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# SELECTION OF PARENTS FOR CROSSING BASED ON GENOTYPING AND PHENOTYPING FOR STRIPE RUST (*PUCCINIA STRIIFORMIS*) RESISTANCE AND AGRONOMIC TRAITS IN BREAD WHEAT BREEDING

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*Bread wheat (Triticum aestivum L.) germplasm consisting of 45 genotypes were clustered phenotypically using ten morphological traits and Area Under Disease Progress Curve (AUDPC) as measure of stripe rust resistance. The clustering was ratified by using twenty three molecular markers (SSR, EST and STS) linked to stripe rust (Puccinia striiformis f. sp. tritici) resistant QTLs. The aim was to asses the extent of genetic variability among the genotypes in order to select the parents for crossing between the resistant and susceptible genotypes with respect to stripe rust. The Euclidian dissimilarity values resulted from phenotypic data regarding morphological traits and AUDPC were used to construct a dendrogram for clustering the accessions. Using un-weighted pair group method with arithmetic means, another dendrogram resulted from the similarity coefficient values was used to distinguish the genotypes with respect to stripe rust. Clustering based on phenotypic data produced two major groups and five clusters (with Euclidian dissimilarity ranging from 2.44 to 16.16) whereas genotypic data yielded two major groups and four clusters (with percent similarity coefficient values ranging from 0.1 to 46.0) to separate the gene pool into highly resistant, resistant, moderately resistant, moderately susceptible and susceptible genotypes. With few exceptions, the outcome of both type of clustering was almost similar and resistant as well as susceptible genotypes came in the same clusters of molecular genotyping as yielded by phenotypic clustering. As a result seven genotypes (Bakhtawar-92, Frontana, Saleem 2000, Tatara, Inqilab-91, Fakhre Sarhad and Karwan) of diverse genetic background were selected for pyramiding stripe rust resistant genes as well as some other agronomic traits after hybridization.*

**Introduction.** Stripe (yellow) rust caused by a fungus *Puccinia striiformis* f. sp. *tritici* (an obligate biotrophic organism) is a devastating disease of wheat worldwide [1]. Grain yield losses from 10 to 70 % have been reported depending upon the cultivar grown and conducive environmental conditions during ear emergence [2, 3]. Cultivation of genetically resistant cultivars is the most effective, environmentally safe, and economical measure to control the disease [4]. In many wheat growing areas of Pakistan, the disease appeared during the year 2004—2005 as the indirect tsunami effect and caused excessive rain fall with associated humid conditions from February till April thereby making environmental conditions highly conducive for the disease development [4]. Yield loses and use of fungicidal control of the disease in the crop can be overcome up to great extent through development and cultivation of resistant wheat cultivars.

Resistant to stripe rust like other metric traits is under control of cumulative effect of both major genes and polygenes [4]. Incorporation of resistant genes into a single genotype is based on the genetic variability of the germplasm to be used as resistant source [5]. It is therefore, imperative to determine the extent of genetic variability among the available germplasm to be utilized in the breeding programme. Smith et al. [6] considered morphological characterization as first step in description and classification of germplasm that needs to be supplemented through the use of molecular characterization as the morphological traits represent few loci and are highly influenced by environmental fluctuations [7].

Several techniques such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), sequence tagged sites (STS), amplified fragment length polymorphism (AFLP), expressed sequence tag (EST), simple sequence repeats (SSR) and others are currently in use for assessment of genetic variability in crop plants including wheat. Of these, the SSR or microsatellites are DNA based short (2—6 bp) tandemly repeated units with high polymorphism even among closely related cultivars with variation due to mutational events [8]. The polymorphism can be easily detected at specific loci using specific primers in the flanking regions of such loci [1] and can be used as an efficient and economical method for the assessment of genetic diversity in both eukaryotes and prokaryotes [9]. The

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present study was organized to assess genetic variability among 45 accessions of bread wheat (*Triticum aestivum* L.) using Area under disease progress curve (AUDPC) for stripe rust and some morphological traits as input for phenotypic clustering. The clustering was further ratified by using molecular markers, including SSR, EST and STS type, linked to 20 different stripe rust resistant QTLs. The aim was to select parents for crossing among the accession for pyramiding stripe rust resistant genes and some economically important agronomic traits from different sources into a single line. The study on crosses which resulted from the selected parents was extended to determine gene action regarding stripe rust resistance [4] including some agronomic traits.

**Materials and methods.** *Plant material and experimental Site*: Forty five genetically diverse bread wheat accessions were collected from Wheat Research Institute (WRI), Faisalabad and NIFA, Peshawar. Origin and source of the genotypes is shown in Table 1. All the genotypes were planted in two separate experimental plots, i.e. one as stripe rust screening nursery and another as stripe rust free condition i.e. no artificial inoculation [4]. Each accession was planted in two replications in two meter long rows per entry with 20 seeds per row in randomized complete block design at experimental field of Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan, situated at latitude 34° 01' N and longitude 71° 40' E, and altitude 347 m AMSL, during October 2003. The plot area per entry in each experimental set was 1.2 m$^2$.

*Field evaluation of stripe rust and agronomic traits:* In stripe rust screening nursery, spreader 'Morocco' (a susceptible check) was sown as border around each entry of the nursery for spreading stripe rust (*Puccinia striiformis* f. sp. *tritici*) spores through the nursery material. Following the methodology of Zadoks et al. [10], the nursery material was inoculated by spraying spores suspension (1 gram urediospores ml$^{-1}$ of distilled water, 30 000 spores ml$^{-1}$ with tween 20 as emulsifier) through turbo air sprayer at tillering stage in late afternoon at the end of February, 2004. The inoculum (urediospores of *Puccinia striiformis* f. sp. *tritici*) was provided by National Wheat Diseases Research Program

(NWDRP) of National Agriculture Research Center (NARC) Islamabad, Pakistan. Stripe rust pathotypes prevalent in Pakistan have not been isolated so far. However the inoculum used in the present study has the virulence against yellow rust resistant genes *Yr1*, *Yr2*, *Yr6*, *Yr7*, *Yr9*, *Yr17*, *YrA* and *Yr27* and avirulance for *Yr3*, *Yr5*, *Yr10*, *Yr15*, *Yrsp* and *YrCv* [11]. The nursery material was covered with plastic sheets for 48 hours to avoid washing of spores by dew drops/rain and uncovered on the third day of inoculation [4]. In order to make conditions conducive for spores multiplication and disease development, spraying of plane water in late afternoon on each second day was conducted on the inoculated material for a period of fortnight till the disease symptoms appeared in the field [4]. Observations on individual plants for disease reaction were started 22 days after inoculation.

Rust severity (percentage of leaf area with symptoms) was determined by phenotypic observation and recorded from 0 to 100 % of rust infection on 5 selected plants with in each population according to the modified Cobb scale [12]. The severity was recorded from 0—9 points disease rating scale on the top three leaves of five randomly selected plants from each accession with little modification to those of Line et al. [13] as suggested by Imtiaz et al. [2]. Second reading of disease incidence on all selected plants was recorded after seven days of the first reading. Observations on response and severity of stripe rust were recorded according to Loegering [14]. The term trace (T) was used below 5 % severity for recording correct readings of severity up to interval 2. Five and 10 percent intervals were used from 5 to 20 percent and higher severity readings, respectively. The procedures regarding the response of individual plants within each population to the type of stripe rust infection are summarized in Table 2. Severity and reaction were recorded together with severity first. The coefficient of infection (CI) for the rust was calculated in the manner used in CIMMYT and IRN (USDA) i.e., by multiplying the response value with the intensity of infection in percent. Average coefficient of infection (ACI) was derived from the sum of CI values of each entry divided by the number of

Table 1

**Pedigrees and origin of 45 wheat genotypes tested
for resistance against stripe rust and other agronomic traits**

| Genotype | Pedigree | Origin | Source |
|---|---|---|---|
| Frontana | Fronteria/Mentana | Brazil | WRI, Faisalabad |
| B-92 | KAUZ 'S' | CIMMYT, Mexico | NIFA, Peshawar |
| Saleem-2000 | CHAM-6//KITE/PGO | CIMMYT, Mexico | NIFA, Peshawar |
| Tatara | JUP/ALD "S"//RLT 'S'/3VEE 'S') | CIMMYT, Mexico | NIFA, Peshawar |
| Fakhre Sarhad | PFAU 'S'/SERI/BOW 'S' | CIMMYT, Mexico | NIFA, Peshawar |
| CT-02009 | PUNJAB-96-0PAK | CIMMYT, Mexico | NIFA, Peshawar |
| CT-02019 | KAUZ//STAR/LUCO-M | CIMMYT, Mexico | NIFA, Peshawar |
| CT-02081 | VEE/TRAP#1//ANGRA/3/PASTOR | CIMMYT, Mexico | NIFA, Peshawar |
| CT-02192 | IRENA//CMH76.176/2*GEN/3/SNB/4/BORL95 | CIMMYT, Mexico | NIFA, Peshawar |
| CT-02266 | SW89.5181/KAUZ | CIMMYT, Mexico | NIFA, Peshawar |
| CT-02267 | SW89.5181/KAUZ | CIMMYT, Mexico | NIFA, Peshawar |
| CT-02204 | KAUZ/PASTOR | CIMMYT, Mexico | NIFA, Peshawar |
| CT-02306 | CMH80A.542/CNO79 | CIMMYT, Mexico | NIFA, Peshawar |
| CT-02248 | ALTAR84/AE.SQUARROSA(219)//SERI | CIMMYT, Mexico | NIFA, Peshawar |
| CT-02390 | FRET2 | CIMMYT, Mexico | NIFA, Peshawar |
| CT-01183 | SITTA/*SKUZ | CIMMYT, Mexico | NIFA, Peshawar |
| CT-01084 | ATTILA/3*BCN | CIMMYT, Mexico | NIFA, Peshawar |
| Inqilab-91 | WL 711/CROW 'S' | CIMMYT, Mexico | WRI, Faisalabad |
| Karwan | C182.2/C166.3/3/CNO/7C2*//CC//TOB/SWM6828 | CIMMYT, Mexico | WRI, Faisalabad |
| CT-99022 | URES/JUN//KAUZ | CIMMYT, Mexico | NIFA, Peshawar |
| Metal Tail | ORE F1 158/FDL//KAL/BB/3/NAC | India | WRI, Faisalabad |
| V-84051 | TAN'S'/3/TI/TOB//ALD | India | WRI, Faisalabad |
| Soleman-96 | (Pedigree not available) | CIMMYT, Mexico | WRI, Faisalabad |
| CB-61 | MILAN/HD.832 PK.3484-3A-3A-500A | CIMMYT, Mexico | WRI, Faisalabad |
| CB-82 | SATLUJ 86CMT/YR//MON 'S' | CIMMYT, Mexico | WRI, Faisalabad |
| CB-148 | WEAVER/TSC//WEAVER/3/WEAVER | CIMMYT, Mexico | WRI, Faisalabad |
| CB-179 | GAMDOW-6/CM79515-044Y... | CIMMYT, Mexico | WRI, Faisalabad |
| CB-185 | PASTOR-2/CM85295-0101TOPY-- | CIMMYT, Mexico | WRI, Faisalabad |
| CB-195 | MAYA74'S'/MON'S' | CIMMYT, Mexico | WRI, Faisalabad |
| CB-196 | MAYA74 'S'/MON CM 29480-20Y0Y | CIMMYT, Mexico | WRI, Faisalabad |
| CB-197 | PF70402/ALD'S'//PAT72/160//ALD'S'/3/PEW 'S' | CIMMYT, Mexico | WRI, Faisalabad |
| CB-289 | BOW'S'*2/PRL'S' | CIMMYT, Mexico | WRI, Faisalabad |
| UQAB-2000 | CROW'S'/NAC//BOW'S'PB 22138 | CIMMYT, Mexico | WRI, Faisalabad |
| CB-325 | TAN'S'/3/TI/TOB//ALD = V-84051 | CIMMYT, Mexico | WRI, Faisalabad |
| DRRM 03-04 | PB-96/V-87094//MH-97 | India | WRI, Faisalabad |
| CM-03-04 | PASTOR/3/VEE#5DOVE/BUC | India | WRI, Faisalabad |
| E-41 | SH-88/PAK-81//MH-97 | India | WRI, Faisalabad |
| V-2156 | Weaver/SH-88 | India | WRI, Faisalabad |
| V-03007 | Pb-96/V-87094//MH-97 | India | WRI, Faisalabad |
| AS-2002 | Pedigree not available | India | WRI, Faisalabad |
| CB-145 | CHOIX/STAR/3/HE1/3*CNO79//2*SERI | India | WRI, Faisalabad |
| Mango | RSK/AZ//PVN/CM 4170-9 | CIMMYT, Mexico | WRI, Faisalabad |
| BANA-4 | (Pedigree not available) | CIMMYT, Mexico | WRI, Faisalabad |
| CB-171 | ABTIN-1ICW92-0717 | CIMMYT, Mexico | WRI, Faisalabad |
| E-29 | SH-88/V-90A 204//MH-97 | CIMMYT, Mexico | WRI, Faisalabad |

Source: [4].

replications. Based on rating scale suggested by Doling [15] for selecting wheat varieties to powdery mildew, little modifications were made and a rating scale for disease resistance as adapted by PARC Islamabad, Pakistan for measuring cereal rusts severity [16] and later adopted by ARC (Agricultural Research Council) of Great Britain for the farmers was followed in this study. Using the following formula [17], AUDPC was calculated for individual plants from the C.I. values of the original rust severity data.

$$\text{AUDPC} = \Sigma\ [(X_i + X_{i+1})/2]t_i,$$

where $X_i$ and $X_{i+1}$ are severity in the form of CI value on date i and date $i + 1$, respectively and $t_i$ is the number of days between date $i$ and date $i + 1$.

Data for ten different agronomic traits as detailed in Table 5 were recorded on five individual plants to the trait's relevant appropriate growth stages with in each entry of the un-inoculated experimental set.

Mean, range, standard deviation, and coefficient of variation [18] were calculated from mean values of AUDPC for resistance against stripe rust and agronomic traits for measuring the genotypic differences among the accessions. Euclidean distance was estimated for all pairs of accessions. The resulting euclidean dissimilarity coefficient matrices were used to established the relationship between the accessions with cluster analysis using ward's method (Statistica version 7.0).

*DNA Extraction, use of molecular markers and genotyping*: Using two weeks old tender leaves (weighing 3 g), DNA samples from 45 wheat accessions were isolated according to the method outlined by Maroof et al. [19] in

Table 2

**Assessment and evaluating of stripe rust reaction and measurement of Coefficient of infection (CI)**

| Reaction | Observation | Response value |
|---|---|---|
| Disease reaction, observation and response value in the manner used in CIMMYT and IRN (USDA) | | |
| No disease | O | 0.0 |
| Traces | Tr | 0.2 |
| Resistant | R | 0.2 |
| Resistant to moderately resistant | R-MR | 0.3 |
| Moderately Resistant | MR | 0.4 |
| Moderately Resistant to Moderately Susceptible | MR-MS | 0.6 |
| Moderately Susceptible | MS | 0.8 |

| Genotype | Rep 1 | Rep 2 | Rep 3 | CI (Total) | CI (Average) |
|---|---|---|---|---|---|
| Procedure for calculating the CI and Average CI values (single observation) | | | | | |
| B-92 | 30S | 10MRMS | 5S | | |
| CI | 30(1) = 30 | 10(0.6) = 6.0 | 5(1) = 5.0 | 41.0 | 13.7 |
| Karwan | TR | 30MRMS | 10MR | | |
| CI | 0.2 | 30(0.6) = 18.0 | 10(0.4) = 4.0 | 22.2 | 7.4 |
| F-Sarhad | 5MSS | 10RMR | 5MR | | |
| CI | 5(0.9) = 4.5 | 10(0.3) = 3.0 | 5(0.4) = 2.0 | 9.5 | 3.2 |

O − No visible infection

R − Resistant. Necrotic areas with or without minute uredia

MR − Moderately resistant. Small uredia present surrounded by necrotic areas

MS − Moderately susceptible. Medium uredia with no necrosis but possibly some distinct chlorosis

S − Susceptible Large uredia and little or no chlorosis present

TR − Trace severity of resistant type infection

10MR − 10 percent severity of a moderately resistant type infection

50S − 50 percent severity of a susceptible type infection

CI: Coefficient of infection, Source: [4]

Table 3

**Reaction to stripe rust, CI values and AUDPC for 45 wheat genotypes**

| Genotype | Reaction to Yr (Single plant data) | | | | wAUDPC (No. of observations = 5) | |
| | (1st reading) | | (2nd reading) | | | |
| | Scoring | CI | Scoring | CI | AUDPC | SD (±) |
|---|---|---|---|---|---|---|
| Frontana | Tr | 0.2 | 20RMR | 6 | 35.10 | 2.14 |
| B-92 | 20MRMS | 12 | 20MSS | 18 | 143.40 | 21.83 |
| Saleem-2000 | 10MSS | 9 | 10MSS | 9 | 103.20 | 10.12 |
| Tatara | O | 0 | 10RMR | 3 | 45.67 | 7.09 |
| Fakhre Sarhad | 5MSS | 4.5 | 20RMR | 6 | 60.29 | 7.66 |
| CT-02009 | O | 0 | 20MR | 8 | 93.06 | 27.02 |
| CT-02019 | 20MRMS | 12 | 5MS | 4 | 99.91 | 13.24 |
| CT-02081 | 10MSS | 9 | 20MRMS | 12 | 96.06 | 15.70 |
| CT-02192 | TMSS | 0.09 | 10RMR | 3 | 175.31 | 31.04 |
| CT-02266 | Tr | 0.02 | 10MRMS | 6 | 69.05 | ±12.44 |
| CT-02267 | 10S | 10 | 5S | 5 | 84.68 | ±15.73 |
| CT-02204 | R | 0.2 | 10MRMS | 6 | 82.61 | 17.46 |
| CT-02306 | 5MRMS | 3 | 10S | 10 | 141.72 | 29.77 |
| CT-02248 | 5MS | 4 | 20MS | 18 | 100.62 | 25.18 |
| CT-02390 | 5S | 5 | 5MS | 4 | 77.22 | 11.87 |
| CT-01183 | 5RMR | 2 | 10S | 10 | 93.66 | 24.81 |
| CT-01084 | 20S | 20 | 50S | 50 | 122.40 | 25.22 |
| Inqilab-91 | 20MR | 8 | 20MRMS | 12 | 244.80 | 32.87 |
| Karwan | O | 0 | 20R | 4 | 70.50 | 7.81 |
| CT-99022 | 20MSS | 18 | 40MSS | 36 | 76.50 | 21.80 |
| Metal Tail | 30MR | 12 | 30MRMS | 18 | 144.53 | 37.64 |
| V-84051 | 20RMR | 6 | 20MRMS | 12 | 194.40 | 42.59 |
| Soleman-96 | Tr | 0 | 10MSS | 9 | 180.60 | 33.58 |
| CB-61 | 10MR | 4 | 20MSS | 18 | 121.80 | 12.36 |
| CB-82 | 5RMR | 1.5 | 30MRMS | 18 | 120.60 | 14.61 |
| CB-148 | 10MRMS | 6 | 20MRMS | 12 | 123.90 | 6.66 |
| CB-179 | 10MRMS | 6 | 20MRMS | 12 | 154.80 | 13.23 |
| CB-185 | 10MRMS | 6 | 10MSS | 9 | 94.44 | 9.73 |
| CB-195 | 10R | 2 | 10MSS | 9 | 129.60 | 13.39 |
| CB-196 | 20S | 20 | 30MSS | 27 | 181.20 | 22.45 |
| CB-197 | 20RMR | 6 | 40MSS | 36 | 237.60 | 24.40 |
| CB-289 | 20MR | 8 | 30MRMS | 18 | 250.80 | 29.69 |
| UQAB-2000 | 20MSS | 18 | 30MRMS | 18 | 157.20 | 11.93 |
| CB-325 | 10MRMS | 6 | 40MRMS | 24 | 177.90 | 8.71 |
| DRRM 03-04 | 10MSS | 9 | 10MRMS | 6 | 149.40 | 7.51 |
| CM-03-04 | 30MRMS | 18 | 30MR | 12 | 121.02 | 19.25 |
| E-41 | 10MR | 4 | 30MRMS | 18 | 165.00 | 14.84 |
| V-2156 | 20RMR | 6 | 50RMR | 15 | 118.94 | 16.62 |
| V-03007 | O | 0 | 30MR | 12 | 69.56 | 14.20 |
| AS-2002 | 30MR | 12 | 20MRMS | 12 | 99.60 | 12.66 |
| CB-145 | O | 0 | 5S | 5 | 85.50 | 14.88 |
| Mango | 10MRMS | 6 | 20MRMS | 12 | 182.70 | 34.52 |
| BANA-4 | 20MRMS | 12 | 60MS | 48 | 130.50 | 26.17 |
| CB-171 | 20MRMS | 12 | 30MSS | 27 | 111.06 | 20.11 |
| E-29 | 10MSS | 9 | 20MSS | 18 | 195.90 | 2.14 |

CI: Coefficient of infection, SD (±): Standard deviation, Source [4].

Table 4

**Mean values of ten different agronomic characters
of 45 bread wheat (*Triticum aestivum* L.) accessions**

| Genotype | Plant height (cm) | Days to heading | Days to maturity | Grain filling duration (days) | Flag leaf area (cm$^2$) | No. of spikes Plant$^{-1}$ | No. of spikelets spike$^{-1}$ | No. of grains spike$^{-1}$ | 1000 grain Wt. (g) | Grain yield plant$^{-1}$ (gm) |
|---|---|---|---|---|---|---|---|---|---|---|
| Frontana | 124.5 | 121.8 | 173.4 | 24.8 | 11.0 | 21.0 | 63.1 | 32.4 | 16.8 | 2.1 |
| B-92 | 83.8 | 112.1 | 158.7 | 25.4 | 8.0 | 22.5 | 69.3 | 32.4 | 15.4 | 2.2 |
| Saleem-2K | 77.8 | 116.7 | 163.6 | 21.7 | 9.5 | 22.6 | 69.6 | 34.0 | 15.0 | 2.3 |
| Tatara | 97.1 | 119.4 | 167.0 | 34.7 | 9.8 | 21.4 | 66.6 | 37.0 | 14.6 | 2.4 |
| F. Sarhad | 84.5 | 125.7 | 173.8 | 34.9 | 10.8 | 22.1 | 64.5 | 36.0 | 11.4 | 2.3 |
| CT-02009 | 94.7 | 121.4 | 164.6 | 20.3 | 5.9 | 20.6 | 76.1 | 26.0 | 14.3 | 2.0 |
| CT-02019 | 94.1 | 122.9 | 165.3 | 21.2 | 7.8 | 20.3 | 45.1 | 45.0 | 12.2 | 2.1 |
| CT-02081 | 94.1 | 122.9 | 165.3 | 21.2 | 7.8 | 20.3 | 45.1 | 45.0 | 12.2 | 2.1 |
| CT-02192 | 92.2 | 121.1 | 162.4 | 22.6 | 6.9 | 21.0 | 46.6 | 37.9 | 11.7 | 1.8 |
| CT-02266 | 97.2 | 123.5 | 168.9 | 24.3 | 8.3 | 22.5 | 48.7 | 37.5 | 11.1 | 1.8 |
| CT-02267 | 97.6 | 124.6 | 169.1 | 24.1 | 9.0 | 21.8 | 49.2 | 35.3 | 10.6 | 1.7 |
| CT-02204 | 93.5 | 126.0 | 164.9 | 22.2 | 5.9 | 21.1 | 49.6 | 37.2 | 11.2 | 1.8 |
| CT-02306 | 102.6 | 125.6 | 168.8 | 21.7 | 5.2 | 21.1 | 37.2 | 36.4 | 7.1 | 1.4 |
| CT-02248 | 92.2 | 119.1 | 160.1 | 20.4 | 8.7 | 20.0 | 48.1 | 34.2 | 8.0 | 1.6 |
| CT-02390 | 101.5 | 121.3 | 165.8 | 22.9 | 8.6 | 20.8 | 51.0 | 48.4 | 11.0 | 2.5 |
| CT-01183 | 96.0 | 124.2 | 161.0 | 20.3 | 5.3 | 20.2 | 63.3 | 31.7 | 10.0 | 2.0 |
| CT-01084 | 102.7 | 126.3 | 165.1 | 23.2 | 6.6 | 24.1 | 67.7 | 34.6 | 11.3 | 2.3 |
| Inqilab-91 | 87.7 | 123.9 | 163.9 | 24.5 | 12.3 | 22.6 | 56.8 | 38.4 | 13.1 | 2.2 |
| Karwan | 93.5 | 122.1 | 167.0 | 25.2 | 10.4 | 22.1 | 59.5 | 33.8 | 10.9 | 2.0 |
| CT-99022 | 101.1 | 125.2 | 167.8 | 22.7 | 9.3 | 24.3 | 62.0 | 44.0 | 10.4 | 2.8 |
| Metal Tail | 108.2 | 112.5 | 150.5 | 21.9 | 22.1 | 20.8 | 51.7 | 34.1 | 13.3 | 1.8 |
| V-84051 | 76.1 | 103.3 | 137.3 | 21.1 | 18.8 | 19.3 | 52.4 | 33.2 | 11.8 | 1.7 |
| Soleman-96 | 107.5 | 111.9 | 152.5 | 22.1 | 7.0 | 22.2 | 58.6 | 33.2 | 10.1 | 1.9 |
| CB-61 | 86.8 | 103.6 | 133.2 | 25.6 | 10.4 | 20.5 | 44.7 | 38.1 | 10.0 | 1.7 |
| CB-82 | 111.5 | 129.7 | 171.0 | 29.6 | 6.8 | 22.3 | 67.7 | 39.4 | 8.6 | 2.1 |
| CB-148 | 108.7 | 125.8 | 169.2 | 31.2 | 10.4 | 25.1 | 65.6 | 32.6 | 15.9 | 2.1 |
| CB-179 | 90.2 | 103.3 | 153.2 | 20.1 | 7.5 | 20.3 | 49.4 | 34.6 | 13.2 | 1.7 |
| CB-185 | 65.4 | 96.7 | 138.0 | 18.3 | 15.6 | 18.1 | 43.1 | 28.9 | 16.0 | 2.1 |
| CB-195 | 96.9 | 105.8 | 131.2 | 20.8 | 6.7 | 20.8 | 43.9 | 31.4 | 11.5 | 1.8 |
| CB-196 | 96.9 | 105.8 | 131.2 | 20.8 | 6.7 | 20.8 | 43.9 | 38.6 | 12.3 | 1.7 |
| CB-197 | 88.8 | 112.5 | 152.5 | 20.1 | 7.0 | 22.5 | 51.0 | 35.7 | 13.3 | 1.8 |
| CB-289 | 111.5 | 118.8 | 157.7 | 31.9 | 15.0 | 22.9 | 68.5 | 38.6 | 16.8 | 2.6 |
| UQAB-2000 | 103.4 | 125.4 | 165.4 | 33.0 | 14.2 | 23.3 | 68.6 | 37.1 | 14.8 | 2.5 |
| CB-325 | 84.7 | 101.5 | 144.8 | 29.0 | 10.2 | 19.6 | 49.6 | 32.2 | 14.2 | 1.6 |
| DRRM 03-04 | 95.0 | 116.0 | 165.8 | 30.7 | 14.2 | 20.6 | 43.0 | 44.4 | 13.5 | 1.9 |
| CM-03-04 | 90.9 | 123.5 | 166.6 | 26.5 | 10.8 | 22.4 | 47.5 | 40.6 | 13.0 | 1.9 |
| E-41 | 94.1 | 111.4 | 144.2 | 21.0 | 13.5 | 19.6 | 44.1 | 36.5 | 12.4 | 1.6 |
| V-2156 | 106.6 | 125.9 | 167.4 | 32.3 | 14.0 | 23.3 | 70.6 | 32.6 | 14.7 | 2.2 |
| V-03007 | 75.6 | 108.5 | 143.7 | 25.1 | 8.9 | 19.7 | 56.7 | 35.0 | 12.5 | 2.0 |
| AS-2002 | 96.3 | 105.2 | 140.2 | 27.0 | 7.8 | 19.8 | 47.5 | 34.5 | 17.4 | 1.6 |
| CB-145 | 100.9 | 89.8 | 168.0 | 25.2 | 8.5 | 20.5 | 55.0 | 34.0 | 17.1 | 1.8 |
| Mango | 106.2 | 125.9 | 163.3 | 23.0 | 9.4 | 20.5 | 57.3 | 36.8 | 18.5 | 2.1 |
| BANA-4 | 75.0 | 130.0 | 164.4 | 24.1 | 9.8 | 21.3 | 56.8 | 33.9 | 15.6 | 1.9 |
| CB-171 | 85.2 | 106.1 | 140.5 | 23.4 | 9.0 | 21.0 | 56.2 | 33.8 | 17.4 | 1.9 |
| E-29 | 100.2 | 120.6 | 162.0 | 22.5 | 9.2 | 21.3 | 51.8 | 38.1 | 19.0 | 2.0 |
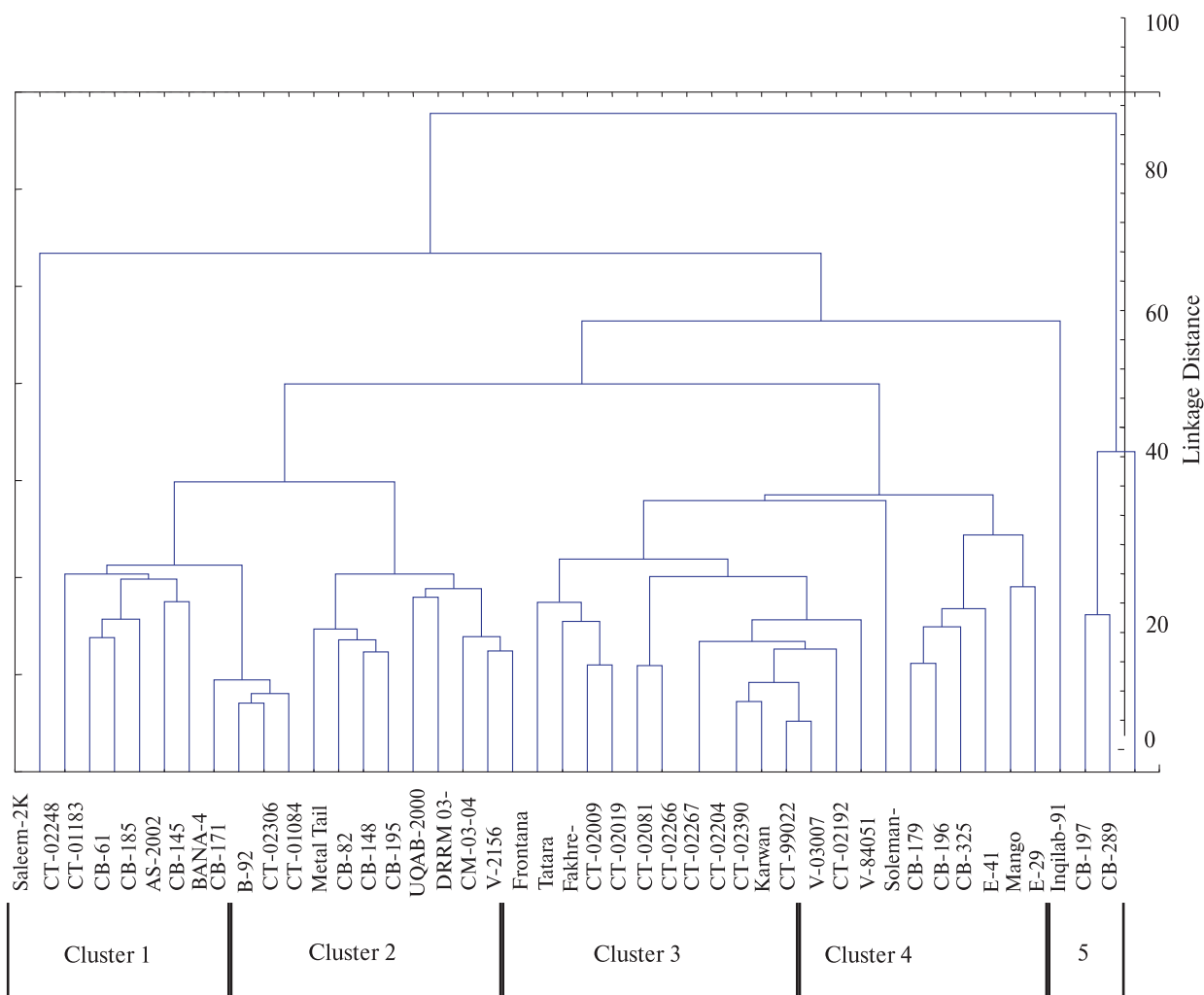
**Fig. 1.** Phenogram based on eleven quantitative traits in 45 wheat genotypes

Institute of Biotechnology, Jiangsu Academy of Agricultural Sciences (JAAS), Nanjing, China. In all 60 primers based on thirty stripes rust resistant genes were surveyed from grain genes and other sources for amplification of DNA samples (3 µl per sample) of forty five genetically diverse accessions. Among these, only 23 primers for 20 different stripe rust resistant genes were selected on the basis of their distinct banding patterns and were manufactured from Shanghai Sangon Biological Engineering Technology and services Co, Ltd. The primers along with stripe rust resistant genes and other necessary information are presented in Table 7. Polymerase chain reaction was performed in the 96 well (0.25 ml)

polycarbonate micro plate using 90 wells for two primers at a time per run. The template DNA (3 µl) in the PCR reaction was mixed with premix at the rate of 17 µl per sample. The Premix was consisted of dd $H_2O$ (9 µl), primer concerned (3 µl), 10X buffer (2 µl), 25 mM $MgCl_2$ (1.2 µl), 10 mM dNTPs (1.6 µl) and Taq polymerase (0.2 µl). The thermocycler was adjusted for three major steps per cycle. After initial denaturation at 94 °C for three minute, the PCR was carried out for 45 cycles. The cycle programme consisted of a denaturation step (94 °C for 3 minutes), an annealing step for 1 minute (the annealing temperature for each primer is shown in Table 7) and an extension step at 72 °C for 2 minutes. The

Table 5

**Clusters vise mean values and standard deviations based on AUDPC and 10 agronomic traits in bread wheat (*Triticum aestivum* L.) accessions**

| Trait | Group A | | | Group B | |
|---|---|---|---|---|---|
| | Cluster 1 | Cluster 2 | Cluster 3 | Cluster 4 | Cluster 5 |
| AUDPC | 104.49 ± 14.31 | 133.88 ± 13.66 | 73.57 ± 18.84 | 178.65 ± 12.94 | 244.40 ± 6.61 |
| Plant height (cm) | 86.18 ± 11.62 | 100.92 ± 8.46 | 96.08 ± 10.97 | 94.23 ± 10.01 | 96.01 ± 13.43 |
| Days to flowering | 110.15 ± 13.19 | 120.78 ± 7.77 | 121.95 ± 4.48 | 111.64 ± 9.00 | 118.40 ± 5.71 |
| Days to maturity | 152.11 ± 13.75 | 161.79 ± 11.5 | 165.89 ± 7.30 | 150.10 ± 11.55 | 158.03 ± 5.71 |
| Flag leaf area (cm$^2$) | 22.88 ± 2.88 | 26.93 ± 4.58 | 24.89 ± 4.67 | 22.46 ± 2.61 | 25.52 ± 5.97 |
| No. of spikes per plant | 9.40 ± 2.75 | 10.82 ± 5.08 | 8.733 ± 1.63 | 9.91 ± 3.98 | 11.42 ± 4.07 |
| No. of spikelets per spike | 20.44 ± 1.22 | 22.39 ± 1.47 | 21.37 ± 1.21 | 20.51 ± 0.93 | 22.65 ± 0.23 |
| No. of grains per spike | 53.8 ± 8.84 | 57.53 ± 12.84 | 56.701 ± 9.50 | 50.41 ± 5.23 | 58.76 ± 8.92 |
| 1000 grain weight (grams) | 33.67 ± 2.44 | 35.96 ± 4.10 | 37.89 ± 6.16 | 35.67 ± 2.43 | 37.52 ± 1.62 |
| Grain weight per spike (g) | 1.89 ± 0.22 | 2.02 ± 0.0 | 2.12 ± 0.30 | 1.77 ± 0.18 | 2.18 ± 0.37 |
| Grain yield per plant (g) | 14.05 ± 3.67 | 12.64 ± 2.80 | 12.22 ± 1.89 | 13.69 ± 3.07 | 14.37 ± 2.08 |

last cycle was followed by a final extension-polymerization of 10 minutes at 72 °C. The amplification products were separated on 1.2 % (W/V) agarose gell in 1 TBE buffer, stained by ethidium bromide, visualized and photographed under UV light through gel electrophoresis images analysis system.

Each DNA fragment amplified by a given primer was taken as a unit and the suggested bands linked to the QTLs were scored as present (1) or absent (0) for each of the primer-accession combination. The molecular size of the amplification product was measured with DNA marker DL 2000. The accessions were scored for the presence or absence of bands linked to the stripe rust resistance QTLs. Polymorphic bands were scored in MS excel programme for windows and used for further analysis. Similarity coefficient between wheat lines was computed using SIMQUAL module of computer software NTSYSpc [20]. The SAHN module was used for cluster analysis with the Unweighted Pair Group Method with Arithmetic mean (UPGMA).

**Results.** *Phenotypic clustering based on agronomic traits and AUDPC for stripe rust.* Mean values of AUDPC (Table 3) and ten agronomic traits (Table 4) were used to construct Euclidean dissimilarity coefficient matrix and phenogram (Fig. 1) was constructed for 45 wheat accessions. The dissimilarity range was from 2.44 to 16.16 among all the accessions. The dendrogram showed five clusters. Group A is consisted on three and group B on two clusters. Cluster vise means and standard deviations of AUDPC and ten agronomic traits are presented in Table 5 whereas grouping based on different clusters along with Euclidean distances is presented in Table 6. In group A, nine genotypes i.e. Saleem-2K, CT-02248, CT-01183, CB-61, CB-185, AS-2002, CB-145 and BANA-4 were in cluster 1 which presents 20 % of the total material (Table 6). The accessions in cluster 1 showed AUDPC in acceptable range (104.49 ± 14.31)

*Genotyping of the germplasm for stripe rust based on molecular markers.* DNA samples of 45 bread wheat accessions were amplified

**Grouping based on different clusters for 45 bread wheat accessions evaluated during rabi 2003–2004**

| Cluster | Frequency | %, age | Accessions with Eucli | | | | |
|---|---|---|---|---|---|---|---|
| | | | Group A | | | | |
| 1 | 9 | 20 | Saleem-2K (6.63) CB-171 (4.83) | CT-02248 (4.34) | CT-01183 (6.65) | CB-61 (8.43) | CB-185 (8.62) |
| 2 | 11 | 24,44 | B-92 (7.51) DRRM 03 (7.14) | CT-02306 (5.89) CM-03-04 (5.44) | CT-01084 (4.19) V-2156 (6.65) | Metal Tail (5.90) | CB-82 (6.21) |
| 3 | 13 | 28,88 | Frontana (13.68) CT-02204 (3.38) | Tatara (7.34) CT-02390 (5.54) | F-Sarhad (7.76) Karwan (2.81) | CT-02009 (5.54) CT-99022 (4.98) | CT-02019 (6.48) V-03007 (8.46) |
| | | | Group B | | | | |
| 4 | 9 | 20 | CT-02192 (5.18) E-29 (5.91) | V-84051 (7.10) | Soleman (4.37) | CB-179 (6.92) | CB-196 (6.50) |
| 5 | 3 | 6,67 | Inqilab-91 (6.46) | CB-197 (9.79) | CB-289 (5.80) | | |

Indications. In Parentheses is the Euclidian distance representing the separation/closeness among the lines including

and short plant height (86.18 ± 11.62). Cluster 2 (Table 6) accounts for 24.44 % of the total material and consists of eleven accessions (Bakhtawar-92 also B-92, CT-02306 CT-01084, Metal Tail, CB-82, CB-148, DRRM-03-04, CM-03-04 and V-2156). The accessions of this cluster exhibited largest flag leaf area (26.93 ± ± 4.58), more spikes per plant (10.82 ± 5.08) and more spikelets per spike (22.39 ± 1.47). Cluster 3 is consisted of 28.88% of the total population and comprised of thirteen accessions (Frontana, Tatara, FS, CT-02009, CT-02019, CT-02081, CT-02266, CT-02267, CT-02204, CT-02390, Karwan, CT-99022 and V-03007). As apparent from the mean values (Table 5), the accessions from this cluster can be picked up for highest Yr resistance (AUDPC: 3.57 ± ± 18.84), broad flag leaf area (24.89 ± 4.67) and larger seed size (1000 grain weight: 37.89 ± ± 6.16). Undesired traits of these accessions are the tendency to lodging because of tall

plant height (96.08 ± 10.97) and late maturity (165.89 ± 7.30).

Group B contains two clusters i.e. cluster 4 and cluster 5 (Table 6). Cluster 4 contains nine accessions (CT-02192, V-84051, Soleman, CB-179, CB-196, CB-325, E-41, Mango and E-29), sharing 20 % with total population. The accessions in this cluster exhibited medium range for all the traits (Table 5). Cluster 5 of group B is the smallest one (Table 6). It has three accessions (Inqilab-91, CB-197 and CB-289) and contributes only 3 % to the total population. The accessions included in this clusters have the highest value regarding yield components such as flag leaf area (25.52 ± ± 5.97), number of spikes per plant (11.42 ± ± 4.07), number of spikelets per spike (22.65 ± ± 0.23), grains per spike (58.76 ± 8.92), 1000 grain weight (37.52 ± 1.62), grain weight per spike (2.18 ± 0.37) and grain yield per plant (14.37 ± 2.08). The accessions of this cluster

Table 6

**accessions evaluated during rabi 2003−2004**

| dean Distances | | |
|---|---|---|
| AS-2002 (7.84) | CB-145 (10.61) | BANA-4 (16.16) |
| CB-148 (4.64) | CB-195 (9.77) | UQAB-2K (6.40) |
| CT-02081 (6.48) | CT-02266 (2.44) | CT-02267 (3.45) |
| CB-325 (4.44) | E-41 (4.24) | Mango (6.28) |

in the same cluster.

(mean AUDPC = 244.40 ± 6.61) were found highly susceptible to yellow rust (Table 5).

*Genotyping of the germplasm for stripe rust based on molecular markers.* DNA samples of 45 bread wheat accessions were amplified for 23 SSR, EST and STS primers linked to yellow rust resistant QTLs (Table 7). The similarity coefficient matrix were calculated (Table 9) and used to construct a dendrogram (Fig. 2) representing two groups (1 and 2) and four distinct clusters (A, B, C and D) which are further detailed in Table 8 with similarity coefficients in parentheses. The cluster A contains ten accessions i.e. Frontana, Saleem-2000, Fakhre-Sarhad (FS), CT-02267, CT-02204, CT-01153, CT-02019, CT-02192 and Bakhtawar-92 (B-92) representing 22.22 % of the total material (Table 8). The lines including in cluster A represent highly resistant material of the germplasm to the stripe rust. All the lines in cluster A are the same as in cluster 3 of Table 6

except Saleem-2000 and B-92 which are lying in cluster 1 and cluster 2, respectively (Table 6), representing moderately resistant lines to stripe rust. Cluster B contains 15 accessions representing 33.33 % of the total germplasm used in the study. The lines showing moderate resistance according to Table 5 fall into this cluster. Most of the lines belonging to cluster B come from cluster 1, 2, 3 and 4 of Table 6. Cluster C contains 16 genotypes i.e. CT-01084, CB-195, CB-82, CB148, Karwan, CB-185, Inqilab-91, CT-99022, E-29 (represented by T in dendrogram), Metal Tail, CB-195, CB-258, CB-197, CB-325, UQAB and V-03007 and comprises 35.55 % of the germplasm. Inqilab 91 and Karwan which are proved to be phonotypically susceptible to yellow rust in the present study are lying in this cluster. Four lines (AS-2002, Tatara, CT-02009 and DRRM-03-04 are included in cluster D representing 8.89 % of the total germplasm. The accessions in this cluster were from cluster 1, 2 and 3 of Table 6, respectively. The genotypes of this cluster (C) are resistant to moderately resistant (R-MR) in accordance with Table 5 for yellow rust because none of these lines belong to cluster 5 of Table 6. The lines with resistant to moderately resistant reaction are lying in clusters A and B and have come from cluster 1, 2, 3 and 4 of Table 6. The highly susceptible lines belonging to cluster 5 of Table 6 such as Inqilab-91, CB-197, CB-289 and UQAB 2000 are falling in cluster C representing the lines to be susceptible to stripe rust.

*Phenotypic clustering.* In the present study by using cluster analysis, 45 genotypes of the gene pool were classified into two distinct groups as well as five clusters (Fig. 1, Table 5, 6) regarding AUDPC and several other agronomic traits. The Euclidian distance ranged from 2.81 to 16.16 based on dissimilarity (Table 6). The lines which showed similarity with respect to stripe rust resistance (AUDPC) as well as agronomic traits were characterized in the same cluster. The distance among the lines of the same cluster helped to select the parents with considerable genetic diversity for crossing. The accessions included in various clusters were different from one another with respect to parentage and phenotypic expression. Seven different pa-
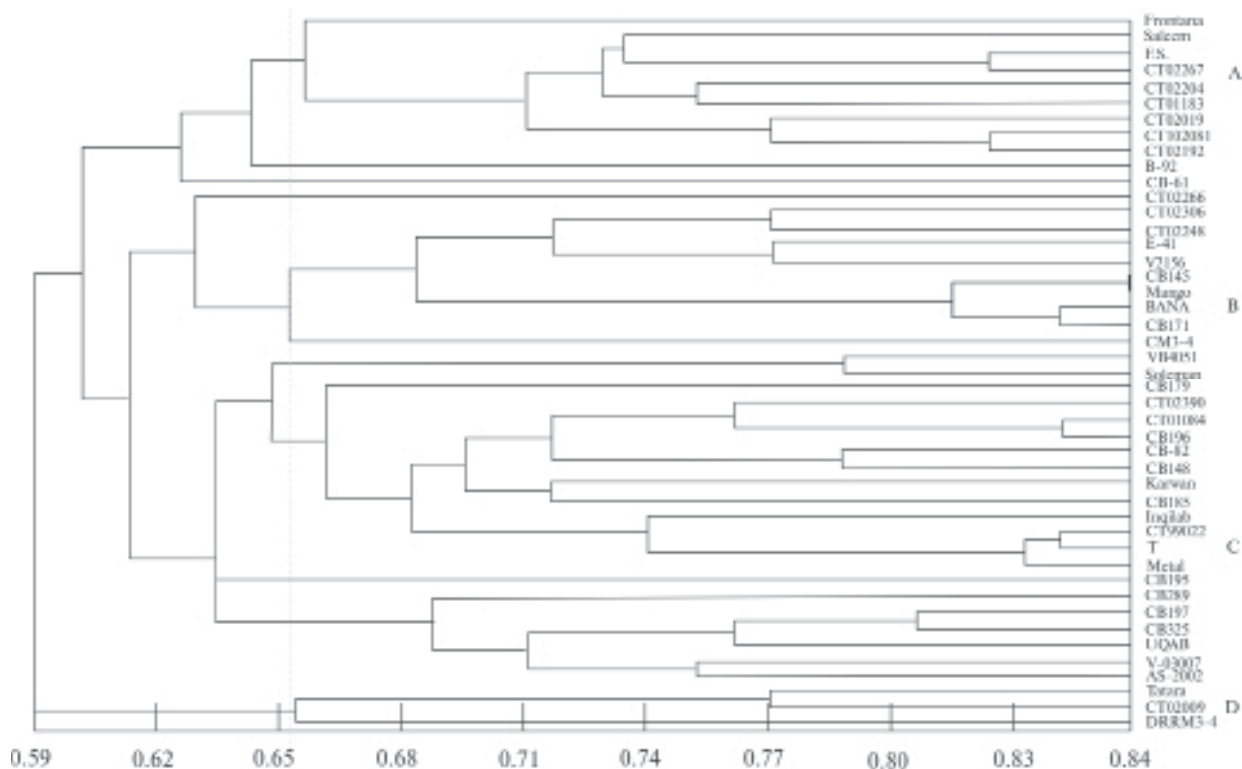
**Fig. 2.** Phenogram of 45 wheat accessions based on banding pattern of 23 molecular markers linked to stripe rust (Yr) resistant QTLs

rents i.e. Frontana, (B-92), Saleem-2000, Tatara, Inqilab-91, FS and Karwan, differing in their pedigree were selected for the crossing. Among these lines, Karwan and FS belonged to a common cluster (Cluster 3 of Table 6) but they still have discrimination by Euclidean distance of 4.95.

*Use of molecular markers and genotyping the accessions.* High level of polymorphism among the SSR primers was observed and more than 750 bands were produced as PCR products for all the accessions. Among these, only 56 scorable and reproducible bands (70.5 % polymorphic) were taken into account. The numbers of bands associated with each primer along with product size are presented in Table 7. These bands were exactly the same as suggested by different researchers to be closely linked to stripe rust resistant genes. The genotyping based on molecular markers (SSR, EST and STS) for stripe rust resistance genes classified the resistant and susceptible genotypes in distinct groups and clusters thereby separating the gene

pool (45 genotypes) into two different groups i.e. 1 and 2 (Fig. 2 and Table 8). Each group in turn was consisted of two clusters i.e. A, B and C, D, respectively. With little deviation, the cluster analysis based on genotyping showed almost the same results as yielded by the analysis of data based on phenotypic observation (Fig. 1, Table 6).

**Discussion.** The objective of the present study was to estimate the extent of genetic variability among 45 accessions of bread wheat in order to select suitable parents for crossing so as to combine genes into single lines from diverse genotypes with respect to stripe rust resistance and some other agronomic traits. The clustering was based on field data (regarding stripe rust resistance and some other agronomic traits as detailed in Table 5). Since the cluster analysis was based on AUDPC as a measure of stripe rust resistance and ten agronomic traits, therefore, the clusters were obtained on the basis of linkage distance and related traits. As the phenotypic observations

Table 7

### Primers sequence and annealing temperature of 23 molecular markers linked to 20 different stripe rust resistance QTLs

| Marker | Gene | Forward primer (5'-3') | Reverse primer (5'-3') | Product size (bp) | Annea-ling, °C | Bands |
|---|---|---|---|---|---|---|
| Xwmc356 | *Yr 3a* | GCCGTTGCCCAA-TGTAGAAG | CCAGAGAAACT-CGCCGTGTC | 245 | 61 | 2 |
| STS-7 & 8 | *Yr5* | GTACAATTCACCT-AGAGT | GCAAGTTTTCT-CCCTATT | 478, 472 | 50 | 2 |
| BF428563-2BL | *Yr7* | GAGGTTTATGCC-ATATCTGC | TCTTGGCCTGC-TGACATAC | 370, 375, 380 | 55 | 3 |
| BE442858 | *Yr7* | ATTTCGTTCTGAT-TAATTCC | CCCAAATAGTT-GTGATTA | 370 | 55 | 1 |
| Xgwm526-2B | *Yr7* | CAATAGTTCTGTG-AGAGCTGCG | CCAACCCAAAT-ACACATTCTCA | 138, 148 | 55 | 3 |
| Xgwm582-1BL | *Yr9* | AAGCACTACGAA-AATATGAC | TCTTAAGGGGT-GTTATCATA | 135 | 55 | 3 |
| WMS295 | *Yr10* | GCAGACCTGTGT-CATTGGTC | GACGGCTGCG-ACGTAGAG | 254, 258 | 60 | 2 |
| WMS11 | *Yr15* | GGATAGTCAGAC-AATTCTTGTG | GTGAATTGTGT-CTTGTATGCTTCC | 213, 202 | 50 | 2 |
| Xgwm130 | *Yr18* | AGCTCTGCTTCAC-GAGGA AG | CTCCTCTTTATA-TCGCGTCCC | 130 | 60 | 3 |
| Xbarc187-1B | *Yr24* | GTGGTATTTCAG-GTGGAGTTGTTTTA | CGGAGGAGCAG-TAAGGAAGG | 121, 126 | 55 | 2 |
| WE171 | *Yr 26* | TCGCAGATCTAA-GCTTTAC | AATCACCGTATT-GACCAAAG | 136, 167 | 55 | 2 |
| M13 2B | *Yr27* | CTAGGGCATAAT-TCCAACA | GATGAGTCCTG-AGTAACGA | 800 | 55 | 3 |
| BE442849 | *Yr28* | GGCCTGTTCAAG-TCGGACC | TACAGTGTTCTG-GCAGTGACATGG | 750 | 55 | 2 |
| WMS259 | *Yr29* | AGGGAAAAGACA-TCTTTTTTTTC | CGACCGACTTCG-GGTTC | 105 | 61 | 2 |
| WMS382 | *Yr32* | GTCAGATAACGC-CGTCCAAT | CTACGTGCACCA-CCATTTTG | 184,118, 108, 86 | 60 | 4 |
| Xgwm410A | *Yr34* | GCTTGAGACCGG-CACAGT | CGAGACCTTGAG-GGTCTAGA | 367, 338, 157, 151 | 55 | 4 |
| Xwmc477-2B | *YrTp-1* | CGTCGAAAACCGT-ACACTCTCC | GCGAAACAGAATA-GCCCTGATG | 156, 152, 115 | 61 | 3 |
| Xgwm493 | *Yrns* | TTCCCATAACTAAA-ACCGCG | GGAACATCATTTC-TGGACTTTG | 179, 171 | 60 | 2 |
| WMS0533 | *Yrns-B1* | AAGGCGAATCAAA-CGGAATA | GTTGCTTTAGGG-GAAAAGCC | 147 | 60 | 2 |
| WMS0802 | *Yrns-B1* | GGTGGACACTATT-CGCAGCT | GGCCCATCGTCA-CACTTACT | 132 | 60 | 2 |
| WMS1015 | *Yrns-B1* | CTTACGTGGCATG-CTTAGCA | TTAAGCTTGGGC-CTCATGTC | 149 | 50 | 2 |
| WMS1329 | *Yrns-B1* | GATCGCGTGGACG-GTCT | GAAAACGCTCAC-GGTCTTCT | 136 | 60 | 3 |
| WMS3087 | *Yrns-B1* | TGTAGTTGAGGGCA-CCTCCT | GTGCCATTGCTT-GGTGTAGA | 229 | 60 | 2 |
| Total | 20 | | | | | 56 |

**Grouping based on 23 molecular markers (SSR, EST and STS) linked to stripe rust resistant QTLs**

| Cluster | Frequency | %, age | Accessions with Eucli | | | | |
|---|---|---|---|---|---|---|---|
| | | | Group 1 | | | | |
| A | 9 | 20.00 | Frontana (10.8) CT-02192 (46.0) | Saleem-2K (18.6) | F-Sarhad (23.7) | CT-02267 (27.1) | CT-02204 (30.3) |
| B | 16 | 35.56 | B-92 (9.2) Mango (20.5) | CB-61 (10.4) BANA-4 (20.5) | CT-02266 (14.1) CB-171 (20.5) | CT-02306 (17.3) CM-03-04 (20.8) | CT-02248 (19.9) V-84051 (22.1) |
| | | | Group 2 | | | | |
| C | 16 | 35.56 | CT-01084 (2.1) E-29 (11.5) | CB-196 (2.7) Metal Tail (12.5) | CB-82 (3.9) CB-195 (13.2) | CB-148 (4.9) CB-289 (13.6) | Karwan (6.7) CB-197 (14.1) |
| D | 4 | 8.89 | AS-2002 (0.11) | Tatara (6.9) | CT-02009 (11.7) | DRRM 3-4 (12.0) | |

Indications. In Parentheses is the percent similarity coefficient representing the separation/closeness among the lines

are highly influenced by the environmental fluctuations, therefore, the grouping of the germplasm was ratified as well by molecular marker based analysis using some markers linked to stripe rust resistant quantitative trait loci (QTLs).

Some SSR, EST and STS molecular markers were included in the present study. Information regarding the primers was searched out from websites graingenes (http://wheat.pw.usda.gov/cgi-bin/graingenes/report.cgi), (http://maswheat.ucdavis.edu/protocols) and other sources. Since DNA samples consisted of extracts from three to five seedlings of hexaploid wheat accessions, a low intensity of any particular fragment may be explained by the lesser representation of that specific sequence in the bulk sample of DNA. Thus the intensity of the band was not taken in to account and the fragments with identical mobility were considered to be identical fragments. Using molecular markers linked to stripe rust resistant QTLs, the methodology for genotypic clustering of the present study was the same as suggested by Sixin et al. [21], McCartney et

al. [22] and Zhuping et al. [23]. They used the methodology for characterization of resistant and susceptible wheat lines by microsatellite markers linked to fusarium head blight (FHB) resistant quantitative trait loci. Using SSR markers linked to stripe rust resistant QTLs, Fahima et al. [1] used similar approach to determine the extent of genetic diversity among Triticum dicoccoides accessions.

*Comparisons between phenotypic and genotypic clustering.* With few exceptions the clustering based on genotyping with molecular markers is in agreement to that based on phenotypic data. The deviation might be due to the reason that the phenotypic clustering was based on AUDPC for stripe wrust as well as ten other agronomic traits. On the other hand, genotypic clustering was based only on molecular markers linked to stripe rust resistant genes in the accessions. Secondly the visual observations with respect to AUDPC and agronomic traits used in phenotypic clustering are highly influenced by environmental variations where as the genotypic clustering is more reliable as the bands appears only when the loci with respect

Table 8

**for 45 bread wheat accessions**

dean Distances

| | | |
|---|---|---|
| CT-01183 (32.7) | CT-02019 (37.4) | CT-02081 (42.0) |
| E-41 (20.1) | V-2156 (20.3) | CB-145 (20.4) |
| Soleman-96 (23.4) | CB-179 (24.3) | CT-02390 (26.9) |
| CB-185 (7.5) | Inqilab-91 (9.5) | CT-99022 (11.1) |
| CB-325 (14.5) | UQAB (14.9) | V-03007 (15.7) |

including in the same cluster.

to the primers are present in genomic DNA of the accessions. The parents of cross B-92 × × Frontana belong to cluster B and A of genotypic clustering with discrimination of 9.47 % similarity coefficient (Table 8). According to phenotypic clustering the same parents belong to cluster 2 and 3 of group A (Table 6). Of these, Frontana has previously known to have durable resistance to leaf rust at adult plant stage Singh et al. [24] whereas B-92 is moderately susceptible to susceptible with AUDPC of 143.40 (Table 3) under field condition in spite of the fact that it has *Yr27* gene [25]. In cross Saleem-2000 × Tatara, the first parent (Saleem-2000) belongs to cluster A of group 1 and $2^{nd}$ parent (Tatara) belongs to cluster D of group 2 with a difference of 11.7 percent similarity coefficient according to clustering based on molecular markers (Table 8). Based on phenotypic clustering, parent 1 is lying in cluster 1 and parent 2 in cluster 3 of group A with a difference of Euclidian dissimilarity of 0.47 (Table 6). In cross Inqilab-91 × × FS, the parents are separated with percent similarity coefficient of 14.2. The parent 1 be-

longs to cluster A of group 1 and parent 2 belongs to cluster C of group 2 (Table 8). As per phenotyping, the parents of this cross are separated with Euclidian dissimilarity of 1.3 where the first parent belong to cluster 4 of group B and the second parent belong to cluster 3 of group A. Though Inqilab-91 is previously reported to have *Yr27* for stripe rust resistance [25], but is now highly susceptible to the disease under field condition (Table 3). The parents of cross Karwan × FS has the separation by 17.0 % similarity coefficient where the first parent (Karwan) belongs to cluster C of group 2 and the second parent (FS) belongs to cluster A of group 1 with respect to genotypic clustering. According to phenotypic clustering, the parents though belong to the same cluster i.e. cluster 3 of group A but they still have the separation of 2.78 % by Euclidian distance.

*Genotypes selected for crossing.* Based on field observations (Euclidian distance) for AUDPC as measure of stripe rust resistance together with molecular characterization (percent similarity coefficients) of the gene pool (Table 1), the present grouping and clustering among the genotypes were used to select the parents of diverse genetic constitution such as Frontana, B-92, Saleem-2000, Tatara, Inqilab-91, FS and Karwan. Six multi-generations ($F_1$, $BC_1$, $BC_2$ and $F_2$) of each the crosses B-92 × Frontana, Saleem 2000 × Tatara, Inqilab-91 × FS and Karwan × FS. Later on, using Joint Segregation Analysis (JSA) as statistical approach, the study was extended to determine the gene action with respect to stripe rust (*Puccinia striiformis* f. species *tritici*) resistance [4]. The genetic effects on stripe rust resistance and other agro-morphological traits for some crosses will be published in other papers to avoid longevity and confusion.

Table 9

**Similarity matrix for 45 wheat genotypes of diverse genetic background**

| Geno-type | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.000 | | | | | | | | | | | | | | | | | | | | | | |
| 2 | 0.643 | 1.000 | | | | | | | | | | | | | | | | | | | | | |
| 3 | 0.714 | 0.714 | 1.000 | | | | | | | | | | | | | | | | | | | | |
| 4 | 0.554 | 0.589 | 0.732 | 1.000 | | | | | | | | | | | | | | | | | | | |
| 5 | 0.696 | 0.625 | 0.732 | 0.607 | 1.000 | | | | | | | | | | | | | | | | | | |
| 6 | 0.536 | 0.607 | 0.679 | 0.768 | 0.696 | 1.000 | | | | | | | | | | | | | | | | | |
| 7 | 0.589 | 0.625 | 0.625 | 0.607 | 0.679 | 0.661 | 1.000 | | | | | | | | | | | | | | | | |
| 8 | 0.679 | 0.607 | 0.750 | 0.589 | 0.732 | 0.679 | 0.804 | 1.000 | | | | | | | | | | | | | | | |
| 9 | 0.750 | 0.679 | 0.750 | 0.589 | 0.768 | 0.571 | 0.732 | 0.821 | 1.000 | | | | | | | | | | | | | | |
| 10 | 0.446 | 0.554 | 0.661 | 0.571 | 0.643 | 0.518 | 0.572 | 0.589 | 0.625 | 1.000 | | | | | | | | | | | | | |
| 11 | 0.625 | 0.662 | 0.732 | 0.571 | 0.821 | 0.589 | 0.643 | 0.696 | 0.768 | 0.714 | 1.000 | | | | | | | | | | | | |
| 12 | 0.589 | 0.696 | 0.732 | 0.536 | 0.678 | 0.554 | 0.714 | 0.661 | 0.696 | 0.607 | 0.786 | 1.000 | | | | | | | | | | | |
| 13 | 0.536 | 0.643 | 0.607 | 0.625 | 0.589 | 0.607 | 0.589 | 0.571 | 0.607 | 0.589 | 0.625 | 0.625 | 1.000 | | | | | | | | | | |
| 14 | 0.482 | 0.589 | 0.517 | 0.607 | 0.643 | 0.589 | 0.571 | 0.554 | 0.661 | 0.679 | 0.679 | 0.679 | 0.768 | 1.000 | | | | | | | | | |
| 15 | 0.589 | 0.661 | 0.696 | 0.607 | 0.714 | 0.625 | 0.714 | 0.732 | 0.768 | 0.607 | 0.678 | 0.678 | 0.625 | 0.643 | 1.000 | | | | | | | | |
| 16 | 0.589 | 0.518 | 0.732 | 0.607 | 0.750 | 0.661 | 0.679 | 0.732 | 0.732 | 0.607 | 0.679 | 0.750 | 0.518 | 0.607 | 0.715 | 1.000 | | | | | | | |
| 17 | 0.625 | 0.625 | 0.732 | 0.714 | 0.714 | 0.661 | 0.643 | 0.696 | 0.732 | 0.572 | 0.643 | 0.678 | 0.661 | 0.678 | 0.785 | 0.750 | 1.000 | | | | | | |
| 18 | 0.625 | 0.482 | 0.732 | 0.643 | 0.678 | 0.625 | 0.643 | 0.625 | 0.661 | 0.536 | 0.607 | 0.643 | 0.589 | 0.571 | 0.643 | 0.750 | 0.750 | 1.000 | | | | | |
| 19 | 0,464 | 0,464 | 0,607 | 0.589 | 0.518 | 0.571 | 0.554 | 0.571 | 0,536 | 0.679 | 0.518 | 0.589 | 0.607 | 0.661 | 0.589 | 0.589 | 0.732 | 0.661 | 1.000 | | | | |
| 20 | 0.536 | 0.536 | 0.714 | 0.554 | 0.661 | 0.536 | 0.554 | 0.602 | 0.643 | 0.661 | 0.625 | 0.661 | 0.500 | 0.589 | 0.661 | 0.696 | 0.696 | 0.643 | 0.732 | 1.000 | | | |
| 21 | 0.571 | 0.571 | 0.644 | 0.589 | 0.696 | 0.500 | 0.589 | 0.536 | 0.607 | 0.554 | 0.661 | 0.661 | 0.571 | 0.625 | 0.667 | 0.589 | 0.732 | 0.732 | 0.678 | 0.821 | 1.000 | | |
| 22 | 0.554 | 0.518 | 0.696 | 0.571 | 0.643 | 0.518 | 0.536 | 0.554 | 0.661 | 0.607 | 0.643 | 0.643 | 0.589 | 0.607 | 0.607 | 0.643 | 0.750 | 0.750 | 0.696 | 0.750 | 0.750 | 1.000 | |
| 23 | 0.589 | 0.482 | 0.661 | 0.571 | 0.643 | 0.554 | 0.607 | 0.554 | 0.589 | 0.536 | 0.571 | 0.607 | 0.589 | 0.500 | 0.607 | 0.664 | 0.678 | 0.678 | 0.661 | 0.661 | 0.696 | 0.786 | 1.000 |

| Genotype | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 24 | 1.000 | | | | | | | | | | | | | | | | | | | | | |
| 25 | 0.786 | 1.000 | | | | | | | | | | | | | | | | | | | | |
| 26 | 0.661 | 0.625 | 1.000 | | | | | | | | | | | | | | | | | | | |
| 27 | 0.589 | 0.643 | 0.783 | 1.000 | | | | | | | | | | | | | | | | | | |
| 28 | 0.589 | 0.589 | 0.732 | 0.661 | 1.000 | | | | | | | | | | | | | | | | | |
| 29 | 0.714 | 0.714 | 0.678 | 0.678 | 0.714 | 1.000 | | | | | | | | | | | | | | | | |
| 30 | 0.482 | 0.696 | 0.589 | 0.589 | 0.589 | 0.589 | 1.000 | | | | | | | | | | | | | | | |
| 31 | 0.643 | 0.786 | 0.714 | 0.714 | 0.678 | 0.678 | 0.732 | 1.000 | | | | | | | | | | | | | | |
| 32 | 0.519 | 0.661 | 0.661 | 0.661 | 0.589 | 0.625 | 0.714 | 0.768 | 1.000 | | | | | | | | | | | | | |
| 33 | 0.536 | 0.607 | 0.643 | 0.643 | 0.643 | 0.464 | 0.625 | 0.714 | 0.732 | 1.000 | | | | | | | | | | | | |
| 34 | 0.589 | 0.696 | 0.625 | 0.625 | 0.821 | 0.589 | 0.678 | 0.661 | 0.750 | 0.625 | 1.000 | | | | | | | | | | | |
| 35 | 0.571 | 0.678 | 0.607 | 0.607 | 0.678 | 0.536 | 0.625 | 0.714 | 0.801 | 0.714 | 0.768 | 1.000 | | | | | | | | | | |
| 36 | 0.536 | 0.571 | 0.571 | 0.571 | 0.589 | 0.393 | 0.554 | 0.607 | 0.554 | 0.678 | 0.554 | 0.678 | 1.000 | | | | | | | | | |
| 37 | 0.643 | 0.571 | 0.607 | 0.607 | 0.643 | 0.572 | 0.483 | 0.571 | 0.589 | 0.607 | 0.589 | 0.607 | 0.571 | 1.000 | | | | | | | | |
| 38 | 0.589 | 0.625 | 0.571 | 0.625 | 0.714 | 0.589 | 0.536 | 0.661 | 0.678 | 0.589 | 0.607 | 0.625 | 0.589 | 0.696 | 1.000 | | | | | | | |
| 39 | 0.571 | 0.571 | 0.607 | 0.607 | 0.750 | 0.428 | 0.554 | 0.714 | 0.732 | 0.643 | 0.553 | 0.607 | 0.643 | 0.678 | 0.768 | 1.000 | | | | | | |
| 40 | 0.518 | 0.625 | 0.625 | 0.696 | 0.714 | 0.554 | 0.536 | 0.732 | 0.750 | 0.696 | 0.607 | 0.696 | 0.661 | 0.625 | 0.643 | 0.732 | 1.000 | | | | | |
| 41 | 0.518 | 0.571 | 0.696 | 0.661 | 0.678 | 0.518 | 0.607 | 0.696 | 0.750 | 0.661 | 0.678 | 0.768 | 0.589 | 0.732 | 0.571 | 0.554 | 0.750 | 1.000 | | | | |
| 42 | 0.696 | 0.625 | 0,661 | 0.625 | 0.518 | 0.643 | 0.696 | 0.643 | 0.625 | 0.536 | 0.589 | 0.625 | 0.625 | 0.643 | 0.661 | 0.643 | 0.678 | 0.857 | 1.000 | | | |
| 43 | 0.732 | 0.696 | 0.625 | 0.625 | 0.661 | 0.678 | 0.768 | 0.678 | 0.625 | 0.571 | 0.661 | 0.661 | 0.661 | 0.714 | 0.696 | 0.643 | 0.714 | 0.821 | 0.785 | 1.000 | | |
| 44 | 0.607 | 0.589 | 0.696 | 0.696 | 0.696 | 0.625 | 0.589 | 0.643 | 0.696 | 0.643 | 0.625 | 0.536 | 0.589 | 0.625 | 0.625 | 0.643 | 0.661 | 0.643 | 0.678 | 0.857 | 1.000 | |
| 45 | 0.571 | 0.714 | 0.643 | 0.643 | 0.643 | 0.857 | 0.625 | 0.678 | 0.625 | 0.571 | 0.554 | 0.571 | 0.643 | 0.607 | 0.661 | 0.679 | 0.589 | 0.589 | 0.839 | 0.804 | 0.839 | 1.000 |

Indicatons. 1 – Frontana; 2 – B-92; 3 – Saleem-2000; 4 – Tatara; 5 – Fakhre Sarhad; 6 – CT-02009; 7 – CT-02019; 8 – CT-02081; 9 – CT-02192; 10 – CT-02266; 11 – CT-2267; 2 – CT-02204; 13 – CT-02306; 14 – CT-02248; 15 – CT-02390; 16 – CT-01183; 17 – CT-01084; 18 – Inqilab-91; 19 – Karwan; 20 – CT-99022; 21 – Metal Tail; 22 – V-84051 and 23 – Soleman-96; 24 – CB-61; 25 – CB-82; 26 – CB-148; 27 – CB-179; 28 – CB-185; 29 – CB-195; 30 – CB-196; 31 – CB-197; 32 – CB-289; 33 – UQAB-2000; 34 – CB-325; 35 – DRRM 03-04; 36 – M-03-04; 37 – E-41; 38 – V-2156; 39 – V-03007; 40 – AS-2002; 41 – CB-145; 42 – Mango; 43 – BANA-4; 44 – CB-171 and 45 – E-29.

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ОТБОР РОДИТЕЛЕЙ ДЛЯ СКРЕЩИВАНИЙ, ОСНОВАННЫХ НА ГЕНОТИПИРОВАНИИ И ФЕНОТИПИРОВАНИИ УСТОЙЧИВОСТИ К ЖЕЛТОЙ РЖАВЧИНЕ ЗЛАКОВ (*PUCCINIA STRIIFORMIS*) И АГРОНОМИЧЕСКИХ ПРИЗНАКОВ, В СЕЛЕКЦИИ МЯГКОЙ ПШЕНИЦЫ

45 генотипов мягкой пшеницы (*Triticum aestivum* L.) были фенотипически кластеризованы по десяти морфологическим признакам и Area Under Disease Progress Curve (AUDPC) как показателя устойчивости к желтой ржавчине. Кластеризация была подтверждена использованием 23 молекулярных маркеров (SSR, EST and STS), связанных с QTL локусами устойчивости к *Puccinia striiformis* f. sp. *tritici*. Целью работы было оценить степень генетической изменчивости, чтобы отобрать родителей для скрещиваний между устойчивыми и чувствительными к желтой ржавчине генотипами. Показатели отклонения, полученные из анализа морфологических признаков и AUDPC, были использованы для построения дендрограмм для кластеризации образцов. С использованием невзвешенного попарно-группового метода со среднеарифметическими значениями  другая дендрограмма, полученная на основе сходства значений коэффициентов, была использована для того, чтобы отличить генотипы по устойчивости к желтой ржавчине. Кластеризация по фенотипическим признакам дала в результате две основные группы и пять кластеров, в то время как генотипические данные дали две основные группы и четыре кластера, что позволило выделить высокоустойчивые, устойчивые, среднеустойчивые, среднечувствительные и чувствительные генотипы. За некоторыми исключениями, результат обоих способов кластеризации был почти одинаков: устойчивые и чувствительные генотипы попали в одни и те же кластеры как в результате молекулярного генотипирования, так и фенотипической кластеризации. В итоге было отобрано семь генотипов (Bakhtawar-92, Frontana, Saleem-2000, Tatara, Inqilab-91, Fakhre Sarhad and Karwan) с разным генетическим фоном для генов устойчивости к желтой ржавчине и некоторых других агрономических признаков после гибридизации.

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