clusters were relatively less divergent and crossing between them cannot produce vigorous offspring (F_1 progenies). These results of genetic diversity study were in agreement with that of Hailegiorgis, et al. [10] who suggested that genotypes of the most diverse cluster may be used as parents in hybridization programmes to develop high yielding varieties. Under the irrigated condition, the maximum intracluster distance was recorded in cluster VI followed by cluster IV and III (Table 3). The least intracluster distance was observed in cluster I indicating a close resemblance between the genotypes presented therein. The maximum inter-cluster distance was observed between cluster IV and VI followed by cluster III and IV and cluster VI and VII indicating high chances of getting high yielding recombinants if crosses are made among the genotypes of these

Table 1. Clustering pattern of 39 genotypes of bread wheat on the basis of D^2 statistic under rainfed condition

| Cluster No. | No. of genotypes within cluster | Genotypes in cluster |
|-------------|---------------------------------|---|
| I | 4 | RWP 2008-31, RWP 2006-35, RWP 2008-26, RWP 2006-15 |
| II | 13 | GW 2007-92, GW 2007-96, GW 2006-17, AKAW-4513, AKAW-4008, KO 501, KO 583, DBW-35, DBW-37, RS 1010, VL-918, VL-812, HD 2733(C) |
| III | 8 | RWP 2008-07, RWP 2008-23, GW 2007-91, AKAW-4189-3, AKAW-4627, WR 2102, KO 604, VW 514 |
| IV | 1 | WR-2099 |
| V | 1 | VW 624 |
| VI | 11 | RWP 2008-28, GW 2007-80, GW 2007-98, WR 2044, WR 1872, WR 1873, WR 1695, NAIW-1607, WSM-1434, NAIW-1342, C 306(C) |
| VII | 1 | WR 1743 |

Table 2. Clustering pattern of 39 genotypes of bread wheat on the basis of D² statistic under irrigated condition

| Cluster No. | No. of genotypes within cluster | Genotypes in cluster |
|-------------|---------------------------------|--|
| I | 5 | RWP 2008-31, RWP 2006-35, RWP 2008-26, RWP 2008-23, RWP 2006-15 |
| II | 10 | GE 2007-92, GW 2007-96, GW 2006-17, AKAW-4513, KO-501, DBW-35, DBW-37, VL-918, VL-812, WSM- 1434 |
| III | 9 | GW 2007-80, WR 2099, WR 1872, WR 1873, WR 1695, NAIW-1607, VW 624, NAIW-1342, HD 2733 |
| IV | 7 | RWP 2008-07, GW 2007-91, AKAW 4627, WR 2102, AKAW-4189-3, KO 604, VW 514 |
| V | 1 | KO 583 |
| VI | 6 | RWP 2008-28, GW 2007-98, AKAW-4008, WR 2044, RS 1010, C 306 |
| VII | 1 | WR 1743 |

Table 3. Mean intra and inter cluster distance (D²) among seven clusters in bread wheat under rainfed condition

| | Cluster I | Cluster II | Cluster III | Cluster IV | Cluster V | Cluster VI | Cluster VII |
|-------------|-----------|------------|-------------|------------|-----------|------------|-------------|
| Cluster I | 24.922 | 100.595 | 86.874 | 229.731 | 196.209 | 249.281 | 167.567 |
| Cluster II | | 46.544 | 201.242 | 69.799 | 73.853 | 114.808 | 135.309 |
| Cluster III | | | 80.480 | 342.046 | 282.944 | 396.518 | 174.061 |
| Cluster IV | | | | 0.000 | 47.220 | 95.296 | 145.876 |
| Cluster V | | | | | 0.000 | 136.980 | 164.499 |
| Cluster VI | | | | | | 125.051 | 233.476 |
| Cluster VII | | | | | | | 0.000 |

Table 4. Mean intra and inter cluster distance (D²) among seven clusters in bread wheat under irrigated condition

| | Cluster I | Cluster II | Cluster III | Cluster IV | Cluster V | Cluster VI | Cluster VII |
|-------------|-----------|------------|-------------|------------|-----------|------------|-------------|
| Cluster I | 26.777 | 79.084 | 194.472 | 110.505 | 96.950 | 182.181 | 164.512 |
| Cluster II | | 44.713 | 83.808 | 205.772 | 69.178 | 115.984 | 155.545 |
| Cluster III | | | 62.962 | 353.161 | 99.957 | 148.198 | 190.519 |
| Cluster IV | | | | 66.466 | 149.705 | 377.326 | 186.550 |
| Cluster V | | | | | 0.000 | 215.468 | 47.045 |
| Cluster VI | | | | | | 154.182 | 337.081 |
| Cluster VII | | | | | | | 0.000 |

groups. The lowest inter-cluster distance was recorded between cluster V and VII followed by cluster II and V, cluster I and II and cluster II and III indicating these clusters were relatively less divergent. Similar results were found by Ribadia, et al. (2007) who acknowledged most diverse cluster based on intra and inter-cluster distance would be used in hybridization programme for achieving high yielding varieties.

Different clusters comprise a unique feature for different characters under investigation. Cluster mean of genotypes under both drought stress and irrigated condition were tabulated in Tables 5 and 6, respectively. Underwater stress

condition, cluster VII had the maximum mean value for flag leaf area, relative water content (RWC), 1000 grain weight and germination percentage. Cluster VII was also suitable for early flowering and maturity and had minimum mean value for canopy temperature and drought susceptible index (DSI). Cluster V may be selected as a donor for dwarf-ness. Cluster VI was suitable for chlorophyll content, number of tillers per plant, number of grains per spike, harvest index (HI) and grain yield per plant as its cluster mean value were maximum for these traits. Cluster I had maximum mean value for spike length, days to fifty per cent flowering and days to maturity. Cluster IV

Table 5. Cluster mean for sixteen characters in bread wheat under rainfed condition

| | DFF | DM | PH | SL | FLA | CHL | CT | RWC | TPP | GPS | TGW | HI | GP | SVI | DSI | GY |
|-------------|------------|-----------|-----------|-----------|------------|------------|-----------|------------|------------|------------|------------|-----------|-----------|------------|------------|-----------|
| Cluster I | 74.08 | 110.75 | 81.13 | 10.39 | 17.38 | 35.66 | 20.67 | 75.82 | 6.27 | 42.20 | 36.48 | 38.65 | 93.17 | 1472.55 | 1.35 | 11.58 |
| Cluster II | 69.72 | 109.90 | 79.02 | 10.14 | 20.43 | 34.35 | 20.48 | 76.88 | 6.13 | 40.07 | 39.36 | 41.54 | 92.92 | 1754.94 | 1.12 | 11.92 |
| Cluster III | 70.46 | 110.00 | 84.18 | 8.67 | 18.30 | 31.39 | 21.48 | 70.92 | 5.59 | 36.26 | 35.99 | 35.97 | 93.29 | 1328.26 | 0.52 | 10.14 |
| Cluster IV | 64.00 | 106.00 | 87.13 | 10.10 | 23.25 | 31.20 | 20.17 | 76.73 | 4.83 | 39.77 | 43.75 | 42.33 | 94.00 | 1957.14 | 1.24 | 11.02 |
| Cluster V | 63.33 | 103.00 | 74.53 | 9.83 | 21.17 | 34.47 | 23.73 | 69.65 | 4.77 | 39.53 | 36.20 | 32.50 | 90.00 | 1843.93 | 1.51 | 9.55 |
| Cluster VI | 68.52 | 108.09 | 79.11 | 10.30 | 23.53 | 36.42 | 19.76 | 82.24 | 8.50 | 44.25 | 42.02 | 44.90 | 93.73 | 1931.42 | 0.81 | 14.34 |
| Cluster VII | 59.00 | 98.67 | 76.87 | 8.10 | 24.48 | 31.63 | 19.17 | 82.79 | 6.87 | 37.10 | 45.74 | 42.73 | 97.33 | 1578.00 | 0.12 | 13.13 |

Table 6. Cluster means for fifteen characters in bread wheat under irrigated condition

| | DFF | DM | PH | SL | FLA | CHL | CT | RWC | TPP | GPS | TGW | HI | GP | SVI | GY |
|-------------|------------|-----------|-----------|-----------|------------|------------|-----------|------------|------------|------------|------------|-----------|-----------|------------|-----------|
| Cluster I | 74.333 | 115.333 | 87.133 | 10.880 | 19.067 | 39.513 | 17.893 | 85.287 | 9.033 | 46.193 | 43.242 | 48.050 | 94.000 | 1473.521 | 16.981 |
| Cluster II | 71.533 | 114.900 | 86.960 | 10.760 | 24.207 | 37.327 | 17.920 | 86.482 | 8.747 | 47.650 | 44.720 | 47.422 | 92.833 | 1743.085 | 17.050 |
| Cluster III | 67.852 | 114.815 | 82.444 | 10.696 | 26.348 | 36.489 | 18.026 | 86.249 | 9.881 | 46.467 | 44.749 | 45.801 | 93.481 | 1957.737 | 16.786 |
| Cluster IV | 71.857 | 117.000 | 91.505 | 8.967 | 20.050 | 32.548 | 20.205 | 79.119 | 7.105 | 39.995 | 40.270 | 37.835 | 92.714 | 1306.952 | 11.100 |
| Cluster V | 66.000 | 111.000 | 82.033 | 10.067 | 21.867 | 32.467 | 18.700 | 83.420 | 7.567 | 38.367 | 40.630 | 44.000 | 94.333 | 1691.230 | 12.703 |
| Cluster VI | 75.889 | 118.444 | 89.322 | 11.578 | 26.739 | 41.200 | 17.728 | 87.673 | 10.161 | 51.128 | 50.196 | 52.306 | 93.167 | 1853.188 | 19.101 |
| Cluster VII | 59.667 | 105.000 | 78.900 | 8.500 | 24.453 | 32.067 | 18.900 | 87.147 | 7.067 | 38.267 | 48.420 | 43.000 | 97.333 | 1578.000 | 13.530 |

Abbreviations: DFF- days to fifty per cent flowering, DM- days to maturity, PH- plant height, SL- spike length, FLA- flag leaf area, CHL- chlorophyll content, CT- canopy temperature, RWC- relative water content, TPP- number of tillers per plant. GPS-number of grains per spike, TGW- 1000 grain weight, HI- harvest index, DSI- drought susceptibility index, GP- germination percentage, GY- grain yield per plant, , SVI- seed vigour index

Table 7. Contribution percentage of sixteen characters towards genetic divergence under rainfed

| Sl. no. | Source | Times ranked 1 st | Contribution % |
|---------|-----------------------------------|------------------------------|----------------|
| 1 | Days to 50% Flowering | 121 | 16.33 |
| 2 | Days to Maturity | 2 | 0.27 |
| 3 | Plant Height (cm) | 6 | 0.81 |
| 4 | Spike Length (cm) | 1 | 0.14 |
| 5 | Flag Leaf Area (cm ²) | 2 | 0.27 |
| 6 | Chlorophyll Content (SPAD) | 8 | 1.08 |
| 7 | Canopy Temperature (°C) | 46 | 6.21 |
| 8 | RWC (%) | 0 | 0.00 |
| 9 | Tillers per Plant | 9 | 1.21 |
| 10 | Number of Grains per Spike | 0 | 0.00 |
| 11 | 1000 Grain Weight | 26 | 3.51 |
| 12 | Harvest Index | 17 | 2.29 |
| 13 | Germination % | 4 | 0.54 |
| 14 | Seed Vigour Index | 467 | 63.02 |
| 15 | DSI | 30 | 4.05 |
| 16 | Grain Yield per Plant | 2 | 0.27 |

Table 8. Contribution percentage of sixteen characters towards genetic divergence under irrigated condition

| Sl. no. | Source | Times ranked 1 st | Contribution % |
|---------|-----------------------------------|------------------------------|----------------|
| 1 | Days to 50% Flowering | 100 | 13.50 |
| 2 | Days to Maturity | 13 | 1.75 |
| 3 | Plant Height (cm) | 4 | 0.54 |
| 4 | Spike Length (cm) | 3 | 0.40 |
| 5 | Flag Leaf Area (cm ²) | 6 | 0.81 |
| 6 | Chlorophyll Content (SPAD) | 9 | 1.21 |
| 7 | Canopy Temperature (°C) | 10 | 1.35 |
| 8 | RWC (%) | 0 | 0.00 |
| 9 | Tillers per Plant | 83 | 11.20 |
| 10 | Number of Grains per Spike | 6 | 0.81 |
| 11 | 1000 Grain Weight | 28 | 3.78 |
| 12 | Harvest Index | 8 | 1.08 |
| 13 | Germination % | 11 | 1.48 |
| 14 | Seed Vigour Index | 420 | 56.68 |
| 15 | Grain Yield per Plant | 40 | 5.40 |

had the genotype with the highest mean value for seed vigour index (SVI) followed by cluster VI and V. For earliness, cluster VII, V and IV were most suited. Therefore, this cluster may be chosen for transferring the traits having high mean values through hybridization programme for water stress condition. Selection of genotypes based on cluster mean for the better exploitation of genetic potential was also reported by Hailegiorgis, et al. [10]. Based on cluster mean in irrigated condition, cluster VI recorded maximum mean values for grain yield per plant, HI, 1000 grain weight, number of grains per spike, number of tillers per plant, RWC, chlorophyll content, flag leaf area and spike length. Cluster VI also exhibited minimum mean value for canopy temperature. For the purpose of earliness in days

to fifty per cent flowering and days to maturity, cluster VII was most suited closely followed by cluster V. Cluster VII had maximum germination percentage. The highest mean value for SVI was recorded in cluster III followed by cluster VI and II. Cluster III, V and VII may be selected as a donor for dwarf-ness. Hailegiorgis, et al. [10] also studied genetic diversity and identified genotypes of different cluster based on cluster mean for the traits plant height, spike length and yield per plant.

4. CONCLUSION

Contribution Percentage of Each Character to Total Divergence: The selection and choice of parents mainly depend upon the contribution of

characters towards divergence. Contribution percentages of each character to total divergence are presented in Tables 7 and 8. The maximum contribution in the manifestation of genetic divergence was exhibited by SVI followed by days to fifty per cent flowering, tillers per plant, grain yield per plant and 1000-grain weight suggesting scope for improvement in these characters. In other words, selection for these characters may be rewarding. A similar observation was recorded by Shoram and Tandon [21] for grain weight per spike, grain yield per plant, 1000 grain weight, plant height, days to flowering and days to maturity contributed the maximum to total divergence.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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