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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

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

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 spike lets $spike^{-1}$	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

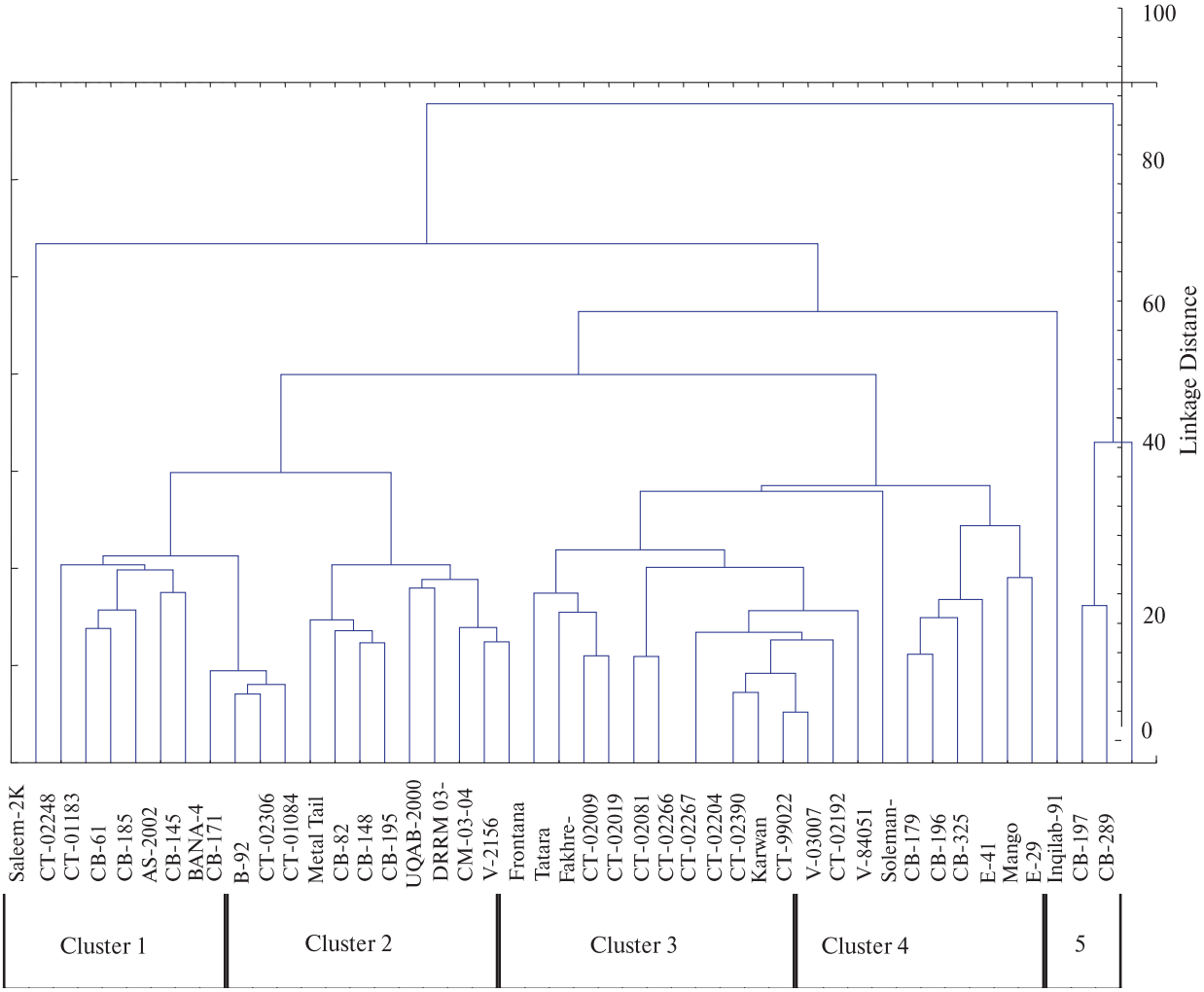

Fig. 1. Phenogram based on eleven quantitative traits in 45 wheat genotypes

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)	CT-02248 (4.34)	CT-01183 (6.65)	CB-61 (8.43)	CB-185 (8.62)
			CB-171 (4.83)				
2	11	24,44	B-92 (7.51)	CT-02306 (5.89)	CT-01084 (4.19)	Metal Tail (5.90)	CB-82 (6.21)
			DRRM 03 (7.14)	CM-03-04 (5.44)	V-2156 (6.65)		
3	13	28,88	Frontana (13.68)	Tatara (7.34)	F-Sarhad (7.76)	CT-02009 (5.54)	CT-02019 (6.48)
			CT-02204 (3.38)	CT-02390 (5.54)	Karwan (2.81)	CT-99022 (4.98)	V-03007 (8.46)
			Group B				
4	9	20	CT-02192 (5.18)	V-84051 (7.10)	Soleman (4.37)	CB-179 (6.92)	CB-196 (6.50)
			E-29 (5.91)				
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-

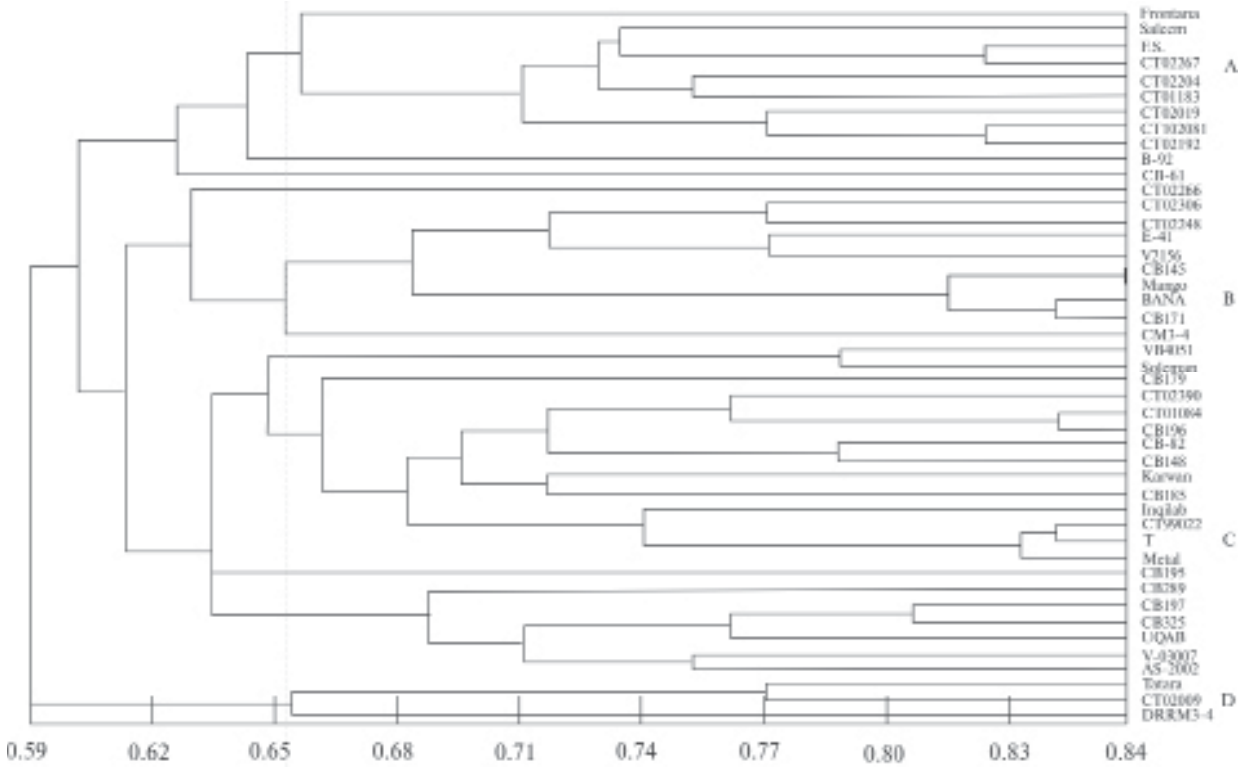

Fig. 2. Phenogram of 45 wheat accessions based on banding pattern of 23 molecular markers linked to stripe rust (Yr) resistant QTLs

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)	Annealing, °C	Bands
Xwmc356	*Yr 3a*	GCCGTTGCCCAATGTAGAAG	CCAGAGAAACTCGCCGTGTC	245	61	2
STS-7 & 8	*Yr5*	GTACAATTCACCTAGAGT	GCAAGTTTTCTCCCTATT	478, 472	50	2
BF428563-2BL	*Yr7*	GAGGTTTATGCCATATCTGC	TCTTGGCCTGCTGACATAC	370, 375, 380	55	3
BE442858	*Yr7*	ATTTCGTTCTGATTAATTCC	CCCAAATAGTTGTGATTA	370	55	1
Xgwm526-2B	*Yr7*	CAATAGTTCTGTGAGAGCTGCG	CCAACCCAAATACACATTCTCA	138, 148	55	3
Xgwm582-1BL	*Yr9*	AAGCACTACGAAAATATGAC	TCTTAAGGGGTGTTATCATA	135	55	3
WMS295	*Yr10*	GCAGACCTGTGTCATTGGTC	GACGGCTGCGACGTAGAG	254, 258	60	2
WMS11	*Yr15*	GGATAGTCAGACAATTCTTGTG	GTGAATTGTGTCTTGTATGCTTCC	213, 202	50	2
Xgwm130	*Yr18*	AGCTCTGCTTCACGAGGA AG	CTCCTCTTTATATCGCGTCCC	130	60	3
Xbarc187-1B	*Yr24*	GTGGTATTTCAGGTGGAGTTGTTTTA	CGGAGGAGCAGTAAGGAAGG	121, 126	55	2
WE171	*Yr 26*	TCGCAGATCTAAGCTTTAC	AATCACCGTATTGACCAAAG	136, 167	55	2
M13 2B	*Yr27*	CTAGGGCATAATTCCAACA	GATGAGTCCTGAGTAACGA	800	55	3
BE442849	*Yr28*	GGCCTGTTCAAGTCGGACC	TACAGTGTTCTGGCAGTGACATGG	750	55	2
WMS259	*Yr29*	AGGGAAAAGACATCTTTTTTTTC	CGACCGACTTCGGGTTC	105	61	2
WMS382	*Yr32*	GTCAGATAACGCCGTCCAAT	CTACGTGCACCACCATTTTG	184,118, 108, 86	60	4
Xgwm410A	*Yr34*	GCTTGAGACCGGCACAGT	CGAGACCTTGAGGGTCTAGA	367, 338, 157, 151	55	4
Xwmc477-2B	*YrTp-1*	CGTCGAAAACCGTACACTCTCC	GCGAAACAGAATAGCCCTGATG	156, 152, 115	61	3
Xgwm493	*Yrns*	TTCCCATAACTAAAACCGCG	GGAACATCATTTCTGGACTTTG	179, 171	60	2
WMS0533	*Yrns-B1*	AAGGCGAATCAAACGGAATA	GTTGCTTTAGGGGAAAAGCC	147	60	2
WMS0802	*Yrns-B1*	GGTGGACACTATTCGCAGCT	GGCCCATCGTCACACTTACT	132	60	2
WMS1015	*Yrns-B1*	CTTACGTGGCATGCTTAGCA	TTAAGCTTGGGCCTCATGTC	149	50	2
WMS1329	*Yrns-B1*	GATCGCGTGGACGGTCT	GAAAACGCTCACGGTCTTCT	136	60	3
WMS3087	*Yrns-B1*	TGTAGTTGAGGGCACCTCCT	GTGCCATTGCTTGGTGTAGA	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)	Saleem-2K (18.6)	F-Sarhad (23.7)	CT-02267 (27.1)	CT-02204 (30.3)
			CT-02192 (46.0)				
B	16	35.56	B-92 (9.2)	CB-61 (10.4)	CT-02266 (14.1)	CT-02306 (17.3)	CT-02248 (19.9)
			Mango (20.5)	BANA-4 (20.5)	CB-171 (20.5)	CM-03-04 (20.8)	V-84051 (22.1)
					Group 2		
C	16	35.56	CT-01084 (2.1)	CB-196 (2.7)	CB-82 (3.9)	CB-148 (4.9)	Karwan (6.7)
			E-29 (11.5)	Metal Tail (12.5)	CB-195 (13.2)	CB-289 (13.6)	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 2nd 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 belongs 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.

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Table 9

Similarity matrix for 45 wheat genotypes of diverse genetic background

Genotype	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	0.696	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.

REFERENCES

1. *Fahima T., Röder M.S., Grama A., Nevo E.* (1998). Microsatellite DNA polymorphism divergence in *Triticum dicoccoides* accessions highly resistant to yellow rust. Theor. Appl. Genet. 96:187–195.
2. *Imtiaz M., Cromey M.G., Hampton J.G., Hill M.J.* (2003). Inheritance of seedling resistance to stripe rust (*Puccinia striifomis* f. sp. tritici) in 'Otan' and 'Tritea' wheat (*Triticum aestivum*). New Zealand J. Crop Hort. Sci. 31:15–22.
3. *Wang X., Zheng W., Buchenauer H., Zhao J., Han Q., Huang L., Kang Z.* (2008). The development of a PCR-based method for detecting *Puccinia striiformis* latent infections in wheat leaves. Eur. J. Plant Pathol. 120:241–247.
4. *Irfaq M., Mir A.K., Khattak G.S.S., Tila M., Shah S.J.A.* (2009). Genetic behavior of controlling area under disease progress curve for stripe rust (*Puccinia striiformis* f. sp. *tritici*) in two wheat (*Triticum aestivum*) crosses. Phytopathology. 99:1265–1272.
5. *Smith S.E., Doss A.A., Warburton M.* (1991). Morphological and agronomic variation in North Africa and Arabian Alfalfas. Crop Sci. 31:1159–1163.
6. *Smith J.S.C., Smith O.S.* (1989). The description and assessment of distances between inbreed lines of maize. The utility of morphological, biochemical and genetic descriptions and a scheme of testing of distinctiveness between inbreed lines. Maydica. 34:151–161.
7. *Ashraf M., Cheema A.A., Qamar Z., Rashid M.* (2007). Detection of polymorphism in rice germplasm using RAPD markers. Pak. J. Bot. 39:2483–2493.
8. *Peter M., Darcatos L.J., Dumsday S., Rhiannon N., Olle O.I., Noel, Cogan P.M., Dobrowolski M., Fujimori H., Roderick V.S., Alan W.S., Smith K.F., John W.F.* (2006). Development and characterization of EST-SSR markers for the crown rust pathogen of ryegrass (*Puccinia coronata* f. sp. *lollii*). Genome. 49:572–583.
9. *Squirrel J., Hollingsworth P.M., Woodhead M., Russell J., Lowe A.J., Gibby M., Powel W.* (2003). How much effort is required to isolate nuclear microsatellits from plants? Mol. Ecol. 12:1339–1348.
10. *Zadoks J.C., Chang T.T., Konzak C.F.* (1974). A decimal code for the growth stages of cereals. Weed Res. 14:415–421
11. *Rattu A.R., Akhtar M.A., Fayyaz M., Bashir M.* (2007). Report on screening of wheats against yellow rusts and leaf rusts under NUWYT and NWDSN during 2005–2006. July, 2006. Crop diseases research programme, Institute of Plant and Environmental Protection, National Agriculture Research Centre, Pakistan Agriculture Research Council, Islamabad, Pakistan, pp. 13–14.
12. *Peterson R.F., Campbell A.B., Hannah A.E.* (1948). A diagnostic scale for estimating rust intensity on leaves and stems of cereals. Can. J. Res. 26:496–500.
13. *Line R.F., Konzak C.F., Alan R.E.* (1974). Evaluating

resistance to *Puccinia striiformis* in wheat. Induced mutation for disease resistance in crop plants. Proceedings of a research Co-ordination meeting, IAEA Austria, pp. 125–132.
14. *Loegering W.Q.* (1959). Methods for recording cereal rust data USDA International Spring Wheat Nursery.
15. *Doling D.A.* (1965). A method for the transformation of field data for comparing the mildew resistance of cereal varieties and the systemic deviation of the values in NIAB farmer's leaflets. J. Nat. Inst. Agric. Bot. 10:169–179.
16. *Aslam M.* (1982). Uniform procedure for development and release of improved wheat varieties. Mimeograph, PARC, Islamabad.
17. *Arama P.F., Parlevliet J.E., Van Silfhout C.H.* (2000). Trangressive segregation for resistance in wheat to septoria tritici blotch. Afric. Crop Sci. J. 8:213–222.
18. *Steel R.J.D., Torrie J.H.* (1980). Duncan's New Multiple Range Test. Principles and Procedures of Statistics. 2nd Ed., Mc Graw Hill Book Comp. Inc., New York.
19. *Maroof M.A.S., Soliman K., Jorgensen R.A., Allerd R.W.* (1984). Ribosomal DNA spacer length lymorphism in barley: Mendelian inheritance, chromosome location and population dynamics. Proc. Natl. Acad. Sci. USA. 81: 8014–8018.
20. *NTSYSpc* (1998). Exeter Software for windows, Version 2.02i, Copuright © 1986–1998. Applied Biostatistics Inc., Setauket, New York.
21. *Sixin L., Anderson J.A.* (2003). Marker Assisted Evaluation of Fusarium Head Blight Resistant Wheat Germplasm. Crop Sci. 43:760–766.
22. *McCartney C.A., Somers D.J., Fedak G., Cao W.* (2004). Haplotype diversity at fusarium head blight resistance QTLs in wheat. Theor. Appl. Genet. 109:261–271.
23. *Zhuping Y., Gilbert J., Procunier L.D.* (2006). Genetic diversity of resistance genes controlling fusarium head blight with simple sequence repeat markers in thirty-six wheat accessions from East Asian origin. Euphytica. 148:345–352.
24. *Singh R.P., Rajaram S.* (1992). Genetics of adult-plant resistance to leaf rust in 'Frontana' and three CIMMYT wheats. Genome. 35:24–31.
25. *McDonald D.B., McIntosh R.A., Wellings C.R., Singh R.P., Nelson J.C.* (2004). Cytogenetical studies in wheat XIX. Location and linkage studies on gene *Yr27* for resistance to stripe (yellow) rust. Euphytica. 136:239–248.

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