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Morphological diversity and yellow rust resistance in bread wheat germplasm lines

Rahul R Nelwadker, MA Khan*, RR Mir, Kamaluddin and M Ashraf Bhat

Faculty of Agriculture and Regional Research Station, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir, India

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***Corresponding author**
Email: *anwarkhan@skuastkashmir.ac.in*

Abstract

Genetic divesity was studied for ten quantitative traits using D^2 statistics in a set of twenty-six wheat germplasm lines studied. The study grouped the genotypes into three clusters. Cluster I comprised of maximum genotypes (16) followed by clustered II (9) and cluster III (1). The mean intra and inter cluster distance ($\sqrt{D^2}$) values revealed that cluster I has the high intra cluster distance value of 16.22 followed by cluster II (16.21). The inter cluster distance D^2 value was highest between cluster II and III (41.20) followed by cluster I and cluster II (30.00). This indicates that the genotypes included in cluster II and III have wide genetic diversity and could be used in hybridization programme aimed at direct selection for the traits or improvement of genotypes through exploitation of heterosis. Traits like 1000 grain weight, Plant height and grain yield had more contribution towards genetic divergence, hence these traits are major determinants of genetic diversity in the present set of genotypes. The fourteen germplasm lines have shown higher levels of resistance to prevalent races of yellow rust at adult plant stage during disease screening experiments seperately conducted in two growing seasons of years 2015 and 2016. The yellow rust resistant genotypes identified in the study could further be tested for their effectiveness over space and time..

Keywords: Wheat, genetic diversity, cluster analysis, stripe rust resistance

1. Introduction

Wheat (*Triticum sp.*) is the most important cereal crop of the world both in terms of area and production. In India, wheat is the second most important cereal crop after rice and is grown on an area of 30.20 million hectares with a production of 88.94 million tones (Anonymous, 2016). In Jammu and Kashmir, it is grown over an area of about 256 thousand hectares with an annual production of about 300 tonnes (Anonymous, 2015). The overall average productivity of wheat is 3093 kg/ha in India and 1541.13 kg/ha in Jammu and Kashmir, respectively (Anonymous, 2015). To meet the demand the option for increasing wheat production, area under cultivation has already been exploited to almost its maximum. So, continuous efforts are required to develop high yielding and disease resistant wheat genotypes.

The morphological characterization of wheat germplasm lines is important to access the diversity of economically important morphological and seed traits like days to flowering, plant height, spike length, awn/awnless character, average number of seed set per spike, average grain yield, seed shape, seed colour, 1000 grain weight and seed dimensions (seed length and seed breadth) etc. Further, the genetic diversity is the most important tool in the hands of plant breeders in choosing the parents for hybridization programme. Narrow genetic diversity is a problem in breeding for adaptation to biotic and abiotic stresses. Therefore, it is necessary to investigate the genetic diversity in wheat germplasm in order to broaden the genetic base for economically important

traits in future breeding programmes (Uddin *et al*., 2008). Understanding of genetic diversity in a crop species is a key to its improvement under changing environments (Sajjad *et al*., 2011). Evaluation of genetic diversity among adapted, elite germplasm can provide predictive estimates of genetic variation among segregating progeny for pure-line cultivar development. Knowledge about germplasm diversity and genetic relationships among breeding materials could be an invaluable aid in crop improvement strategies (Mohammadi and Prasanna, 2003) and study of the genetic diversity in bread wheat is important to breeding and genetic resource conservation programs (Zhang *et al*., 2011). Hence, a technique which can provide direct and reliable estimates of diversity at genotypic level will be more useful. D^2 proposed by Mahalanobis (1936) based on multivariate analysis is most appropriate method for selecting the parents as it furnishes a measure of actual divergence between any pair of population (Rao, 1952).

The stripe rust caused by *Puccinia striiformis f. sp. tritici* is a widely distributed and dangerous (Chen, 2005) disease of wheat crop. This disease affects the crop through damaging its systems, most importantly reduces grain yield by shrivelling grains and also affects respiration that in turn makes growth of plant stunted leading to reducing weight and affecting its quality (Chen, 2005; Line, 2002). The timely application of fungicides against this obligate parasite can provide some control but their use adds to the production costs. Moreover, the use of fungicides is considered unfriendly to the environment. Thus breeding for resistance is the most effective and efficient control strategy, (Yang and Liu, 2004). The long term and economical strategy could thus be resistance breeding through deployment of effective rust resistance genes over space and time, for which screening of wheat germplasm for identification of novel sources of resistance against yellow rust is of utmost importance. The present study was conducted to determine genotypic divergence for different characters in wheat germplasm lines and to screen these wheat germplasm lines for resistance against yellow rust.

2. Materials and methods

The studies of twenty-six wheat germplasm lines for morphological diversity and yellow rust resistance were seperately undertaken in Research Fields of Division of Genetics & Plant Breeding, Faculty of Agriculture and Regional Research Station, Wadura, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (Jammu and Kashmir) during main season of years 2015, and 2015 -2016, respectively. Experimental materials for both the studies consisted of 26 wheat (*Triticum aestivum* L.) germplasm lines including two check varieties Shalimar wheat-1 and Shalimar wheat-2 (Table 5). For morphological diversity studies, the experimental materials were grown under randomized block design (RBD) with three replications, Seeds of each genotype were hand dibbled in five rows of three meter length and row to row spacing of 22 cm and plant to plant spacing of 10cm, during main season of year 2015. Normal agronomic and cultural practices were applied to the experiment throughout the growing season according to local practices. The observations were recorded on five randomly selected competitive plants in each entry of each replication for the characters such as average plant height (cm), spike length (cm), average number of seed set per spike, seed size and seed length. Days to germination, days to flowering, days to maturity, awn/awnless character, seed size (test weight) and grain yield/ha were recorded on plot basis. Qualitative seed traits such as seed shape, seed colour, seed germ width, seed crease, seed brush hair length and seed dimensions (length and breadth) were recorded on five representative seed of each entry.

2.1 Genetic Divergence Analysis : In order to quantify the genetic distance between any two genotypes, Mahalanalobis (1936) D^2 statistics as described by Rao (1952) was employed. The variance and covariances were subjected to multivariate analysis. The original inter-mated variables (x's) were first transformed into set of mutually uncorrelated variable (y's as linear function of x's) and the D^2 values were worked out. Pivotal condensation method was used to compute inverse matrix of the error dispersion matrix (Rao, 1952).

2.2 Group Constellation : Tocher method was used for assigning various varieties to different clusters (Rao, 1952). The two varieties having small distance from each other were considered first to which a third variety having smallest average D^2 value for the first two varieties was added. Next came the nearest fourth variety and the process continued till the average D^2 value increased. The remaining varieties were then considered for the next cluster and the process was continued till all varieties were included in various clusters. The spatial distances between clusters were arrived at by taking square root of

average intra-cluster D^2 values. For each combination (pair of genotypes) the mean deviation (d^2i) i.e., y1-y1 with i = 1,2,3….p was computed and D^2 values was calculated as sum of the squares of these deviations i.e., (yi1 – yi2), where yi is the transformed variable from the original variable xi. Accordingly D^2 values for all combinations were calculated. The D^2 values so obtained for each pair of population were treated as X_2 and were tested against the tabulated values of X_2 degrees of freedom, where p is the number of traits considered. In all combinations each character was ranked on the basis of di = yij – yik values. Rank 1 was given to the highest mean difference and rank p to the lowest mean difference. Where, p is the total number of characters. In this manner contribution of each character to the total divergence was computed. The Tocher method is detailed in a simplified way by Rao (1952) and Singh and Chaudhary (1985).

2.3 Screening of yellow rust resistance : The yellow rust screening experiment was conducted during main season of years 2015 and 2016. The wheat germplasm lines were planted in five rows each of 3m length, with row to row distance of 22cm and plant to plant spacing of 10cm. National susceptible check 'Agra local' was planted as susceptible check, which was planted after every five entries as well as all around the experimental plot to ensure uniform spread of disease. The lines were scored for yellow rust disease severity at adult plant stage under field conditions following modified Cobb scale (Peterson *et al*., 1948; Roelfs, 1992).

The modified Cobb scale gives scores for level of disease severity on the basis of percent leaf area covered. For example; on average if 10% of leaf area is covered by susceptible infection type it is scored as 10S; on average if 25% of leaf area is covered both by susceptible and resistant infection types it is scored as 25MS and on average if 5% of leaf area is covered only by resistant infection types it is scored as 5R. where; R= resistant, MS= moderately susceptible and S= susceptible. The zero to 40 % disease is categorized as resistant and 50 to 100% disease indicates susceptibility.

3. Result and discussion

3.1 Estimation of genetic divergence and cluster analysis of wheat genotypes : In the present study the genotypes under study expressed significant diversity for all the traits. Based on the expression of traits the 26 genotypes (including 2 check Shalimar wheat-1, Shalimar wheat-2) got grouped into 3 clusters (Table 1) as per the Mahalonobis D^2 analysis employing Tocher's method (Rao, 1952). Cluster I comprised of maximum genotypes (16) under study, followed by clustered II (9) and cluster III (1). Cluster I grouped in BISA 11057, BISA-11044, BISA-11041, BISA-3029, BISA-6012, BISA-11058, BISA-7049, BISA-11046, BISA-3049, BISA-11045, BISA-4041, BISA-5031, BISA-11047, Shalimar Wheat-1, Fw-920, *Yr*10 reference. In cluster II Fw-226, Shalimar wheat-2, FLW-16, Fw-1142, FW-921, Fw-943, Selection-42, *Yr*18 reference, FW-638 were grouped whereas FW-1306 was alone included in cluster III.

The clustering of genotypes into different groups through D^2 statistics has also been reported by (Yadav *et al*., 2001). Fang *et al.* (1996) clustered 120 genotypes of durum wheat into five groups based on maturity date, plant height, spike length, number of seed per spike, 1000-seed weight and spike seed yield. Contrary to this, the grains/spike, 1000 gain weight, spike length, biological yield and gain yield were minimum in these genotypes. Gashaw *et al.* (2007) clustered indigenous durum wheat genotypes of diverse origin into homogenous groups based on estimates of genetic divergence (D^2) for the hybridization programme.

They found that there was no correspondence between geographic and genetic distances i.e. germplasm collected from the same geographic area were placed into different cluster groups and those collected from different geographic regions were placed into the same cluster. Ali *et al.* (2008) and Singh and Dwivedi (2002) also reported that cluster analysis can be useful for finding high yielding wheat genotypes, the results of this study showed the presence of a high genetic divergence among wheat genotypes.

The mean intra and inter cluster distance (D^2) values (Table 2) revealed that cluster I has the high intra cluster distance value of 16.22 followed by cluster II (16.21). The inter cluster distance D^2 value was highest between cluster II and III (41.20) followed by cluster I and cluster II (30.00). The results clearly indicate that tremendous potential exists for introgressing the allelic resources present among the genotypes under study that is possible by following a systematic breeding approach so as to recover superior high yielding recombinants.

Table 1. Distribution of different wheat genotypes into clusters based on D^2 statistics

Cluster	Number of genotypes in the cluster	Variety/accession number of the genotypes
I	16	BISA-11057, BISA-10044, BISA-10041, BISA-3029, BISA-6012, BISA-11058, BISA-7049, BISA-10046, BISA-3049, BISA-11045, BISA-4041, BISA-5031, BISA-11047, Shalimar Wheat-1, Fw-920, *Yr*10 reference
II	9	Fw-226, Shalimar Wheat-2, FLW-16, Fw-1142, FW-921, Fw-943, Selection-42, Y18 reference, FW-638
III	1	FW-1306

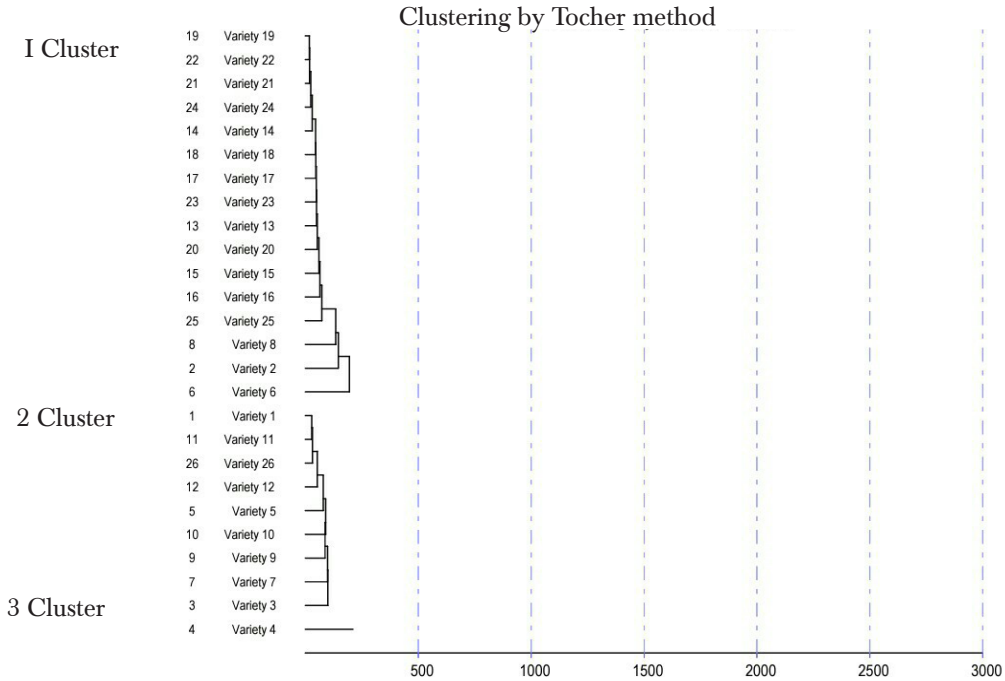

Fig. 1 Clustering of genotypes based on morphological parameters

The cluster means for the traits under study (Table 3) revealed that cluster-I having maximum genotypes, took 23 days for germination, 166 days for flowering and 205 days for maturity on an average. The plant height for cluster-I on an average was 70 cm, spike length 9cm, and this group of genotypes (cluster-I) possessed on an average of 25 number of grains/spike, had average 1000 grain weight of 50 grams, possessed average seed length of 6.5mm and seed breadth was on an average 3.56 mm. The average grain yield/ha of this group of genotypes was 3576.71 kg/ha.

Table 2. Average inter-cluster (above diagonal) and intra-cluster (diagonal) D^2 values among different wheat genotypes

Cluster	Cluster 1	Cluster 2	Cluster 3
1	16.22	30.00	29.75
2		16.21	41.20
3			0.00

Authors The cluster-II included 9 genotypes who took on an average of 23 days for germination, 183 days on an average for days to 50% flowering and 222 days on an average for maturity. The average plant height (77.73cm), spike length (9.81cm), number of grains/spike (37.08), 1000 grain weight (48.16gms), seed length (6.74mm), and seed breadth (3.51mm). This group of genotypes recorded on an average yield of 2154.32kg/ha. The lone genotype in cluster III (FW-1306) showed average days to germination (14.33), days to flowering (188) and average days to maturity (227.67). The genotype showed average plant height of (99.40cm), spike length (10.20cm), number of grains/spike (41.13), 1000 grain weight (43.66gm), seed length (7.3mm) and seed breadth (3.40mm). The genotype FW-1306 showed highest average yield per hectare estimated as 4913.52 kg/ha. Also, the observed that cluster means and coef-

Table 3. Cluster means for morphological and seed traits in different clusters of wheat genotypes

Clusters	Days to germination	Days to flowering	Days to maturity	Plant height (cm)	Spike length (cm)	No.of grains/spike	1000grain weight (gm)	Seed length (mm)	Seed breadth (mm)	Yield/ha (kg)
1	23.48	166.71	205.77	70.80	9.02	25.66	50.88	6.75	3.56	3576.71
2	22.96	183.33	221.85	77.73	9.81	37.08	48.16	6.74	3.51	2154.32
3	14.33	188.00	227.67	99.40	10.20	41.13	43.66	7.13	3.40	4913.52

Table 4. Per cent contribution of individual traits towards total divergence in wheat (*Triticum aestivum L.)*

Traits	Number of times appearing first in ranking	Per cent contribution towards total divergence
Days to germination	3	0.92
Days to flowering	5	1.54
Days to maturity	1	0.31
Plant height (cm)	49	15.08
Spike length (cm)	10	3.08
No of grains/spike	18	5.54
1000 grain weight (cm)	33	10.15
Seed length (mm)	14	4.31
Seed breadth (mm)	10	3.24
Yield/ha (kg)	172	55.83
Total	315	100

Table 5. Average performance of wheat germplasm lines for earliness, days to maturity and yellow rust scores over replications

Genotypes	Average Days to Germination	Average Days to Flowering	Average Days to Maturity	Average yellow rust scores (Modified Cobb Scale)
Fw-226	20 days	182 days	219 days	5MS
Fw-920	25 days	181 days	213 days	5S
FLW-16	26 days	185 days	225 days	10MS
FW-638	28 days	186 days	229 days	5S
FW-1306	14 days	189 days	229 days	Immune
FW-921	20 days	182 days	219 days	5MR
*Yr*10 reference	21 days	183 days	219 days	20S
Y18 reference	32 days	187 days	226 days	10R
Shalimar Wheat-1	22 days	176 days	212 days	30S
Selection-42	22 days	181 days	220 days	10MS
FW-943	21 days	179 days	219 days	5MS
Shalimar Wheat-2	23 days	190 days	229 days	20S
Fw-1142	16 days	177 days	211 days	5MR
BISA-3049	20 days	166 days	206 days	5R
BISA-6012	17 days	163 days	204 days	5R
BISA-4041	24 days	167 days	204 days	5R
BISA-5031	30 days	162 days	199 days	50S
BISA-7049	28 days	166 days	208 days	10R
BISA-11058	26 days	167 days	207 days	5MR
BISA-11057	20 days	163 days	204 days	5MR
BISA-11045	20 days	163 days	205 days	10R
BISA-11041	24 days	160 days	192 days	20S
BISA-11044	29 days	162 days	202 days	10R
BISA-11046	27 days	162 days	205 days	5MR
BISA-3029	22 days	161 days	202 days	5MR
BISA-11047	20 days	159 days	208 days	50S
Agra Local (check)	22 days	165 days	225 days	90-100S

ficient of variation are an interacting picture of diversity. The characters contributing to divergence are reported to vary from crop to crop (Murty and Arunachalam, 1967). The percent contribution of morphological and seed traits under study towards total divergence (Table 4) revealed that yield/ha was the main factor contributing towards divergence (58.96%) followed by plant height (15.08%), 1000 grain weight (10.15%), number of grains/spike (5.54%), seed length(4.31%), seed breadth (3.24%), spike length (3.08%), days to flowering (1.54%), days to germination (0.92%) and days to maturity (3.31%). De *et al*., (1988) proposed that the traits contributing maximum towards the D^2 value need to be given greater emphasis for deciding on the clusters to be choosen for the purpose of further selection and choice of parents for hybridization.

3.2 *Average performance of wheat germplasm lines against yellow rust disease* : Among biotic stresses, wheat is affected by over 40 fungal, 32 viral and 81 bacterial diseases. Rust diseases caused by *Puccinia* species particularly P. striiformis (stripe rust/ yellow rust) is the most serious of the wheat diseases. In the present investigation twenty-six (26) wheat germplasm lines were screened for stripe rust (Table 5) using modified cobb scale (Peterson *et al*., 1948). The performance of the genotypes under study against yellow rust averaged over years 2015 and 2016 depicted that all the wheat genotypes under study showed different levels of resistance to yellow rust except FW-26, FW-920, FW-16, FW-638, *Yr*10 reference line, Shalimar wheat-1, Selection-42, FW-943, Shalimar Wheat-2, BISA-5031, BISA-11041, BISA-11047 which expressed varying levels of susceptibility against yellow rust, showed average plant height of (99.40cm), spike length.

The germplasm lines were evaluated under field conditions only when the yellow rust susceptible check (Agra local) expressed susceptibility (90S-100S). So far, seventy stripe rust resistance genes named as *Yr*1 through *Yr*70 have been designated from both wild and cultivated wheats at International level (Bansal *et al*., 2017). Alsaleh *et al*. (2016) studied diversity in Durum wheat (*Triticum turgidum*) 'Kunduru landraces' 35 genotypes based on few morphological characters, yellow rust score was also evaluated among landraces to judge their resistance.

Seven landraces were very susceptible, 13 were susceptible, five landraces were moderately susceptible, and eight were moderately resistant, whereas none of the accessions was 100% resistant to yellow rust. The identification of novel sources of resistance against prevalent yellow rust race 78S84 will not only add to genetic diversity but it will also give enough indication of stripe rust resistance genes available to wheat breeders for taking future breeding programmes. In conclusion, twenty six genotypes based on various morphological traits were divided into three clusters with sixteen germplasm lines in Cluster-I, nine germplasm lines in Cluster-II and a lone line FW-1306 grouped in Cluster-III. The wheat germplasm lines under study were also screened for yellow/stripe rust resistance over years 2015 and 2016 using modified cobb scale that could classify fourteen lines as resistant, while other twelve lines showing different levels of susceptibility to yellow rust were grouped under susceptible category. The study inferred the understanding of these diverse genotypes which can be helpful in selection of parental lines to breed for agronomic traits. The present study can serve as basis for improvement of these agronomic and yellow rust resistance traits in future wheat breeding programmes.

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