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Multivariate analysis for qualitative traits among wheat (*Triticum aestivum* L.) germplasm under temperate Himalayas

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Abstract

Genotype of a cultivar is very important for the expression of quality attributes and the actual grain yield. In the present study, 101 elite wheat genotypes were examined using RCBD for 17 qualitative traits under temperate conditions to explore genetic variation among genotypes. Statistical analysis was conducted to determine the kinship of the studied genotypes and their grouping based on important qualitative traits, which revealed 23.82% and 16.87% for the first two principal components that were further used to draw the GT bi-plot based on each genotype and each trait by plotting the PC1 score on the PC2. Positive correlation was observed for plant growth habit with flag leaf plant parts whereas negative correlation was observed between plant growth habit and flag leaf length. The overall PC analysis evaluated that maximum difference between waxes in plant parts such as Waxy Ear, Wax Lower Shaller, Waxy Blade and Waxy Peduncle with descriptors selected on each PC. Based on this study, it can be suggested that the important morphological traits could be effective phenotypic markers while practicing selection for good quality and high yielding wheat varieties that these traits include waxiness on plant parts, flag leaf shape and growth habit.

Keywords: Wheat, multivariate analysis, qualitative traits, plant descriptors, genetic divergence

Introduction

Bread wheat (*Triticum aestivum* L.) is one of the major food sources of protein and calories for consumes. Wheat provides 45% of protein and 55% of calories. Rate of consumption of bread wheat for each person is over 160 kg and requires about 11 million tons annually (Ramadas *et al.*, 2019) [23]. Wheat has an average cultivation area of 216 million hectares throughout the world and the most important cereal crop with annual yield of 766 million tons (FAOSTAT, 2019) [10]. Breeding programs is the main thing to use highest yield producing genotype through selection and application. The newly developed germplasm can be used directly for cultivation by farmers or as a suitable source for breeding and production of new hybrids. Since, diversity is the raw material of plant breeding, it is important to explore genetic diversity, classification of germplasm for proper management, conservation of genetic resources and selection of appropriate parents to perform crossbreeding (Chandra *et al.*, 2007) [8]. In breeding programs, selection is based on a number of traits that may have a positive or negative correlation between them. Therefore, analytical methods that reduce the number of effective traits without eliminating a large amount of useful information are valuable for researchers (Chandra *et al.*, 2007) [8]. Common method for estimating genetic diversity and determining fluctuation patterns in genetic source collections is multivariate analysis. PCA and cluster analysis are the best and most appropriate multivariate tools that can be used to group genotypes based on their morphological characteristics and similarities (Banda & Kumarasamy, 2020; Dallastra *et al.*, 2014) [6, 9]. Combining these two methods provides comprehensive information on traits that critically contribute to the genetic diversity of crops (Rachovska *et al.*, 2002) [22]. Cluster analysis is one of the multivariate statistical techniques used to evaluate the diversity of various animal and plant communities, etc. and classify them into various groups according to genetic distance and genetic similarity.

Previously, multivariate analysis has been found effective method to assess genetic diversity in wheat germplasm. For instance, in a study of 64 wheat genotypes using multivariate analysis, wheat genotypes were divided into four groups. The first five PCs with Eigen values > 1 contributed 86.95% of the genetic variability amongst genotypes (Ali *et al.*, 2021) [4]. In another study, the results of two multivariate analyses such as Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) indicated the formation of four diverse clusters with different compositions of accessions, thus not supporting each other in discerning diversity (Al Lawati *et al.*, 2021) [3]. Few other studies have explored the genetic diversity among different wheat germplasm collected from different parts of world (Bibi *et al.*, 2017; Zulkiffal *et al.*, 2018) [7, 25]. The genetic diversity could be the result of geographical effect through evolution, therefore diverse traits could be reasoned as playing key role in variety's development (Rasheed *et al.*, 2019) [24]. In the study of wheat phenotypic variability, twelve quantitative and ten qualitative traits were used. The variation among germplasm was investigated based on both univariate and multivariate statistics. Also, the dendrogram was constructed based on average linkage and Euclidian distance to describe the relationship among the landraces, resulted in six clusters at 73.7% of similarity (Ghimire & Magar, 2017) [12]. The current study was aimed at assessing genetic diversity of wheat genotypes for different agro-morphological traits of agronomic importance under temperate Himalaya's region.

Materials and Methods

101 elite wheat genotypes received from CIMMYT, ICAR-IWBR Karnal exotic nurseries including four checks varieties Shalimar Wheat1, Shalimar Wheat 2, HS-562, VL 907 were used to estimate the genetic variability. Randomized block design (RBD) with two replications was employed for sowing of these wheat genotypes during winter season of 2019-20. A plot size of 1 x 1 m with five rows of 20 cm each was implemented. The experiment was conducted at Mountain Research Centre for Field Crops, Khudwani, SKUAST-Kashmir (Alt: 1590 m amsl). Endorsed crop geometry and agronomic supervision practices were adopted in the experimental layout for accurate assessment. Selected wheat genotypes were scored for 17 different qualitative traits which are mentioned here as OGP: Outer Glume Pubescence; FC: Foliage Color; FLA: Flag Leaf Attitude; FLAn: Flag leaf anthocyanin; FLL: Flag Leaf Length; FLW: Flag Leaf Width; HA: Hairs on Auricles; AL: Awn Length; ED: Ear Density; PGH: Plant Growth Habit; LGSS: Lower Glume Shaller Shape; EC: Ear colour; SAT: Spike attitude; ASP: Awns /Scurs present At: Awn attitude; ESP: Ear shape in Profile; AC: Awn Colour.

Data were recorded for a total of 17 qualitative descriptors used for variation analysis. Numerical data sets were generated by assigning a discrete number of values to the score values for each state of the selected descriptors based on Guideline for the conduct of tests for distinctness, uniformity and stability on bread wheat (*Triticum aestivum* L.) (Anonymous, 2007) [5]. UPGMA cluster analysis was used to determine the kinship of the studied genotypes and their grouping based on important qualitative traits. Different methods were used to determine the appropriate number of clusters and their accuracy was evaluated with the detection function and finally the appropriate number of clusters was

determined. Principal component analysis was also used to group the lines. To show the genetic diversity between wheat genotypes, GT biplots were produced based on PC1 and PC2 and the diversity was shown at the phenotypic level (Gabriel, 1971) [11]. The proliferation of PC1 and PC2 caused the values to be distributed symmetrically between genotype and trait scores. All calculations were performed using Past Ver. 4.05 (Hammer *et al.*, 2001) [14].

Results and Discussion

The qualitative descriptors of 17 traits were scored as the number value for 101 wheat varieties. The cultivation habits of wheat were very variable and in this study the genotypes had the habit of erect to semi-erect and most of these genotypes showed the habit of semi-erect state. Most of the genotypes used in this study (60% of them) had medium flag leaf length (20-30 cm). Genotypes indicated significant fluctuations in the presence of FLA, HA and OGP and there were different levels, and these levels ranged from absence to very strong. Almost similar distributions were observed for DUS descriptors across wheat genotypes.

PC1 and PC2 accounted for 23.82% and 16.87% of the total variance, respectively, and their cumulative variance was 40.69% (Table 1). Principal component analysis is appropriate when a small number of components justify a high percentage of total variation (For example, the first two to five components justify more than 60% of the total variation, and if the first two components justify this amount, and the smaller the number of components, it is much better) or components are selected whose eigenvalues are higher than one. Therefore, in this study, according to the results, components whose values were higher than one was selected and according to the results, they also justified a very good percentage of the total variation (Groth *et al.*, 2013) [13].

In this study, FC, FLA and FLAn as traits were closely related to the first PC and reflected high contributing factors leading to FC, FLA and FLAn. Also, the FC, FLAn, HA, FLA, AL and LGSS traits had high contributing factor loadings for second principal component (PC2). Traits that have a high positive or negative load and are among the qualitative characters have a great role in diversity and are one of the most distinctive descriptions in clusters. The principal component analysis divided the studied lines into three groups (Fig 1.).

In this study, the scores of PC1 versus the scores of PC2 were used to construct and plot the GT biplot (Fig 1) for each genotype (101) and each trait (17) and the 40.69% of the total variation was explained by biplot genotype with trait. Qualitative traits that were placed at short vectors in GT biplot and had the most discrimination for wheat included traits such as AL, HA, FLL, FLA, etc. Characters like FLAt, FLL, FLW, FC at an acute (<90°) angle with AL, HA, ED indicated almost similar variations. A negative correlation was found between PGH and FL while a positive correlation was observed between flag leaf plant components.

Cluster analysis was performed based on the mean of the original data for all traits, the results of which are shown as a dendrogram in Fig 2. Squared Euclidean Distance and Ward's method were used for cluster analysis to determine the distance between genotypes. According to the cut-off point, the studied lines were divided into four groups with similar in-group and dissimilar group characteristics (Fig. 1), and the grouping accuracy was 100% based on the discriminant

analysis.

The study of genetic diversity of wheat and the use of qualitative traits in studies based on statistical methods and identification of superior genotypes are the goals of breeding programs. The studied qualitative characteristics showed a significant difference between genotypes and therefore the use of morphological agronomic traits for grouping genotypes is very effective and efficient. Similar studies have been conducted in wheat germplasm accessions employing multivariate analysis to study the genetic diversity pattern using agro-morphological and qualitative (plant descriptor) traits (Al Lawati *et al.*, 2021; Lodhi *et al.*, 2017; Phogat *et al.*, 2021) [3, 16, 21]. In this study, it was observed that most of the genotypes had variable plant height (medium to tall height), different growth habits (erect to intermediate) and medium flag leaf length and width. There was also a great variety in leaf attitude, green foliage and medium size seeds stem and seed size for genotypes. Qualitative descriptors of approximately the same pattern were observed for wheat genotypes developed in areas with different agricultural climates. This shows that selection criteria for high-yield genotypes, regardless of demographics or climate, are linked by choosing similar qualitative descriptors. The only exception observed is the presence of wax on flower stalks where only genotypes grown in India were detected. Plant parts wax descriptors such as plant height, leaf color, growth habits of plant, length and width of flag leaf, and size of seeds were established to be important in contributing to the genetic diversity for Indian wheat. An important feature that has been identified in the study of diversity and related studies in previous reports is the phenotypic traits that have been studied in the genetic diversity of rice (Moukoubi *et al.*, 2011) [19]. Studies related to PC analysis have shown that the variables that have the most loads in the evaluation of each PC are the first two components. Thus, the waxy properties of plant parts were important for the study of wheat genetic diversity as they exhibited the greatest variability among the descriptors selected for each PC. Therefore, according to these results, in the study of wheat diversity in India, these traits should be considered and used in future phenotypic studies. Leaf posture, ear length, Shaller width, beak length, leaf color and letters such as PGH showed low PC and less phenotypic changes. Therefore, these traits need to be considered when choosing a matrilineal wheat expansion program to integrate genetic diversity. The grouping based on PCA estimates and cluster analysis for genotypes in this study was very similar and in line with previous studies to investigate the genetic diversity of Indian wheat genotypes based on cluster analysis (Malik *et al.*, 2013) [17]. In their study, clustering was found to be a useful method that could be used to find morphological

variations in wheat and to report the causes of new variations for reproductive purposes. Other studies on wheat genetic diversity have reported that gluten, semolina, and grain softening are the most important characteristics of durum's quality traits (Mangova & Petrova, 2007) [18]. Previous studies have explained the importance of genotype using a traits biplot-based evaluation of the morphological traits of potatoes (Afuape *et al.*, 2011) [1] and wheat (Malik *et al.*, 2013) [17]. The waxy plant part was observed as a feature of the most variable phenotype with a long vector of an Indian wheat cultivar, supporting the PCA observations. Technicians such as beak length, plant growth habit, Shaller_Shape, and bran puberty found that relatively short vectors show little fluctuation. Group 1 contained older or older tall varieties primarily cultivated before India's Green Revolution, while the remaining three groups contained clustered varieties developed in the period after India's Green Revolution. In wheat breeding programs conducted in India, high yields of semi-dwarf wheat genotypes have been observed mainly, including genotypes such as WL 711, WH 147, Lok 1, HD 2329, PBW 343 and PBW373 (Jain & Yadav, 2009) [15]. Other studies on other plants have shown that genotype×trait biplot is an effective multivariate approach to assessing phylogenetic diversity and the relationship between genotypes for genetic selection and improvement. Regarding bi-plot studies, we can mention the studies on 40 rice cultivars (Ogunbayo *et al.*, 2005) [20] as well as on barley (Ahmad *et al.*, 2008) [8] which were based on qualitative and quantitative traits.

In this study, it was found that DUS traits have sufficient and necessary ability to detect genetic diversity of Indian wheat genotypes and can be used in breeding programs. Agronomical and morphological characteristics such as wax on parts of the plant, height of the plant, and growth patterns are considered significant phenotypic markers for assessing genetic variation and assessing genetic material. These traits and descriptors must essentially be taken into account for the program of selection, varietal differentiation and constitution of Indian wheat seeds. In this study, it was found that multivariate statistical methods such as PCA and GT bi-plot have effectively and appropriately identified variation of qualitative descriptors in different wheat genotypes and have shown good diversity. The varieties were grouped according to descriptive factors such as height and tenderness of the plant using the GT bi-plot diagram which delineated the green revolution eras (pre and post) of the breeding program of wheat. In this study, for six different climatic conditions of India, almost similar descriptive patterns have been developed in the selected cultivars that can be used in other studies.

Table 1: The result of principal components in wheat genotypes based on 20 qualitative traits.

Qualitative Descriptor	PC1	PC2
PGH	-0.03028	0.022923
FCol	0.95164	0.11022
FLAn	-0.15454	0.69723
HA	-0.01107	0.3421
FLAt	0.10421	-0.1284
FLL	0.223	-0.02675
FLW	0.060272	-0.01245
ESP	-0.02232	-0.01188
ED	-0.0211	0.24717
ASP	-0.00041	-0.0032

AL	0.017563	0.16709
AC	0.002962	0.032979
AAAt	-0.02178	-0.02976
OGP	-0.00998	0.002997
EC	0.003244	0.026997
LGSS	-0.05372	-0.52386
Sat	0.022432	0.022471
% Variance	23.82	16.87
% Cumulative Variance	23.82	40.69

OGP: Outer Glume Pubescence; FC: Foliage Color; FLA: Flag Leaf Attitude; FLAn: Flag leaf anthocyanin; FLL: Flag Leaf Length; FLW: Flag Leaf Width; HA: Hairs on Auricles; AL: Awn Length; ED: Ear Density; PGH: Plant Growth Habit; LGSS: Lower Glume Shaller Shape; EC: Ear colour; SAT: Spike attitude; ASP: Awns /Scurs present; AAAt: Awn attitude; ESP: Ear shape in Profile; AC: Awn Colour

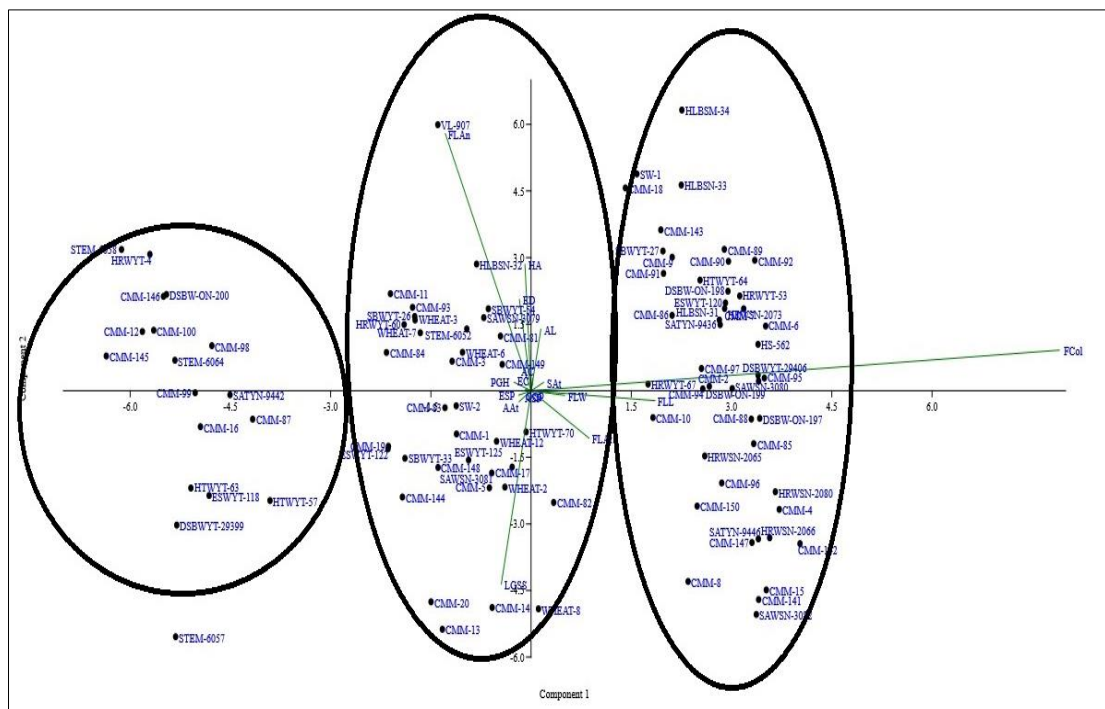


Fig 1: The biplot diagram of Genotype × trait for 101 wheat genotypes and 17 qualitative traits

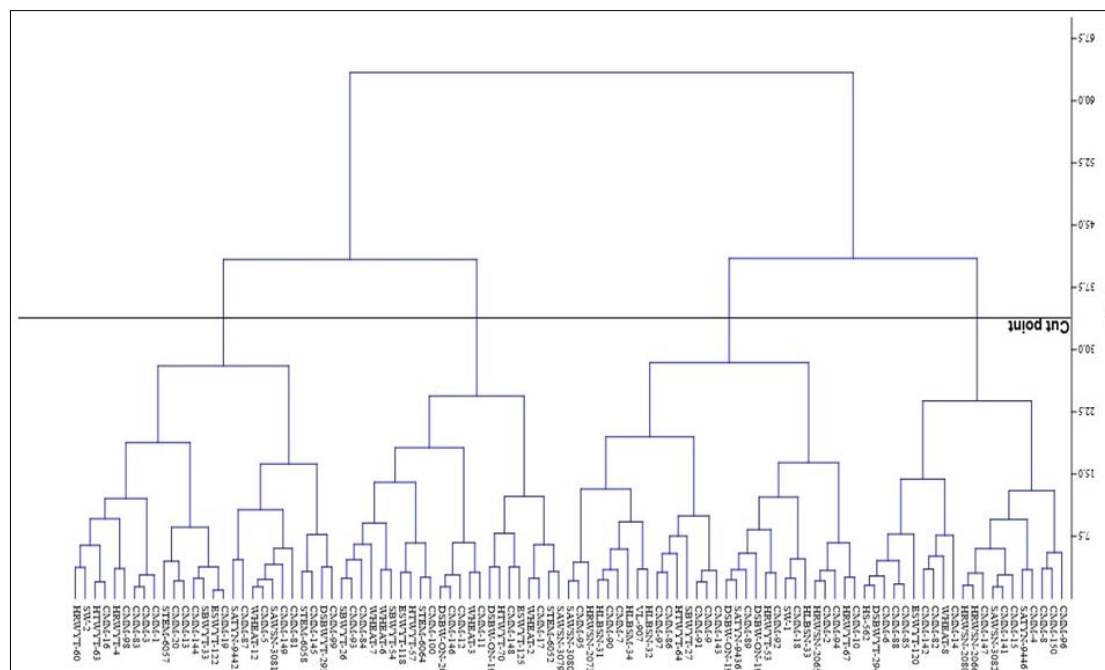


Fig 2: Analysis of clusters of studied lines by Ward method

Conclusion

This study provided important information useful for genetic improvement of common wheat. Genotypes classified into four clusters showed the greatest inter-cluster diversity. There is significant genetic variation between the genotypes tested, suggesting that there is an excellent opportunity for improvement through extensive hybridization by crossing genotypes in different clusters.

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