INVESTIGATING THE ALLELIC VARIATION OF LOCI CONTROLLING RUST RESISTANCE GENES IN WHEAT (*Triticum aestivum* L.) LAND RACES BY SSR MARKER

KHATTAK, S. H.[1,2*] – BEGUM, S.[2] – AQEEL, M.[2] – FAYYAZ, M.[3] – BANGASH, S. A. K.[4] – RIAZ, M. N.[2] – SAEED, S.[5] – AHMED, A.[5] – ALI, G. M.[2*]

[1]*Quaid-i-Azam University, Islamabad, Pakistan*

[2]*National Institute for Genomics & Advanced Biotechnology, National Agricultural Research Centre, Islamabad 45500, Pakistan*

[3]*Crop Disease Research Institute (CDRI), Plant and Environmental Department, National Agricultural Research Centre, Islamabad 45500, Pakistan*

[4]*Institute of Biotechnology and Genetic engineering (IBGE), The University of Agriculture, Peshawar, Pakistan*

[5]*Department of Botany, University of Balochistan, Quetta, Pakistan*

[*]*Corresponding authors*
e-mail: sahir_999@hotmail.com (phone: +92-331-9201-636); drgmali5@gmail.com

(Received 6th Jul 2020; accepted 17th Sep 2020)

Abstract. A study was conducted to determine the status of gene combination for rust (*Puccinia spp.*) resistance in 100 indigenous wheat land races also yield related traits were evaluated at two different locations during the 2016-17 periods with Randomized Complete Block Design. Variations for the studied traits were conferred by Principal Component Analysis (PCA), PC1 with 80.1% variation for the Peshawar, and 93.8% for the Islamabad location. WMC419, XGWM120, GWM174, XGWM140, XGWM410, XGWM111, WMC773, XWMC170, XWMC405, XWMC348 and XWMC407 has no allelic variation because of monomorphic nature while XGWM35 (200&225 bp), GWM 148 (190&200 bp), Barc 86 (200&210 bp), BARC 114 (105&200 bp), CSLV34 (150&190 bp), PSP3000 (300&350 bp), XGWM493 (150&300 bp), XGWM153 (100&300 bp), XGWM44 with 120,285,500 & 700 bp, XBARC4 (90&200 bp), XGWM125 (150&190 bp) show variation in alleles. Analysis of Yellow rust (Yr5,7,10,15,18,19,26,29,34, P138 and Sr13,) and leaf rust (Lr19,34 and 49) revealed A and B resistance groups. Group A comprised of 36% of total accessions, while rest of the accessions (64%) were present in group B. The study revealed high level of diversity assessed through PCA and cluster analysis. Based on marker results, 23 wheat lines ranging 17-21 alleles in combination were identified. These lines have great potential to be used in rust resistance breeding programs.
Keywords: *indigenous germplasm, yield related traits, principal component analysis, cluster analysis*

Introduction

Wheat (*Triticum aestivum* L.) is an essential cereal crop with a variety of uses and is considered the major component of bread. It belongs to the family *Poaceae* and genus *Triticum* and ranks first in cereals. It is a staple diet and provides more nourishment for the people of the world than any crop. There are two kinds of wheat species on which the world wheat production depends. The common/ bread wheat, which is mostly used today, has 42 (42=2n =6x) chromosomes containing AA, BB and DD genomes (Goutam et al., 2013). Wheat has high nutritional value and it gives nearly 21% of calories along with 20% of the protein (Waqar et al., 2018). During

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

2018 it was grown on an 8.7 mha area with 250 million tones production (FAO, 2020). This yield is still not enough to meet global requirements and climatologists also predicted that if existing/available cultivars and agronomic practices are used there is high chance that nearly 30% yield reduction will occur in South Asia's zone (Cgiar, 2017). One of the main factor in decline of yield are wheat diseases particularly Rust which is a common name for *Puccinia* spps i.e. fungal pathogens affecting lots of cereal crops especially wheat (Brown and Hovmøller, 2002; Waqar et al., 2018). Rust disease slows the growth of plant and decrease forage quality, also it results in poor seedling germination, foliar damage and reduced grain size (Chen et al., 2012). Seriousness of this disease can be depicted by its ability to grow everywhere provided that suitable substrate that may be wheat or other species in favorable environmental conditions. It can also pose a high risk to a lot of sustainable wheat production zones (Singh et al., 2004). Pandemic of rusts disease caused for example the 19^{th} century famine that covered large area of the world and affected human. This famine nearly cost 5 billion US dollars losses to cereals especially wheat for many years (Waqar et al., 2018). The severity of the disease affects the yield attributing traits. Therefore, appropriate selection of rust resistance parents is important in crossing nurseries/programs to enhance the genetic recombination for capability of yield enhancement by reducing rust losses (Ajmal et al., 2013).

In Pakistan not much studies have been conducted in order to characterize rust resistant genes using DNA markers particularly SSR for developing rust resistant varieties through gene pyramiding. Developed varieties must be resistant and high yielding. This can be achieved by using the best available technique/designs like ANOVA, PCA, cluster analysis and other statistical tools. PCA is the best tool and is used worldwide for exploring similarities; dissimilarities along with hidden patterns between genotypes especially in land races or if the association on data and grouping is not very clear (Granato et al., 2018). Similarly for markers there are lots of categories but two main groups are broadly used i.e. PCR-based SSRs and AFLPS (Granato et al., 2020). Long term fertility building requires a combined methodology instead of short range approach and targeted way out instead of conventional agriculture approaches, therefore combined efforts of agriculture scientists (of diverse discipline) specially plant breeders and geneticist is required to overcome loss inflicted by lethal disease i.e. rust by incorporation of resistance alleles in various high yielding crops through breeding programs (Waqar et al., 2018). Considering the situation of our country this study was done to evaluate the rust resistance alleles/genes at various loci in indigenous Pakistani wheat germplasm and also to evaluate their performance across different yield related traits for their future incorporation in different breeding programs.

Materials and Methods

The breeding material was collected from BCI, National Agriculture Research Center (NARC) Islamabad, Pakistan (official germplasm of Pakistan). For experimental study and to screen huge breeding material, germination test (15 seeds accession^{-1}) was performed at lab for the selection of 100 elite performer land races (out of 1000) using Filter paper Whatman No. 1 sheet (Punjabi and Basu, 1982). Accessions having higher germination rate i.e. above 70% were registered for further experimental analysis (*Table 1*).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

Table 1. *Performance of 100 selected accessions on the basis of germination test*

Accessions name	Accessions coding	Germinated seed	Germination %age	Accessions name	Accessions coding	Germinated seed	Germination %age
11123	1	12	80	11222	51	14	93
11126	2	11	73	11223	52	11	73
11144	3	14	93	11224	53	13	87
11145	4	13	87	11225	54	11	73
11152	5	14	93	11226	55	12	80
11154	6	12	80	11227	56	14	93
11155	7	11	73	11228	57	14	93
11160	8	11	73	11229	58	15	100
11161	9	15	100	11231	59	15	100
11162	10	14	93	11233	60	11	73
11163	11	13	87	11236	61	14	93
11164	12	15	100	11237	62	12	80
11166	13	14	93	11239	63	12	80
11168	14	12	80	11240	64	13	87
11170	15	13	87	11242	65	14	93
11171	16	11	73	11243	66	14	93
11173	17	11	73	11244	67	15	100
11174	18	13	87	11246	68	14	93
11177	19	14	93	11248	69	12	80
11178	20	15	100	11249	70	13	87
11179	21	13	87	11250	71	14	93
11181	22	12	80	11252	72	12	80
11183	23	14	93	11253	73	11	73
11184	24	11	73	11255	74	13	87
11185	25	12	80	11256	75	15	100
11186	26	11	73	11259	76	14	93
11187	27	12	80	11261	77	12	80
11188	28	15	100	11265	78	12	80
11189	29	15	100	11272	79	11	73
11190	30	12	80	11274	80	14	93
11192	31	15	100	11275	81	14	93
11193	32	14	93	11278	82	13	87
11194	33	12	80	11288	83	14	93
11195	34	11	73	11290	84	13	87
11197	35	14	93	11292	85	13	87
11198	36	12	80	11293	86	13	87
11200	37	13	87	11294	87	12	80
11202	38	15	100	11295	88	14	93
11205	39	12	80	11296	89	15	100
11207	40	13	87	11297	90	15	100
11208	41	12	80	11299	91	14	93
11209	42	15	100	11304	92	11	73
11210	43	14	93	11317	93	14	93
11211	44	12	80	11553	94	12	80
11214	45	12	80	11558	95	13	87
11215	46	11	73	12087	96	14	93
11216	47	13	87	12100	97	11	73
11217	48	11	73	12231	98	12	80
11218	49	13	87	18668	99	13	87
11221	50	12	80	24740	100	11	73

* Germination test was done prior to field evaluation

** Accessions with less than 70% germination were not selected in present study

For the evaluation morphological parameters RCB-Design was used with 3 replications across two locations i.e. in Islamabad (located at 33.6844°N and 73.0479°E) and in Peshawar region (located at 34.01^0N and 71.35^0E). The climate of the Islamabad region is a humid subtropical climate (Köppen climate classification. The temperature

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

ranges from a minimum of -3.9 °C (25.0 °F) in January to a maximum of 46.1 °C (115.0 °F) in June. The *rainy* period of the year lasts for *12 months*, from *November 19* to *November 6*, with a sliding 31-day rainfall of at least *0.5 inches*. *Most rain* falls during the 31 days centered on *July 29*, with an average total accumulation of *7.4 inches*.

Peshawar region lies 331 m above sea level and climate it's referred as a local steppe climate because there is not much rainfall all year long. The Köppen-Geiger climate classification is BSh. The temperature here averages 22.7 °C | 72.8 °F. Precipitation here is about 384 mm |15.1 inch per year. In Peshawar sowing was done on the 20th November while in Islamabad it was on the 1st December. Furthermore, proper agronomic practices (one ploughing with soil turing plough followed by 2-3 harrowing) were followed in order to have data accuracy. Crop was planted when soil was in proper water condition. Plot size was 5 m^2 while plant to plant distance was 10 cm and row to row distance was 18 cm. Seed drill method with 5 cm depth with seed rate of 100 kg/ha was used. Fertilizer treatment was 150 kg N + 60 kg P_2O_5 + 40 kg K_2 O/ha. Weeding was done when required by manual method in between the intervals while 2.4-D @ 500 g ai/ha in 700 liters of water was applied at 35-40 days after sowing.

Data was recorded by taking 5 samples from each replication on days to germination, 1st leaf stage, 2nd leaf stage, 3rd leaf, booting, half boot, heading, fertilization leaf length and width, plant height, peduncle length, 1000 g seed weight and seed plant^{-1} were determined for principal component analysis.

SSR markers (Primer Invitrogen) were used for finding out rust genetic diversity on molecular basis. DNA extraction was done by commercially available kit (Fermintas) and Polymerase Chain Reaction (Applied Biosystems 96 well USA, model 9902) was carried out by the protocol as mentioned in (Begum et al., 2014). Twenty-two (11 each monomorphic and polymorphic) previously reported DNA markers XGWM35 (200&225 bp), WMC419, XGWM120, GWM174, XGWM140, XWMC170, XWMC405, XWMC348, XWMC407 (*Figure 1*), GWM 148 (190&200 bp), Barc 86 (200&210 bp), WMC 773, BARC 114 (105&200bp), CSLV34 (150&190bp), PSP3000 (300&350bp), XGWM493 (150&300bp), XGWM153 (100&300bp), XGWM111, XGWM44 with 120,285,500 & 700bp (*Figure 2*), BARC4 (90&200bp), XGWM125 (150&190bp) and XGWM410 were employed to amplify PCR products (*Table 2*). PCR products were resolved in gel tank (Cleaver scientific LtD, made in UK serial Number MS 130711) on 4% agarose gel and were stained with Ethidium Bromide. The fragments were visualized under UV light in the gel documentation system (Syngene) at NIGAB for identification. For proper assessment of data all data was compiled with recommended guidelines necessary for statistical analysis. The data were analyzed using Web-based software using SaaS application (McNee, 2007) statistical packages "R" version 4.0.2 (R Core Team, 2014).

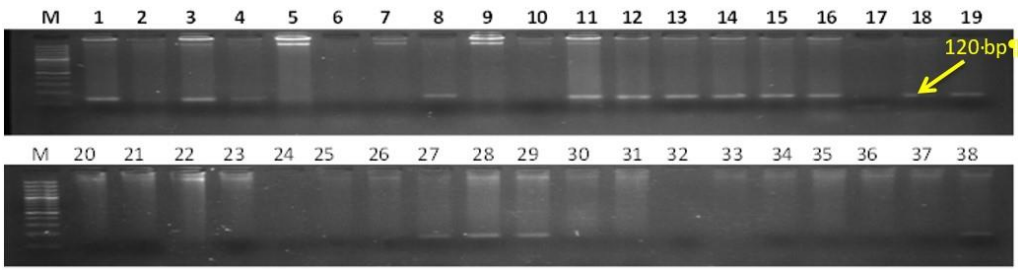

Figure 1: *PCR confirmation of 1-38 accessions with XWMC 407 SSR marker(monomorphic) indication presence of 120 bp bands size*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

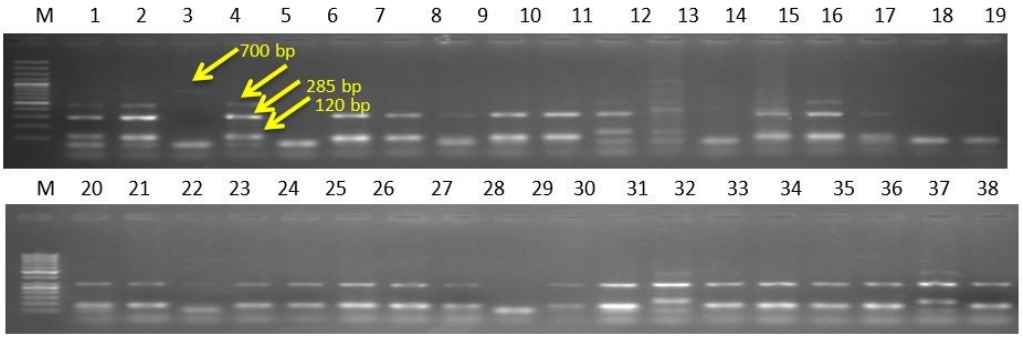

Figure 2: *PCR confirmation of 1-38 accessions with XGWM 44 SSR marker (polymorphic) indication presence of 120-700 bp bands size*

Table 2: *List of applied markers with band size and target site used to detect rust resistance genes*

S.No	Marker	Allelic variant (Band size)	Target Gene/site	Polymorphism	Reference
1	Barc 86	200 & 210	Yr-26	Yes	(Wang et al., 2008)
2	WMC773	298	Yr-26/Lr 38		(Mebrate et al., 2008)
3	BARC114	105 & 200	4D chromosome	Yes	(Båga et al., 2007)
4	XGDM125	150 & 190		Yes	(Tékeu et al., 2017)
5	XGWM35	200 & 225		Yes	(Abbasabad et al., 2017)
6	GWM174	220			(Li et al., 2005)
7	CSLV34	150 & 190	Yr-18/Lr34	Yes	(Begum et al., 2014)
8	PSP3000	300 & 350	Yr-10	Yes	(Begum et al., 2014)
9	WMC419	200	Yr-29		(Begum et al., 2014)
10	XGWM140	120	YrH52		(Peng et al., 2000)
11	XGWM44	120,285,500 &700	Lr-19/Yr18	Yes	(Li et al., 2005)
12	XGWM410	140	YrCN19		(Luo et al., 2006)
13	XGWM120	150	Yr-5		(Begum et al., 2014)
14	xwmc 170	170	2A/stripe rust		(Chhuneja et al., 2008)
15	XWMC405	220	7D		(Suenaga et al., 2003)
16	XWMC348	130	Lr 49		(Bansal et al., 2008)
17	XWMC407	120	Lr17		(Wang et al., 2010)
18	XGWM493	150 & 300	3B/stripe rust	Yes	(Börner et al., 2000)
19	XGWM153	100 & 300	YrP138	Yes	(Yue et al., 2010)
20	GWM148	190 & 200	Lr60	Yes	(Carter et al., 2009)
21	XGWM111	185	Yr33/ yr 26		(Dawit et al., 2019)
22	BARC4	90 & 200	Lr34/Yr18 & Lr46/Yr29	Yes	(Lillemo et al., 2008)

Results

Analysis revealed highly significant variation for many of the traits, furthermore genotype by location interaction were also highly significant (*Table S1-S8*). For genotype results of days to germination, 1st leaf emergence, 2nd leaf, leaf width, days to plant maturity, height and peduncle length showed highly significant results. While significant results were observed for traits like 2nd leaf, days to booting, days for half opening of spike, days to heading, days to fertilization, leaf length and seeds spike^{-1}. Moreover, traits like spike length, number of spikelet spike^{-1}, infertile spikelet spike^{-1}, awn length, spike plant^{-1} (fertile tillers) and seeds plant^{-1} showed non-significant variation. Similarly for genotype by location interaction results showed that highly

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

significant variation were observed due to the environment for 1st leaf emergence, 3rd leaf, days to booting, half opening of spike, days to heading, days to fertilization, days to plant maturity and plant height traits, while non-significant variation were reported for traits like days to germination, 1st leave emergence, 2nd leaf, leaf length, leaf width, peduncle length, spike length, number of spikelet spike^{-1}, infertile spikelet spike^{-1}, awn length, seeds spike^{-1}, spike plant^{-1} (fertile tillers) and seeds plant^{-1}.

All the significant environmental interactions are further explained in order to get the exact picture of genotypes performance for the selected locations. It's often difficult for a plant breeder to explain the extent of variation among large number of accession when assessed together. In order to effectively and reliably explain the extent of variation we have used statistical methods like PCA which will not only help us to explain the extent of variation but will help in the grouping of these accessions based on similarities and dissimilarities.

Islamabad region

The PCA was performed to find out the genetic base of accession (*Figures 3*, *4* and *Table S9*). Analysis revealed that PC1 was covering 96.7% variation while PCA component 2 (PC2) was only accommodating 2.1% variation (*Figure 3*). PCA distributed all the lines in to three major clusters based on genetic diversity. Cluster III comprised of only one line (2), cluster II was having two lines (6 and 46), and the rest of the accessions (97%) were clustered in cluster I based on their performance for the studied traits. Accessions 69, 7, 80, 29, 30, 91, 83, 92, 75, 81, 56, 82, 96, 93, 5, 65, 84, 27, 82, 96, 93, 73, 95, 19, 94, 31, 11, 97, 62, 54, 100, 18 and 4 are all in quadrant (Plot C) and contained high diversity level.

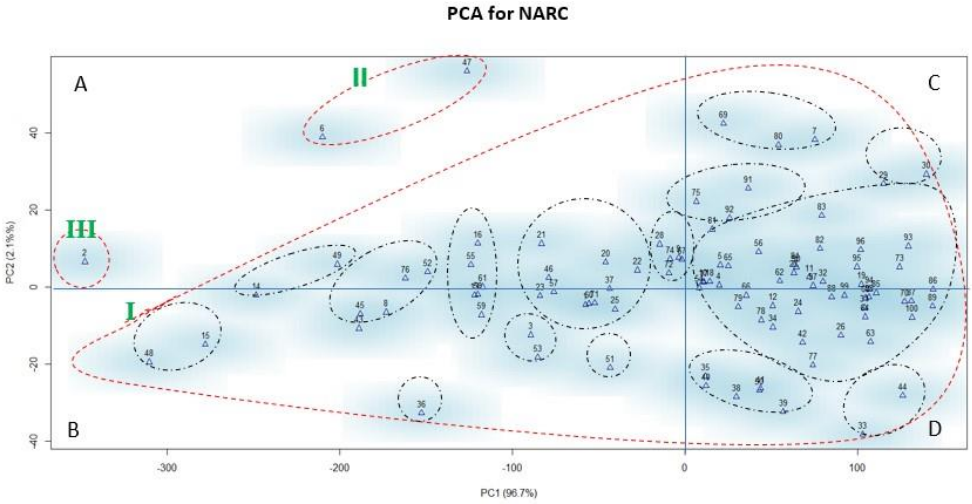

Figure 3. *PCA analysis of yield related traits for100 indigenous lines for Location A (NARC Islamabad field)*

Peshawar region

Analysis revealed that PC1 was explaining 80.1% variation while component 2 (PC2) was having 15.6% variation (*Figure 4*). PCA grouped all lines into five clusters. Cluster IV and V comprised of only one line i.e. 36 and 47 respectively, cluster III was

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

having two lines (52 and 92), cluster II was having 8 accessions (6, 7, 30, 69, 80, 91, 95 and 93), while the rest of the accessions (87%) were clustered in cluster I based on their performance for the studied traits.

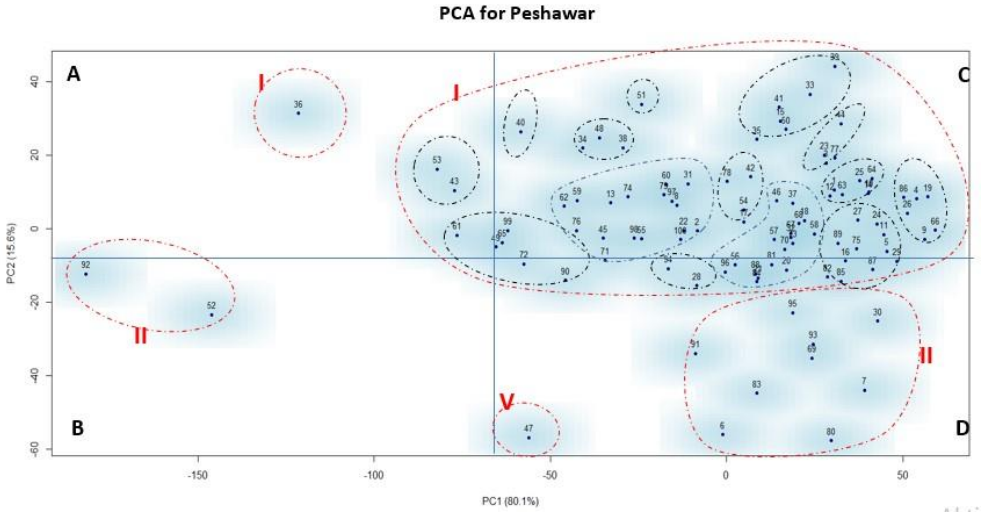

Figure 4. *PCA analysis of yield related traits for 100 indigenous lines for Location B (Peshawar)*

Furthermore, PCA analysis at both locations showed diversification of accession along with random pattern confirming broad genetic base and least similarity among the accessions with desired performance for most of the accessions in 3rd quadrant (Plot C) along with high genetic diversity compared to other quadrants.

Most of the accessions were found in positive quadrant (C) proving them to have broad genetic base with ideal performance for all traits except those where lower mean values are preferred. Each quadrant accessions were different from other quadrant. Remaining germplasm had random pattern confirming broad genetic base. The germplasm in plot B and D also exhibited the diversity were poor in performance for studied traits where higher mean value is required. Moreover, accessions were randomly arranged in quadrant B and C containing some outliers as well proving that more data of these particular accessions are required in order to link them clearly to quadrant C.

Cluster analysis based on SSR

A total of 22 SSR (mono/polymorphic) were used to detect rust resistance genes/alleles. A total of 36 reproducible bands were reported. Data was documented on the bases of detection of specific band sizes (*Tables S9* and *S10*). Bands frequency ranged from 5-21. A total of 23 land races were identified with the presence of nearly 50% bands frequency for SSR markers. Accessions 4, 8, 12, 26, 49, 68, 83 and 84 were having 17 score able bands out of 36. Similarly accessions 2, 6, 11, 14, 22, 27, 30, 31, 32, 38, 51 and 98 were having 18 score able bands, while accessions 24, 25 and 29, 52 had 19 and 21 score able bands respectively. A dendrogram was constructed on the basis of coefficient of dissimilarity to find out genetic diversity in indigenous germplasm. High level of diversity was observed i.e. two main groups were revealed (A and B). Group A was only having 36% of the total accessions while the rest of the accessions were grouped in B shown in (*Table 3*). Both groups were dissimilar with

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

Euclidian value of 10. Both the groups were divided into two sub-groups i.e. A-1, A-2 and B-1 and B-2 respectively (*Table 3* and *Figure 5*). A-1 and A-2 were 80 percent dissimilar (Euclidian value 0.8) while sub-groups B-1 and B-2 were 90% distinct. Apart from sub-group A-1, all were subdivided into 2 clusters each.

Table 3: *Distribution of 100 accessions into groups and clusters by cluster analysis on the basis of 22 SSR (mono/polymorphic) markers*

S.No	Groups	Clusters/Subcluster		Accessions number	Total (%)
1	A	A-1*		81, 84, 48, 50	4
		A-2	A-2-1	79, 92, 75, 33, 91, 35, 80, 77, 21, 34, 44, 78, 88, 89, 55, 25, 36	17
			A-2-2	28, 85, 32, 87, 94, 26, 23, 30, 95, 7, 93, 16, 19, 27, 57	15
2	B	B-1	B-1-1	61, 66, 99, 100, 11, 8, 98, 97, 73, 59, 63, 62, 65, 83, 20, 10, 22, 29, 60, 68, 86	21
			B-1-2	4, 56, 9, 74, 69, 18, 17, 13, 1, 5, 15, 14, 2, 12, 96, 31, 37, 76, 6, 58, 64, 3, 38, 82, 72, 24, 70, 67, 71	29
		B-2	B-2-1	43, 39, 90, 40, 41, 46	6
			B-2-2	49, 53, 51, 42, 45, 52, 47, 54	8

**Grouping of accessions are based on similarity in terms of the presence of same band sizes for each SSR marker
*Cluster A-1 has no sub-cluster due to least of all accessions

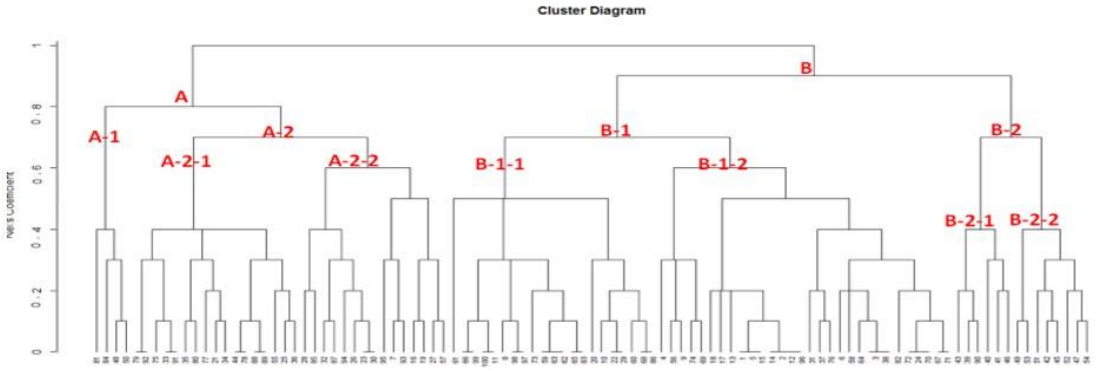

Figure 5: *Dendrogram showing Distribution of 100 accessions in to groups and clusters on the basis of 22 SSR markers*

Cluster B-1-1, B-1-2 and B-2-2, B-2-2 were almost 70% dissimilar to each other. B-1 was the largest subgroup containing 50% of the total accessions. Similarly cluster B-1-1 was the biggest of all with 29 accessions. The cluster grouped different accessions in terms of the presence of multiple alleles detected by 24 SSR markers for rust resistance (*Figure 5*).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

Discussion

Most agronomic traits are affected by the varying environment, due to which genetic basis dissection becomes very difficult (Wang et al., 2017). Genetic diversity is partially depleting as the breeding only focuses on yield related parameters (Ren et al., 2013; Mengistu et al., 2016b) which is of serious concern as narrowing the genetic base of wheat could be disastrous in fighting climate change and other yield related parameters or disease related issues. Present study was performed in order to find out genetic diversity on morphological traits as wells as for rust resistance genes. Similarly results effect due to location, varieties and G×E was also addressed for the indigenous population. Pooled ANOVA for RCBD showed highly significant variations in all three compartments (location, varieties and G×E) proving the locations and germplasm diversity. Principal Component Analysis can be used for multi-location trials effectively for genetic diversity (Bhanupriya et al., 2014; Mengistu et al., 2016a; Devesh et al., 2019). The PCA results are a bit different from Mengistu and Dvesh as their results showed 80% variation by PC compartments. PC plot proved high genetic diversity but it also suggested that the landrace lot of variability structure and consisted of exceedingly admixed lineages. Results of Gordon et al. (2019) and Meena et al. (2014) were slightly different as PC1 to PC7 showed 79.85% of the total variation. Though PCA explained the variation among germplasm in both the studies the difference in the detected variation depends on the source of the dataset used in the study. Similarities or dissimilarities among germplasm can be identified by cluster analysis on the basis of genetic distance between groups and clusters. All the accessions within one cluster are genetically similar while genetically dissimilar to other cluster. Results of our study are also in line with Chen et al. (2012) and Islam et al. (2012) who found different clusters and subgroups according to geographical zones of ninety wheat accessions with 269 SSR. Rust species specific alleles in crop can be easily recognized by comparing their lethality or resistance to different pathotypes. The accessions that are having more resistance gene combination must be explored further in breeding germplasm with available rust markers (Sadiq, 2019). In order to increase the biodiversity of Pakistani germplasm, present study was design to broaden the genetic base of our existing varieties by identifying accessions with rust resistant genes.

Yellow rust resistance

Rust resistance gene *Yr5* is present on 2BL chromosome and was transferred to bread wheat from spelt wheat. *Xgwm120* marker, that is present at a 12 cM distance from *Yr5* (Begum et al., 2014), showed a 150-bp fragment in 54% of wheat land races. Similarly, *Yr10* (stripe rust resistance gene) is dominant as well as race specific and was mapped on 2BS chromosome of wheat. At present *psp3000* marker is the only one available for *Yr10.* The distance between both marker and gene is 1.2 cM (Begum et al., 2014). Amplification of PCR product produced polymorphic band i.e. 300bp and 350 bp fragment in 43% of population. Moreover, the co-dominant nature of this marker is considered ideal for *Yr10* gene identification in segregating population. Australian germplasm was also successfully screened by this marker (Bariana et al., 2002).

Locus *Yr18* and *Lr34* confer slow rusting to stripe and leaf rust (Begum et al., 2014). The *csLV34* marker (Lagudah et al., 2006) amplified 150bp and 190bp fragment in 16% of the genotypes proving presence of *Yr18* gene, whereas a 215bp size was associated with the nonappearance of *Yr18* was successfully able to amply in 53% of total

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

population. The rest of land races didn't amplify any of the bands. This marker is co-dominant which makes them suitable for use in early segregating generations. The frequency of this gene is quite low in Pakistani population as previously reported (Begum et al., 2014) hence there is dire need to broaden the race-nonspecific resistance for yellow rust. Similarly, results were also observed previously (Begum et al., 2019) where all advanced wheat lines were checked by CSLV34 for presence of Lr34/Yr18 that yielded a PCR product of 150 bp amplification in 43% of the accessions.

Two markers Barc 86 (200bp) and Wmc 773 (298bp) were used to detect the presence of *Yr26* gene. Former showed its presence in 94 accessions while the latter was detected in 74 accessions out of the total population. Wheat genotypes with *Yr26* gene were found resistant to majority of *Pst* races in virulent tests (Wang et al., 2008). However, virulence against this gene has recently been detected in Australia. Pyramiding of this gene with other *Yr* genes may be helpful to broaden the genetic base of wheat against *Pst*. Three other markers were used to detect presence of *Yr* 29 gene. Wmc 419 (200bp) showed its presence in 55% of the population while xgwm 140 (120bp) and xgwm 410 (140 bp) were present in 56 and 45% of the landraces, respectively. Similar results were obtained by Begum et al. (2014) for *Yr26* and 29 and genes. The *Yr* resistance for barc4 (90-100bp) is located on arm 5BS of the chromosome (Law and Worland, 1997), similarly Maccaferri et al. (2015) reported that it is associated with genetic resistance for yellow rust but Lillemo et al. (2008) was able to find a significant Quantitative trait locus (QTL) for Powdery mildew resistance on 5BS,close to the SSR marker Xbarc4. STS markers *Xgwm111* with a band size of 185bp was detected in 70% of the population. This marker is considered more reliable for *Yr* identification (Sadiq, 2019) that is linked to *Yr* 15 and 26 with a distance of 1.9 cM (Ma et al., 2001). This marker has been also reported to be linked with *Yr33* as well (Dawit et al., 2019).

The marker Xgwm148 (190-200 bp) is associated with high-temperature adult-plant resistance to stripe rust resistance in spring wheat, present on 2BS chromosome in between a 16.9 cM region (Carter et al., 2009). Its 190 bp and 200 bp allele was detected in 63% and 13% population respectively in present study. Marker for locus xwmc170-2A is associated to stripe rust resistance. It was maped on chromosome 2A of *T. monococcum* within 3.6 cM distance for Xwmc407 and Xwmc170 marker (Chhuneja et al., 2008). In the present study Xwmc407 was identified in 60 out of 100 land races proving that the majority of germplasm was having resistant genes. Microsatellite marker xwmc348 (130bp) was screened in 31 of the land races confirming the presence of *Lr* 49 in this population. Previously the same was reported by Bansal et al. (2008) as well. Xgwm153 (100bp and 300bp) was present in almost 82% of the population. It is 8.2 cM away from the YrP138 resistance gene. This is quite different from other known resistance as it shows resistance to the prevailing Chinese Pst raceCYR32 at seedling stage (Yue et al., 2010). The marker Xgwm493 is present on the short arm of chromosome 3BS indicating the location of the stripe rust resistance gene (Börner et al., 2000) was able to detect the presence of Sr resistance gene in 57% (150 bp) and 66% (300bp) of land races.

Leaf rust resistance

Microsatellite markers barc 114 is present on chrosome 4D (Båga et al., 2007) amplified 105-200 bp band, xgwm 165 (225 bp), gwm 174 (220bp) and xgwm 125 (150-190 bp) were used to detect this gene. These markers identified 85, 62, 1, 55 land

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

races with *Lr* 34 gene, respectively. Xgwm 125 marker has been previously used for genetic diversity as well (Tékeu et al., 2017). Similarly, marker Xgwm35 has been previously used for genetic diversity as well (Abbasabad et al., 2017). Xgwm44 (185 bp) is closely linked to *Lr* 19 (Li et al., 2005) gene and is broadly used for its detection and is also used for maping of gene (Xing et al., 2006) was able to screen 66% of the population. This marker was also reported to be linked with *Yr18* gene (Imtiaz et al., 2004). Marker Xwmc407 (120 bp) was used to detect *Lr* 17 in 42% of the population used in present study, which is flanked by this marker that is on the short-arm of wheat 2A chromosome (Wang et al., 2010). But it is also widely used to examine the presence of Yr17 gene (Sadiq, 2019). Suenaga et al. (2003) mentioned the linkage between stripe and leaf rust severity detected by the marker Xwmc405 which is present on chromosome 7D. Its allele of 120bp was in 81 land races proving that these lines can be used for developing both leaf and stripe rust resistant varieties. But reports suggest that this marker is also linked to other traits as well (Golabadi et al., 2011).

Conclusion and Recommendations

Results indicated high level of diversity in studied pool of indigenous lines which can be used for creating varieties for any desired traits. Based on marker results, 23 wheat lines ranging 17-21 alleles in combination were identified. Durable resistance in the present study was a bit low making it compulsory that these selected land races should be used in future breeding programs as donor parents to increase gene frequency for broadening the available genetic base of Pakistani varieties that will be newly developed. Over the last three decades, rust has destroyed yield of wheat crop significantly therefore, there is a need to develop lines by pyramiding of gene pool in our varieties. This study will ultimately help in yield enhancement, moreover the study discovered the presence of variability among the tested genotypes for rust resistance genes and confirmed possibility for increasing wheat productivity in the target area by incorporating these diversified lines in future breeding programs. Combined PCA and SSR provides sufficient power in identifying diversity at phenotypic and genetic level, thus providing a better chance to plant breeders and geneticists to select desirable genotypes out of base population with unknown diversity or variability. Our results highly recommended that these land races must be evaluated with more number of markers linked to all available pool of rust resistant genes. Furthermore, gene sequencing of these lines for the marker that showed allelic variations are also recommended for finding our desirable gene combination that will be a great assistance in developing future wheat varieties.

Acknowledgements. The Government of Pakistan funded the study through NIGAB project ‘National Institute for Genomics and Advanced Biotechnology’. All the authors are thankful to the National Agricultural Research Centre and Crop Diseases Research Program Islamabad Pakistan for providing seeds and guidance. The authors are obliged to Assistant Professor Dr Sajid Ali (Institute of Biotechnology & Genetic Engineering (IBGE) for assistance in rust validation and Mr. Zabih Ullah (Department of Plant Breeding & Genetics, Faculty of Crop Production Sciences, The University of Agriculture, Peshawar, Pakistan) for assisting statistical analysis in the present study.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

REFERENCES

[1] Abbasabad, E. Z., Mohammadi, S. A., Moghaddam, M., Kamali, M. R. J. (2017): Analysis of genetic diversity, population structure and linkage disequilibrium in Iranian wheat landraces using SSR markers. – Plant Genetic Resources 15: 327.

[2] Ajmal, S. U., Minhas, N. M., Hamdani, A., Shakir, A., Zubair, M., Ahmad, Z. (2013): Multivariate analysis of genetic divergence in wheat (*Triticum aestivum*) germplasm. – Pak. J. Bot. 45: 1643-1648.

[3] Båga, M., Chodaparambil, S. V., Limin, A. E., Pecar, M., Fowler, D. B., Chibbar, R. N. (2007): Identification of quantitative trait loci and associated candidate genes for low-temperature tolerance in cold-hardy winter wheat. – Functional & Integrative Genomics 7: 53-68.

[4] Bansal, U. K., Hayden, M. J., Venkata, B., Khanna, R., Saini, R., Bariana, H. S. (2008): Genetic mapping of adult plant leaf rust resistance genes Lr48 and Lr49 in common wheat. – Theoretical and Applied Genetics 117: 307-312.

[5] Bariana, H., Brown, G., Ahmed, N., Khatkar, S., Conner, R., Wellings, C., Haley, S., Sharp, P., Laroche, A. (2002): Characterisation of Triticum vavilovii-derived stripe rust resistance using genetic, cytogenetic and molecular analyses and its marker-assisted selection. – Theoretical and Applied Genetics 104: 315-320.

[6] Begum, S., Iqbal, M., Ahmed, I., Fayyaz, M., Shahzad, A., Ali, G. M. (2014): Allelic variation at loci controlling stripe rust resistance in spring wheat. – Journal of genetics 93: 579-586.

[7] Bhanupriya, B., Satyanarayana, N., Mukherjee, S., Sarkar, K. (2014): Genetic diversity of wheat genotypes based on principal component analysis in Gangetic alluvial soil of West Bengal. – J. Crop Weed 10: 104-107.

[8] Börner, A., Röder, M., Unger, O., Meinel, A. (2000): The detection and molecular mapping of a major gene for non-specific adult-plant disease resistance against stripe rust (Puccinia striiformis) in wheat. – Theoretical and Applied Genetics 100: 1095-1099.

[9] Brown, J. K., Hovmøller, M. S. (2002): Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. – Science 297: 537-541.

[10] Carter, A. H., Chen, X., Garland-Campbell, K., Kidwell, K. K. (2009): Identifying QTL for high-temperature adult-plant resistance to stripe rust (Puccinia striiformis f. sp. tritici) in the spring wheat (*Triticum aestivum* L.) cultivar 'Louise'. – Theoretical and Applied Genetics 119: 1119-1128.

[11] Cgiar, I. A. U. (2017): Project Management, Good Practice Note, Smb-Arc-08. – Project Management, Good Practice Note, Smb-Arc-08, Version: July2017. Https://www. CGIAR. Org/Wp/WpContent/Uploads/2018/01/Gpn-Project-Management-July-2017. Pdf.

[12] Chen, X., Min, D., Yasir, T. A., Hu, Y.-G. (2012): Genetic diversity, population structure and linkage disequilibrium in elite Chinese winter wheat investigated with SSR markers. – PLOS one 7: e44510.

[13] Chhuneja, P., Kaur, S., Garg, T., Ghai, M., Kaur, S., Prashar, M., Bains, N., Goel, R., Keller, B., Dhaliwal, H. (2008): Mapping of adult plant stripe rust resistance genes in diploid A genome wheat species and their transfer to bread wheat. – Theoretical and Applied Genetics 116: 313-324.

[14] Dawit, W., Badebo, A., Hunde, B., Kassa, D., Röder, M. (2019): Haplotype analysis of Ethiopian bread wheat (*Triticum aestivum*) cultivars and elite lines for yellow rust resistance genes using linked molecular markers. – African Journal of Biotechnology 18: 37-57.

[15] Devesh, P., Moitra, P., Shukla, R., Pandey, S. (2019): Genetic diversity and principal component analyses for yield, yield components and quality traits of advanced lines of wheat. – Journal of Pharmacognosy and Phytochemistry 8: 4834-4839.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

[16] Golabadi, M., Arzani, A., Maibody, S. M., Tabatabaei, B. S., Mohammadi, S. (2011): Identification of microsatellite markers linked with yield components under drought stress at terminal growth stages in durum wheat. – Euphytica 177: 207-221.
[17] Gordon, E., Kaviani, M., Kagale, S., Payne, T., Navabi, A. (2019): Genetic diversity and population structure of synthetic hexaploid-derived wheat (*Triticum aestivum* L.) accessions. – Genetic Resources and Crop Evolution 66: 335-348.
[18] Goutam, U., Kukreja, S., Tiwari, R., Chaudhury, A., Gupta, R., Dholakia, B., Yadav, R. (2013): Biotechnological approaches for grain quality improvement in wheat: present status and future possibilities. – Australian Journal of Crop Science 7: 469.
[19] Granato, D., Santos, J. S., Escher, G. B., Ferreira, B. L., Maggio, R. M. (2018): Use of principal component analysis (PCA) and hierarchical cluster analysis (HCA) for multivariate association between bioactive compounds and functional properties in foods: A critical perspective. – Trends in Food Science & Technology 72: 83-90.
[20] Granato, D., Barba, F. J., Bursać Kovačević, D., Lorenzo, J. M., Cruz, A. G., Putnik, P. (2020): Functional foods: Product development, technological trends, efficacy testing, and safety. – Annual Review of Food Science and Technology 11: 93-118.
[21] Imtiaz, M., Ahmad, M., Cromey, M., Griffin, W., Hampton, J. (2004): Detection of molecular markers linked to the durable adult plant stripe rust resistance gene Yr18 in bread wheat (*Triticum aestivum* L.). – Plant Breeding 123: 401-404.
[22] Islam, S., Haque, M., Emon, R., Islam, M., Begum, S. (2012): Molecular characterization of wheat (*Triticum aestivum* L.) genotypes through SSR markers. – Bangladesh Journal of Agricultural Research 37: 389-398.
[23] Lagudah, E., McFadden, H., Singh, R., Huerta-Espino, J., Bariana, H., Spielmeyer, W. (2006): Molecular genetic characterization of the Lr34/Yr18 slow rusting resistance gene region in wheat. – Theoretical and Applied Genetics 114: 21-30.
[24] Law, C., Worland, A. (1997): The control of adult-plant resistance to yellow rust by the translocated chromosome 5BS-7BS of bread wheat. – Plant breeding 116: 59-63.
[25] Li, X., Yang, W.-X., Li, Y.-N., Liu, D.-Q., Yan, H.-F., Meng, Q.-F., Zhang, T. (2005): A SSR marker for leaf rust resistance gene Lr19 in wheat. – Scientia Agricultura Sinica 38: 1156-1159.
[26] Lillemo, M., Asalf, B., Singh, R., Huerta-Espino, J., Chen, X., He, Z., Bjørnstad, Å. (2008): The adult plant rust resistance loci Lr34/Yr18 and Lr46/Yr29 are important determinants of partial resistance to powdery mildew in bread wheat line Saar. – Theoretical and Applied Genetics 116: 1155-1166.
[27] Luo, P., Zhang, H., Zhang, H., Ren, Z. (2006): Diagnostic detection and genetic analysis of wheat stripe rust resistant gene YrCN19. – Fen zi xi bao sheng wu xue bao = Journal of Molecular Cell Biology 39: 217-222.
[28] Ma, J., Zhou, R., Dong, Y., Wang, L., Wang, X., Jia, J. (2001): Molecular mapping and detection of the yellow rust resistance gene Yr26 in wheat transferred from Triticum turgidum L. using microsatellite markers. – Euphytica 120: 219-226.
[29] Maccaferri, M., Zhang, J., Bulli, P., Abate, Z., Chao, S., Cantu, D., Bossolini, E., Chen, X., Pumphrey, M., Dubcovsky, J. (2015): A genome-wide association study of resistance to stripe rust (Puccinia striiformis f. sp. tritici) in a worldwide collection of hexaploid spring wheat (*Triticum aestivum* L.). – G3: Genes, Genomes, Genetics 5: 449-465.
[30] McNee, W. S. (2007): SaaS 2.0. – Journal of Digital Asset Management 3: 209-214.
[31] Mebrate, S., Oerke, E., Dehne, H., Pillen, K. (2008): Mapping of the leaf rust resistance gene Lr38 on wheat chromosome arm 6DL using SSR markers. – Euphytica 162: 457-466.
[32] Meena, N., Mishra, V., Baranwal, D., Singh, A., Rai, V., Prasad, R., Arun, B., Chand, R. (2014): Genetic evaluation of spring wheat (*Triticum aestivum* L.) recombinant inbred lines for spot blotch (Bipolaris Sorokiniana) resistance and yield components under natural conditions for South Asia. – Journal of Agricultural Science and Technology 16: 1429-1440.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

[33] Mengistu, D., Kidane, Y. G., Fadda, C., Pè, M. E. (2016a): Genetic diversity in Ethiopian durum wheat (Triticum turgidum var durum) inferred from phenotypic variations. – Plant Genetic Resources 16(1): 39-49.
[34] Mengistu, D. K., Kidane, Y. G., Catellani, M., Frascaroli, E., Fadda, C., Pè, M. E., Dell'acqua, M. (2016b): High-density molecular characterization and association mapping in Ethiopian durum wheat landraces reveals high diversity and potential for wheat breeding. – Plant Biotechnology Journal 14: 1800-1812.
[35] Peng, J. H., Fahima, T., Röder, M., Li, Y., Grama, A., Nevo, E. (2000): Microsatellite high-density mapping of the stripe rust resistance gene YrH52 region on chromosome 1B and evaluation of its marker-assisted selection in the F2 generation in wild emmer wheat. – New Phytologist 146: 141-154.
[36] Punjabi, B., Basu, R. (1982): Testing germination and seedling growth by an inclined glass plate blotter method. – Indian Journal of Plant Physiology 25: 289-295.
[37] Ren, J., Sun, D., Chen, L., You, F. M., Wang, J., Peng, Y., Nevo, E., Sun, D., Luo, M.-C., Peng, J. (2013): Genetic diversity revealed by single nucleotide polymorphism markers in a worldwide germplasm collection of durum wheat. – International Journal of Molecular Sciences 14: 7061-7088.
[38] Sadiq, M. (2019): Molecular screening of stripe rust resistance genes in segregating wheat populations and its evaluation in the field. – University of the Punjab, Lahore.
[39] Singh, R. P., William, H. M., Huerta-Espino, J., Rosewarne, G. (2004): Wheat rust in Asia: meeting the challenges with old and new technologies. – Proceedings of the 4th International Crop Science Congress, The Regional Institute Ltd Gosford, Australia.
[40] Suenaga, K., Singh, R., Huerta-Espino, J., William, H. (2003): Microsatellite markers for genes Lr34/Yr18 and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. – Phytopathology 93: 881-890.
[41] Tékeu, H., Ngonkeu, E. M., Djocgoué, F. P., Ellis, A., Lendzemo, V., Springfield, L., Moulin, L., Klonowska, A., Diouf, D., Botes, W. C. (2017): Genetic diversity of Cameroonian bread wheat (*Triticum aestivum* L.) cultivars revealed by microsatellite markers. – African Journal of Biotechnology 16: 1832-1839.
[42] Wang, C., Zhang, Y., Han, D., Kang, Z., Li, G., Cao, A., Chen, P. (2008): SSR and STS markers for wheat stripe rust resistance gene Yr26. – Euphytica 159: 359-366.
[43] Wang, Y., Peng, H., Liu, G., Xie, C., Ni, Z., Yang, T., Liu, Z., Sun, Q. (2010): Identification and molecular mapping of a leaf rust resistance gene in spelt wheat landrace Altgold. – Euphytica 174: 371-375.
[44] Wang, X., Luo, G., Yang, W., Li, Y., Sun, J., Zhan, K., Liu, D., Zhang, A. (2017): Genetic diversity, population structure and marker-trait associations for agronomic and grain traits in wild diploid wheat Triticum urartu. – BMC plant biology 17: 112.
[45] Waqar, A., Khattak, S. H., Begum, S., Rehman, T., Shehzad, A., Ajmal, W., Zia, S. S., Siddiqi, I., Ali, G. M. (2018): Stripe Rust: A Review of the Disease, Yr Genes and its Molecular Markers. – Sarhad Journal of Agriculture 34.
[46] Xing, L., Yang, W.-X., Li, Y.-N., Liu, D.-Q., Yan, H.-F., Meng, Q.-F., Zhang, T. (2006): A SSR marker for leaf rust resistance gene Lr19 in wheat. – Agricultural Sciences in China 5: 111-115.
[47] Yue, Y.-L., Yao, Z.-J., Ren, X.-X., Li, W. (2010): Molecular mapping of a gene for resistance to stripe rust in wheat variety PIW138. – Agricultural Sciences in China 9: 1285-1291.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

SUPPLEMENTAL DATA

Table S1. *Analysis of variance (RCB design) of Germination for 100 selected accessions with three replications across two locations for environmental interactions*

SOV	DF	SS	%SS	MS	F-Cal	F-Tab @ 0.05	F-Tab @ 0.01
Locations	1	3788.85	0.51	3788.85	47.942364	7.71	21.1976895
Reps w/n Loc	4	316.117		79.0292			
Genotypes	99	2181.08	0.30	22.0312	9.2770415	1.28	1.42312627
GxL	99	159.097	0.021	1.60704	0.6767063	1.28	1.42312627
Pool Error	396	940.424	0.127	2.37481			
Total	599	7385.57					

*F-Cal ≥ F-Tab 0.01= Highly Significant, F-Cal ≥ F-Tab 0.05= Significant, F-Cal < F-Tab 0.05= Non-significant

Table S2. *Analysis of variance (RCB design) of Days to heading for 100 selected accessions with three replications across two locations for environmental interactions*

SOV	DF	SS	SS%	MS	F-Cal	F-Tab @ 0.05	F-Tab @ 0.01
Locations	1	87865.8	0.669094	87865.8	370.12083	7.71	21.19768
Reps w/n Loc	4	949.591		237.3977			
Genotypes	99	35162.3	0.267759	355.175	27.961729	1.28	1.423126
GxL	99	2312.83	0.017612	23.3619	1.8392072	1.28	1.423126
Pool Error	396	5030.06	0.038304	12.7021			
Total	599	131320.					

*F-Cal ≥ F-Tab 0.01= Highly Significant, F-Cal ≥ F-Tab 0.05= Significant, F-Cal < F-Tab 0.05= Non-significant

Table S3. *Analysis of variance (RCB design) of Days to maturity for 100 selected accessions with three replications across two locations for environmental interactions*

SOV	DF	SS	SS%	MS	F-Cal	F-Tab @ 0.05	F-Tab @ 0.01
Locations	1	188659.8	0.823797	188659.8	547.3080002	7.71	21.19768958
Reps w/n Loc	4	1378.82		344.705			
Genotypes	99	29695.4	0.12966	299.953	19.713824	1.28	1.4231262
GxL	99	3253.07	0.01420	32.8593	2.1596064	1.28	1.4231262
Pool Error	396	6025.29	0.02631	15.2154			
Total	599	229012.4					

F-Cal ≥ F-Tab 0.01= Highly Significant, F-Cal ≥ F-Tab 0.05= Significant, F-Cal < F-Tab 0.05= Non-significant

Table S4. *Analysis of variance (RCB design) of spike length for 100 selected accessions with three replications across two locations for environmental interactions*

SOV	DF	SS	SS%	MS	F-Cal	F-Tab @ 0.05	F-Tab @ 0.01
Locations	1	0.000417	0.00000028	0.000417	1.70869E-05	7.71	21.19768958
Reps w/n Loc	4	97.54047		24.38512			
Genotypes	99	184.7316	0.126064852	1.865976	0.802466252	1.28	1.423126275
GxL	99	262.2779	0.178984093	2.649272	1.139323862	1.28	1.423126275
Pool Error	396	920.8195	0.628387058	2.325302			
Total	599	1465.37					

F-Cal ≥ F-Tab 0.01= Highly Significant, F-Cal ≥ F-Tab 0.05= Significant and F-Cal < F-Tab 0.05= Non-significant

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

Table S5. *Analysis of variance (RCB design) of Number of Spikelets Spike^{-1} for 100 selected accessions with three replications across two locations for environmental interactions*

SOV	DF	SS	SS%	MS	F-Cal	F-Tab @ 0.05	F-Tab @ 0.01
Locations	1	62.08167	0.010075	62.08167	0.44953597	7.71	21.19768958
Reps w/n Loc	4	552.4067		138.1017			
Genotypes	99	904.965	0.146867	9.141061	0.972838307	1.28	1.423126275
GxL	99	921.4183	0.149537	9.307256	0.990525658	1.28	1.423126275
Pool Error	396	3720.927	0.60387	9.396279			
Total	599	6161.798					

F-Cal ≥ F-Tab 0.01= Highly Significant, F-Cal ≥ F-Tab 0.05= Significant and F-Cal < F-Tab 0.05= Non-significant

Table S6. *Analysis of variance (RCB design) of seeds spike^{-1} for 100 selected accessions with three replications across two locations for environmental interactions*

SOV	DF	SS	SS%	MS	F-Cal	F-Tab @ 0.05	F-Tab @ 0.01
Locations	1	0.06	8.0795E-07	0.06	2.1501E-05	7.71	21.19768958
Reps w/n Loc	4	11162.25		2790.563			
Genotypes	99	13507.33	0.18188918	136.4377	1.3613606	1.28	1.4231262
GxL	99	9903.94	0.133366	100.0398	0.9981861	1.28	1.4231262
Pool Error	396	39687.75	0.53444	100.2216			
Total	599	74261.33					

F-Cal ≥ F-Tab 0.01= Highly Significant, F-Cal ≥ F-Tab 0.05= Significant and F-Cal < F-Tab 0.05= Non-significant

Table S7. *Analysis of variance (RCB design) of spike plant^{-1} for 100 selected accessions with three replications across two locations for environmental interactions*

SOV	DF	SS	SS%	MS	F-Cal	F-Tab @ 0.05	F-Tab @ 0.01
Locations	1	45.375	0.031941	45.375	1.50447612	7.71	21.19769
Reps w/n Loc	4	120.64		30.16			
Genotypes	99	226.4183	0.159384	2.287054	1.052260193	1.28	1.423126275
GxL	99	167.4583	0.11788	1.6914	0.77824854	1.28	1.423126
Pool Error	396	860.6933	0.605872	2.1734			
Total	599	1420.585					

F-Cal ≥ F-Tab 0.01= Highly Significant, F-Cal ≥ F-Tab 0.05= Significant and F-Cal < F-Tab 0.05= Non-significant

Table S8. *Analysis of variance (RCB design) of seeds plant^{-1} for 100 selected accessions with three replications across two locations for environmental interactions*

SOV	DF	SS	SS%	MS	F-Cal	F-Tab @ 0.05	F-Tab @ 0.01
Locations	1	16727.0	0.00321829	16727.04	0.85684897	7.71	21.19768958
Reps w/n Loc	4	78086.2		19521.57			
Genotypes	99	838221	0.16127461	8466.884	0.95298555	1.28	1.423126275
GxL	99	746147	0.14355956	7536.848	0.84830581	1.28	1.423126275
Pool Error	396	3518297	0.67692364	8884.588			
Total	599	5197479					

F-Cal ≥ F-Tab 0.01= Highly Significant, F-Cal ≥ F-Tab 0.05= Significant and F-Cal < F-Tab 0.05= Non-significant

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

Table S9. *Combined morphological data for 13 parameters used for PCA*

S.No	Acc.	Germination	1st leaf (days after germination)	2st leaf (days)	3rd leaf (days)	Booting (days)	Half open (days)	Heading (days)	Fertilization (days)	Leaf length (cm)	Width (cm)	Plant height (cm)	Peduncle (cm)	No of seed / spike
1	11123	12.33	7.33	6.33	4.33	118.00	127.67	131.00	136.00	21.00	1.43	75	24.00	33.00
2	11126	11.67	7.33	6.33	4.33	115.50	124.33	129.00	134.00	20.33	1.43	70	26.33	42.00
3	11144	13.33	8.00	7.00	4.67	125.00	128.33	132.67	140.33	17.33	1.37	90	28.00	49.00
4	11145	11.33	6.33	6.33	4.67	109.00	124.33	128.33	136.67	22.00	1.40	68	22.33	35.00
5	11152	11.00	6.33	6.33	4.33	113.50	121.33	125.00	130.33	18.00	1.43	75	27.00	30.00
6	11154	16.00	8.67	7.67	4.67	97.00	107.33	111.67	118.33	20.33	1.67	69	25.67	46.00
7	11155	10.00	7.00	6.00	4.33	91.00	105.67	110.67	117.67	21.33	1.27	103	25.00	23.00
8	11160	12.33	7.67	6.67	4.33	115.50	128.33	133.00	140.00	21.33	1.27	79	28.33	42.00
9	11161	7.00	6.67	6.00	4.33	109.00	120.33	124.67	131.00	19.33	1.30	77	27.67	34.00
10	11162	12.00	7.00	6.33	4.00	113.00	123.33	128.00	134.67	21.00	1.37	98	26.00	23.00
11	11163	12.67	7.67	6.67	4.33	111.67	120.67	125.67	134.00	21.00	1.40	75	27.67	32.00
12	11164	12.67	7.33	6.33	4.33	117.00	126.00	130.00	135.67	18.67	1.43	72	24.00	30.00
13	11166	12.00	7.00	6.67	4.00	117.33	125.00	128.67	136.00	20.67	1.40	78	29.67	46.00
14	11168	13.33	7.33	6.67	4.33	114.67	125.33	130.33	138.00	19.67	1.30	105	32.33	65.00
15	11170	12.67	7.33	7.00	4.33	125.33	132.33	136.00	143.67	16.33	1.40	75	28.67	48.00
16	11171	13.33	7.33	6.67	4.33	111.67	121.00	125.00	129.67	21.67	1.43	68	26.67	33.00
17	11173	13.33	7.67	6.67	4.67	114.33	122.33	127.00	134.67	16.00	1.47	68	26.67	44.00
18	11174	13.33	7.00	6.33	4.33	114.00	122.00	127.33	135.00	19.00	1.63	65	24.67	38.00
19	11177	14.67	7.00	7.00	4.67	114.33	122.33	127.33	133.33	17.67	1.43	72	27.33	23.00
20	11178	14.67	6.67	6.67	4.33	113.33	121.33	125.33	131.33	19.00	1.37	109	21.67	43.00
21	11179	13.00	6.33	7.00	4.67	110.33	118.67	123.67	130.00	20.00	1.17	75	25.00	37.00
22	11181	11.00	6.33	7.00	4.67	114.00	122.33	126.33	132.00	23.67	1.33	67	26.33	46.00
23	11183	13.67	6.33	6.67	4.33	117.67	125.00	129.00	136.00	22.00	1.63	73	32.00	31.00
24	11184	16.00	6.67	7.33	5.00	117.67	126.33	130.00	136.33	24.67	1.53	108	25.00	22.00
25	11185	14.33	7.67	6.67	4.67	116.33	125.33	129.67	136.67	23.33	1.43	85	30.67	33.00
26	11186	12.33	7.67	6.67	4.67	119.33	127.67	132.00	138.33	23.33	1.33	96	28.67	19.00
27	11187	14.67	7.33	7.00	5.00	114.00	121.67	127.00	131.67	19.67	1.37	97	32.33	21.00
28	11188	12.00	7.00	6.00	4.00	110.67	118.00	122.33	128.00	20.33	1.40	92	34.33	34.00
29	11189	12.67	7.00	6.33	4.33	101.00	109.67	114.00	121.00	22.67	1.33	89	32.00	23.00
30	11190	11.33	7.00	6.00	4.00	98.00	108.67	113.00	121.33	23.67	1.40	98	28.00	21.00
31	11192	12.33	7.00	6.00	4.00	115.00	125.67	129.67	135.00	19.67	1.40	84	29.00	26.00

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

32	11193	13.33	7.33	7.00	5.00	110.00	122.67	127.00	134.00	26.33	1.50	69	28.33	24.00
33	11194	13.67	7.67	6.67	4.33	132.67	140.00	143.67	148.67	27.00	1.30	85	27.67	31.00
34	11195	13.00	7.33	6.33	4.33	118.00	127.33	131.00	138.67	26.33	1.53	87	27.33	38.00
35	11197	13.67	8.00	6.00	5.00	128.67	134.00	138.00	142.33	26.67	1.27	78	45.67	33.00
36	11198	12.67	7.33	6.33	4.33	132.67	138.33	142.67	146.67	27.33	1.33	103	32.00	45.00
37	11200	13.67	7.67	6.67	4.67	118.00	125.33	129.33	133.00	25.67	1.33	75	28.33	19.00
38	11202	12.33	7.33	6.33	4.33	128.67	135.67	139.67	144.00	23.67	1.50	81	27.00	50.00
39	11205	11.67	7.33	6.33	4.33	132.33	140.00	143.67	148.67	24.33	1.43	74	31.00	31.00
40	11207	14.33	8.00	7.00	5.00	127.33	134.67	139.00	143.67	25.00	1.43	88	32.67	45.00
41	11208	12.67	7.67	6.67	4.67	127.00	134.67	138.33	144.67	22.00	1.30	83	34.33	39.00
42	11209	11.33	7.67	6.67	4.67	122.67	129.33	133.33	138.33	21.33	1.30	86	24.00	32.00
43	11210	10.67	7.00	6.00	4.67	125.67	130.00	133.67	138.67	21.33	1.10	78	22.33	40.00
44	11211	12.00	7.67	6.67	4.67	127.33	135.67	140.00	145.67	26.00	1.37	75	22.67	25.00
45	11214	12.00	7.33	6.33	4.33	118.67	128.67	133.33	140.33	24.33	1.27	75	22.33	39.00
46	11215	7.00	7.00	6.00	4.67	113.33	122.33	127.33	133.00	21.00	1.30	91	35.67	38.00
47	11216	11.33	7.67	6.67	4.67	88.67	98.33	103.00	109.33	22.67	1.40	77	33.67	43.00
48	11217	8.00	7.00	6.00	4.67	128.00	135.00	138.67	143.67	22.67	1.53	88	28.67	47.00
49	11218	5.67	6.33	5.33	4.67	113.00	122.67	127.33	133.00	23.00	1.67	83	30.00	39.00
50	11221	11.00	7.67	6.67	4.67	126.33	134.67	139.67	145.33	23.67	1.43	86	29.00	34.00
51	11222	10.33	6.67	5.67	4.33	129.33	131.67	136.33	142.00	23.33	1.37	82	27.67	39.00
52	11223	9.00	6.67	5.67	4.33	111.00	122.67	127.00	133.33	26.00	1.40	96	36.33	44.00
53	11224	10.33	7.00	6.00	4.67	125.67	133.00	136.67	141.33	18.33	1.27	86	27.33	38.00
54	11225	9.67	7.00	6.00	4.67	114.33	124.33	128.00	133.00	23.00	1.20	84	30.33	34.00
55	11226	10.00	7.00	6.00	4.67	112.33	122.00	125.67	131.67	22.33	1.37	81	31.00	56.00
56	11227	13.00	7.67	6.67	4.67	110.00	119.67	124.00	128.67	21.00	1.50	89	26.33	35.00
57	11228	11.67	6.67	6.67	4.67	114.00	125.00	129.33	137.00	22.67	1.50	73	25.00	41.00
58	11229	14.33	8.00	7.00	5.00	115.00	125.33	129.67	135.33	24.00	1.43	86	31.33	42.00
59	11231	13.33	7.00	6.67	4.67	119.67	128.00	132.00	139.33	23.00	1.33	55	31.33	52.00
60	11233	13.67	6.67	7.33	5.33	117.33	125.00	129.33	135.33	24.67	1.70	91	33.33	52.00
61	11236	14.67	8.00	7.00	5.00	114.00	123.33	129.33	136.00	20.00	1.47	81	28.33	53.00
62	11237	10.67	7.00	6.00	4.00	113.33	121.33	126.67	132.00	22.33	1.53	85	30.00	44.00
63	11239	13.33	7.67	6.67	4.67	119.00	127.33	132.67	140.00	30.33	1.60	90	32.00	34.00
64	11240	11.67	7.67	6.67	4.67	118.00	126.00	131.33	137.67	21.67	1.30	93	20.33	32.00
65	11242	17.67	7.67	6.67	4.67	109.67	120.67	124.67	132.67	20.67	1.33	86	28.00	38.00
66	11243	11.33	7.33	6.33	4.33	113.00	123.67	129.00	136.00	22.33	1.60	85	26.67	29.00

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

67	11244	11.00	7.33	6.33	4.33	111.67	120.33	124.67	130.33	20.67	1.37	84	25.33	27.00
68	11246	11.00	7.00	6.00	4.00	115.67	124.00	128.00	133.33	22.00	1.27	84	30.67	25.00
69	11248	12.67	7.33	6.33	4.33	93.33	104.33	109.33	117.00	21.67	1.33	72	22.67	24.00
70	11249	12.33	7.33	6.33	4.33	114.33	125.67	129.67	135.33	22.67	1.57	77	29.33	21.00
71	11250	11.33	7.33	6.33	4.33	115.00	124.67	129.00	136.00	28.67	1.63	96	36.00	45.00
72	11252	12.00	7.33	6.33	4.33	112.67	122.33	127.33	133.67	22.67	1.40	73	24.33	34.00
73	11253	16.33	8.33	5.33	5.33	111.67	121.33	125.33	133.00	19.00	1.20	63	22.00	22.00
74	11255	18.00	9.00	5.33	5.33	112.67	119.33	123.33	130.67	21.67	1.60	76	31.00	39.00
75	11256	13.67	7.00	5.67	4.67	102.67	113.33	117.00	126.00	22.67	1.50	72	21.33	36.00
76	11259	11.67	7.67	6.67	4.67	114.67	123.33	127.67	137.00	22.00	1.33	72	25.67	50.00
77	11261	11.33	7.33	6.33	4.33	124.33	132.67	137.33	142.67	21.33	1.37	93	32.33	19.00
78	11265	12.00	8.00	6.00	5.00	118.67	128.33	132.00	136.67	21.33	1.23	72	29.00	26.00
79	11272	18.33	9.00	5.00	6.00	117.00	124.33	128.33	136.67	21.33	1.17	76	27.67	46.00
80	11274	12.33	7.67	5.67	4.67	93.67	106.00	109.67	119.33	26.00	1.43	85	29.33	25.00
81	11275	12.33	7.67	5.67	4.67	107.33	116.67	120.67	126.33	21.67	1.37	87	27.33	33.00
82	11278	13.33	8.00	6.00	5.00	107.67	117.33	122.67	129.67	19.67	1.40	94	30.00	35.00
83	11288	8.67	7.33	5.33	5.00	104.00	114.33	119.00	126.67	25.33	1.47	72	23.00	34.00
84	11290	12.00	8.00	6.00	5.00	113.33	121.33	125.33	129.00	17.33	1.40	72	31.00	32.00
85	11292	12.00	8.00	5.33	5.00	114.33	124.00	128.00	135.33	19.33	1.33	72	23.67	22.00
86	11293	12.33	7.33	6.33	4.33	114.67	123.33	127.00	134.00	17.33	1.30	77	29.33	16.00
87	11294	11.33	7.33	6.00	4.33	116.00	125.33	129.00	133.67	19.33	1.57	80	25.33	23.00
88	11295	10.67	7.67	5.67	4.67	113.33	124.67	129.33	136.67	19.33	1.60	96	28.00	19.00
89	11296	11.00	7.67	5.67	4.67	116.33	125.33	130.00	136.00	19.33	1.40	72	27.33	9.00
90	11297	10.33	7.67	5.67	4.67	112.33	121.67	125.33	130.00	20.00	1.53	80	25.00	35.00
91	11299	11.67	7.67	5.67	4.67	101.00	111.67	115.67	122.67	16.67	1.33	78	28.33	39.00
92	11304	12.67	7.67	5.67	4.67	104.67	115.00	120.00	127.67	19.67	1.37	79	25.33	31.00
93	11317	15.33	8.00	6.33	5.00	107.67	117.00	121.67	129.00	22.33	1.50	82	27.33	28.00
94	11553	12.33	7.67	6.00	4.67	111.00	122.67	127.33	134.67	21.00	1.33	86	32.33	29.00
95	11558	18.00	9.00	5.67	5.00	110.33	120.33	125.00	132.67	20.33	1.37	76	23.33	24.00
96	12087	12.00	7.67	6.67	4.67	108.00	119.33	123.33	129.00	22.00	1.43	82	31.33	28.00
97	12100	13.67	8.00	7.00	5.00	114.33	122.00	126.67	132.67	20.00	1.90	81	26.67	44.00
98	12231	12.00	7.33	6.33	4.33	116.00	124.33	128.67	133.67	20.33	1.43	82	28.00	25.00
99	18668	13.00	7.67	6.67	4.67	117.00	123.67	127.33	133.00	23.67	1.50	87	28.67	26.00
100	24740	13.33	7.67	7.00	4.67	118.00	126.67	130.33	136.67	21.00	1.37	101	25.67	13.00

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

Table S10. *PCR confirmation of 22 SSR along with band size for indigenous germplasm*

Acc No.	XGWM35 225bp	XGWM35 200bp	WMC419 200bp	XGWM120 150bp	GWM174 220bp	XGWM140 120bp	XWMC170 200bp	XWMC405 220bp	XWMC348 130bp	XWMC407 120bp	GWM 148 190bp	GWM148 200bp
1	0	0	1	0	0	1	0	0	0	1	1	0
2	0	0	1	0	0	1	0	1	0	1	1	0
3	0	0	1	0	0	1	1	1	0	1	1	0
4	0	0	1	1	0	1	1	0	0	1	1	0
5	0	0	1	0	0	1	0	0	0	1	1	0
6	1	0	1	0	0	1	1	1	0	0	1	0
7	1	0	0	0	0	0	0	1	0	0	1	0
8	1	0	1	1	0	1	0	1	0	1	1	0
9	0	0	0	1	0	1	1	0	0	0	1	0
10	0	0	1	1	0	0	1	1	0	0	1	0
11	1	0	1	1	0	1	1	1	0	1	1	0
12	0	0	1	0	0	1	0	1	0	1	1	0
13	0	0	1	0	0	1	0	0	0	1	1	0
14	0	0	1	0	0	1	0	1	0	1	1	0
15	0	0	1	0	0	1	0	1	0	1	1	0
16	0	0	0	0	0	0	0	1	0	1	1	0
17	0	0	1	0	0	1	0	0	0	0	1	0
18	0	0	1	0	0	1	0	0	1	1	1	0
19	0	0	0	0	0	0	0	0	0	1	1	0
20	0	0	1	1	0	0	1	1	1	0	1	0
21	0	0	1	0	0	0	0	1	1	0	0	0
22	0	0	1	1	0	0	1	1	0	0	1	0
23	0	0	0	0	0	0	1	1	1	0	1	0
24	0	0	1	0	0	0	1	1	0	0	1	0
25	0	0	1	1	0	0	0	1	0	0	1	0
26	0	0	1	0	0	0	1	1	1	0	1	0
27	0	0	0	0	0	0	1	1	1	1	1	0
28	0	0	0	1	0	0	1	0	1	1	1	0
29	0	0	1	1	0	0	1	1	1	1	1	0
30	0	0	0	0	0	0	1	1	1	0	1	0
31	0	0	0	0	0	1	1	1	1	0	1	0
32	0	0	0	1	0	0	1	1	1	0	1	0
33	0	0	0	0	0	0	1	1	0	0	0	0

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

34	0	0	1	0	0	0	1	1	1	0	0	0
35	0	0	0	0	0	0	0	1	1	0	0	0
36	0	0	1	1	0	0	0	1	0	0	0	0
37	0	0	0	0	0	1	1	1	0	0	1	0
38	0	0	1	0	0	1	1	1	0	1	1	0
39	0	0	0	0	0	0	0	1	0	1	0	0
40	0	0	0	0	0	1	0	1	1	1	0	1
41	0	0	1	1	0	1	0	1	0	1	0	1
42	0	0	0	1	0	1	1	1	1	1	0	1
43	0	0	0	1	0	0	0	1	0	1	0	1
44	0	0	0	1	0	0	0	1	0	0	0	0
45	0	0	0	1	0	1	1	0	1	1	0	1
46	0	0	1	1	0	0	0	1	1	1	0	1
47	0	0	0	0	0	1	1	1	1	0	0	1
48	1	0	0	1	1	0	1	1	1	0	0	0
49	0	0	1	1	0	1	1	1	1	0	0	1
50	1	0	0	1	0	0	1	1	1	0	0	0
51	0	0	0	1	0	1	1	0	1	0	0	1
52	0	0	0	1	0	1	1	1	0	0	0	1
53	0	0	1	1	0	1	0	1	1	0	0	1
54	0	0	0	1	0	1	1	1	1	0	0	1
55	0	0	1	1	0	0	0	1	0	0	0	1
56	0	0	1	1	0	0	1	0	0	1	1	0
57	0	0	0	0	0	0	1	1	0	1	1	0
58	0	0	1	0	0	1	1	1	0	0	1	0
59	0	0	1	1	0	1	1	1	0	0	1	0
60	0	0	1	1	0	0	1	1	0	1	1	0
61	0	0	0	1	0	1	1	1	0	1	1	0
62	0	0	1	1	0	1	1	1	0	1	1	0
63	0	0	1	1	0	1	1	1	0	0	1	0
64	0	0	1	0	0	1	1	1	0	1	1	0
65	0	0	1	1	0	1	1	1	0	1	1	0
66	0	0	0	1	0	1	1	1	0	1	1	0
67	0	0	1	0	0	0	1	1	0	1	1	0
68	0	0	1	1	0	0	1	1	0	1	1	0
69	0	0	1	0	0	1	1	0	0	1	1	0
70	0	0	1	0	0	0	1	1	0	0	1	0

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

71	0	0	1	0	0	0	1	1	0	1	1	0
72	0	0	1	0	0	0	1	1	0	0	1	0
73	0	0	1	1	0	1	1	1	0	0	1	0
74	0	0	1	1	0	1	1	0	0	0	1	0
75	0	0	1	0	0	0	1	1	0	0	0	0
76	0	0	1	0	0	1	1	1	0	0	0	0
77	0	0	1	1	0	0	0	1	1	0	0	0
78	0	0	0	1	0	0	0	1	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	1	0	0	0	1	1	0	0	0
81	1	1	1	1	0	0	0	1	1	0	0	0
82	0	0	1	0	0	0	0	1	0	0	1	0
83	0	0	1	1	0	0	0	1	1	1	1	0
84	1	1	0	1	0	0	1	1	1	0	1	0
85	0	0	0	1	0	0	1	0	1	0	0	0
86	0	0	1	1	0	0	1	0	0	0	0	0
87	0	0	0	1	0	0	1	1	1	0	0	0
88	0	0	0	1	0	0	1	1	0	0	0	0
89	0	0	0	1	0	0	1	1	0	0	0	0
90	0	0	0	1	0	0	0	1	0	1	0	0
91	0	0	0	0	0	0	1	1	0	0	0	0
92	0	0	0	0	0	0	0	0	0	0	0	0
93	1	0	0	1	0	0	0	1	0	0	1	0
94	0	0	0	0	0	0	1	1	0	0	1	0
95	1	0	0	0	0	0	1	0	0	0	1	0
96	0	0	0	0	0	1	0	1	0	0	1	0
97	0	0	1	1	0	1	0	1	0	0	1	0
98	1	0	1	1	0	1	0	1	0	1	1	0
99	0	0	0	1	0	1	0	1	0	1	1	0
100	0	0	0	1	0	1	0	1	0	1	1	0
Acc. No.	**Barc 86 200bp**	**Barc 86 210bp**	**WMC773 298bp**	**BARC114 105bp**	**BARC 114 200bp**	**CSLV 34 215bp**	**CSLV 34 190bp**	**CSLV 34 150bp**	**PSP 3000 350bp**	**PSP 3000 300bp**	**XGWM493 150bp**	**XGWM 493 300bp**
1	1	0	0	1	0	1	0	0	0	0	0	0
2	1	0	1	1	0	1	0	0	1	0	0	0
3	1	0	1	1	0	1	0	0	1	0	0	0
4	1	0	1	1	0	1	0	0	1	0	0	0
5	1	0	1	1	0	1	0	0	1	0	0	0

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

6	1	0	1	1	0	0	1	0	0	0	0	1
7	1	0	1	1	0	1	0	0	1	0	0	1
8	1	0	1	1	0	1	0	0	0	0	1	0
9	1	0	1	1	0	1	0	0	0	0	0	0
10	0	0	0	1	0	1	0	0	1	0	0	1
11	1	0	1	1	0	0	0	0	0	0	0	1
12	0	0	1	1	0	0	0	0	1	1	0	1
13	1	0	1	1	0	0	0	0	0	0	0	1
14	1	0	1	1	0	0	1	0	1	0	0	1
15	1	0	1	1	0	1	0	0	0	0	0	1
16	1	0	1	1	0	1	0	0	0	1	1	0
17	1	0	1	1	0	1	0	0	1	0	0	1
18	1	0	1	1	0	1	0	0	0	1	0	1
19	0	0	1	1	0	0	0	0	1	0	0	1
20	1	0	1	0	0	1	0	0	0	0	1	1
21	1	0	1	0	0	1	0	0	0	1	1	1
22	1	0	1	1	0	1	0	0	0	0	1	1
23	0	0	1	1	0	1	0	0	0	0	1	1
24	1	0	1	1	0	0	0	0	1	1	1	1
25	1	0	1	1	0	0	1	0	1	1	1	1
26	1	0	1	0	0	1	0	0	0	0	1	1
27	1	0	1	1	0	0	1	0	0	0	1	1
28	1	0	1	0	0	0	1	0	0	0	1	1
29	1	0	1	1	1	0	1	0	0	0	1	1
30	1	0	1	1	0	0	1	0	0	1	1	1
31	1	0	1	1	1	1	0	0	0	0	1	1
32	1	0	1	1	1	0	1	0	0	0	1	1
33	1	0	1	1	0	0	1	0	1	1	1	1
34	1	0	1	1	0	0	0	0	0	0	1	1
35	1	0	1	1	0	0	0	0	1	1	1	1
36	1	0	1	1	0	0	0	0	1	1	1	1
37	1	0	1	1	0	0	1	0	0	0	1	1
38	1	0	1	1	0	0	1	1	0	0	1	1
39	0	0	1	1	0	1	0	0	1	1	0	0
40	1	0	1	1	0	1	0	0	0	0	1	1
41	1	0	1	1	0	0	1	0	0	0	0	1
42	1	0	1	1	0	1	0	0	1	1	1	1

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

43	1	0	1	1	0	1	0	0	0	0	1	1
44	1	0	1	1	0	1	0	0	0	0	1	1
45	1	0	1	1	0	1	0	0	0	0	1	1
46	1	0	1	1	0	1	0	0	0	1	1	1
47	1	0	1	1	0	1	0	0	0	0	1	1
48	1	0	1	1	0	1	0	0	0	0	1	1
49	1	0	1	1	0	1	0	0	0	0	1	1
50	1	0	1	1	0	1	0	0	0	0	1	1
51	1	0	1	1	0	1	0	0	0	0	1	1
52	1	0	1	1	0	1	0	0	0	0	1	1
53	1	0	1	1	0	1	0	0	0	0	1	1
54	1	0	1	1	0	0	0	0	0	0	1	1
55	1	0	1	1	0	1	0	0	0	0	1	1
56	1	0	1	1	0	1	0	0	0	0	1	1
57	1	0	1	1	0	1	0	0	0	0	1	1
58	0	0	0	1	0	1	0	0	0	0	1	0
59	1	0	0	1	0	1	0	0	0	0	1	0
60	1	0	0	1	0	1	0	0	0	0	1	0
61	1	0	0	1	0	0	1	0	0	0	1	0
62	1	0	0	1	0	0	0	0	0	0	1	0
63	1	0	0	1	0	1	0	0	0	0	1	0
64	1	0	0	1	0	0	0	0	0	0	1	0
65	1	0	0	1	0	0	0	1	0	1	1	0
66	1	0	0	1	0	1	0	0	0	0	1	0
67	1	0	0	1	0	1	0	0	0	0	1	0
68	1	0	0	1	0	1	0	0	0	1	1	0
69	1	0	0	1	0	1	0	0	0	0	1	0
70	1	0	0	1	0	1	0	0	0	0	1	0
71	1	0	0	1	0	0	0	0	0	0	1	0
72	1	0	0	1	0	0	0	0	0	0	1	0
73	1	0	0	1	0	0	0	0	0	0	1	0
74	1	0	0	1	0	0	0	0	0	0	1	0
75	1	0	0	1	0	0	0	0	0	0	1	0
76	1	0	0	1	0	0	0	0	0	0	1	0
77	1	0	1	1	0	0	0	0	0	0	0	1
78	1	1	1	1	0	0	0	0	0	0	0	1
79	1	0	1	1	0	0	0	0	0	1	0	1

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

80	1	0	1	1	0	0	0	0	0	0	0	1
81	1	1	0	1	0	0	0	0	0	0	0	1
82	1	1	1	0	0	0	0	0	0	0	0	1
83	1	1	1	1	0	0	0	0	1	1	0	1
84	1	0	1	1	0	0	0	0	0	0	0	1
85	1	1	1	1	0	1	0	0	1	1	0	1
86	1	0	0	0	0	0	0	0	1	1	0	0
87	1	1	1	0	0	0	0	0	0	0	0	1
88	1	0	1	0	0	0	0	0	0	0	0	1
89	1	1	1	0	0	0	0	0	1	0	0	1
90	1	1	1	0	0	0	0	0	1	1	0	1
91	1	0	0	0	0	0	0	0	0	0	0	0
92	1	0	0	0	0	1	0	0	0	0	0	0
93	1	0	0	0	0	1	0	0	0	0	0	0
94	1	0	1	0	0	0	1	0	0	0	0	0
95	1	0	1	1	0	0	0		0	1	0	0
96	1	0	1	0	0	1	0	0	0	0	0	1
97	1	0	1	1	0	1	0	0	0	0	0	1
98	1	0	1	1	0	1	0	0	0	0	0	1
99	1	0	1	1	0	1	0	0	0	0	0	1
100	1	0	1	1	0	1	0	0	0	0	0	1
Acc No.	**XGWM 153 100bp**	**XGWM 153 300bp**	**XGWM 111 185bp**	**XGWM 44 120bp**	**XGWM 44 285bp**	**XGWM44 500bp**	**XGWM44 700bp**	**XBARC4 90bp**	**XBARC4 200bp**	**XGDM 125 150bp**	**XGDM 125 190bp**	**XGWM 410 140bp**
1	1	1	1	1	1	1	0	1	0	0	1	1
2	1	1	0	1	1	1	0	1	0	0	1	1
3	1	0	1	0	0	0	1	1	0	0	1	0
4	1	0	1	1	1	0	0	1	0	0	1	0
5	1	0	1	0	0	0	1	1	0	0	1	1
6	1	0	1	1	1	0	0	1	0	0	1	1
7	1	0	1	1	1	0	0	1	0	0	1	1
8	1	0	1	1	1	0	0	0	0	0	1	0
9	1	0	1	1	1	0	0	1	0	0	0	1
10	1	0	1	1	1	0	0	0	0	0	0	1
11	1	0	1	1	1	1	0	0	0	0	0	1
12	1	0	1	1	1	1	1	0	0	0	0	1
13	0	0	1	0	0	1	1	1	0	0	1	1

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

14	1	0	1	1	1	0	0	1	0	0	1	1
15	1	0	0	1	1	1	0	1	0	0	1	0
16	1	0	1	1	1	0	0	0	0	0	1	1
17	0	0	1	0	0	0	0	1	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	1	0
19	1	0	1	1	1	0	0	1	0	1	0	0
20	0	0	1	1	1	0	0	1	0	1	0	0
21	1	0	1	1	1	0	0	1	0	0	0	0
22	1	0	1	1	1	0	0	1	1	1	0	0
23	1	0	1	1	1	0	0	0	1	1	0	0
24	1	0	1	1	1	0	0	1	1	1	0	1
25	1	0	1	1	1	0	0	1	1	0	0	1
26	1	0	1	1	1	0	0	1	0	1	0	1
27	1	0	1	1	1	0	0	1	1	0	0	1
28	1	0	1	1	1	0	0	1	0	1	0	0
29	0	0	1	1	1	0	0	1	1	1	0	1
30	1	0	1	1	1	0	0	1	0	1	0	1
31	0	0	1	1	1	0	0	1	0	1	0	1
32	1	0	0	1	1	0	0	1	0	1	0	1
33	0	0	0	1	1	0	0	1	0	1	0	1
34	1	0	1	1	1	0	0	1	0	1	0	0
35	1	0	1	1	1	0	0	1	0	1	0	1
36	1	0	0	1	1	0	0	1	0	1	0	1
37	0	0	0	0	0	0	0	1	0	1	0	1
38	0	0	1	0	1	0	0	1	0	1	0	1
39	1	0	1	0	1	0	0	0	0	0	0	1
40	1	0	1	0	0	0	0	1	0	1	0	0
41	1	0	1	0	1	0	0	1	0	0	0	0
42	1	0	1	0	1	0	0	1	0	1	0	1
43	1	0	1	0	0	0	0	1	0	1	0	1
44	1	0	1	0	0	0	0	1	0	1	0	0
45	1	0	1	0	0	0	0	1	0	0	0	0
46	1	0	0	0	0	0	0	1	0	0	0	0
47	1	0	0	0	1	0	0	1	0	1	0	0
48	1	0	0	0	1	0	0	1	0	0	0	0
49	1	0	0	0	1	0	0	1	0	1	0	0
50	1	0	1	0	1	0	0	1	0	0	0	0

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

51	1	0	1	0	1	0	0	1	1	1	0	1
52	1	0	1	0	1	0	0	1	0	1	0	0
53	1	0	1	0	0	0	0	1	0	0	0	1
54	1	0	1	0	0	0	0	1	0	1	0	1
55	1	0	0	0	0	0	0	0	1	0	0	0
56	1	0	1	0	1	0	0	0	1	0	0	1
57	1	0	0	0	1	0	0	0	1	1	0	1
58	1	0	0	0	0	0	0	0	1	1	0	1
59	1	0	0	0	1	0	0	0	1	0	0	1
60	1	0	0	0	1	0	0	0	1	0	0	1
61	1	0	0	0	1	0	0	0	1	1	0	0
62	0	0	0	0	1	0	0	0	1	0	0	0
63	0	0	0	0	1	0	0	0	1	1	0	1
64	1	0	1	0	1	0	0	0	1	1	0	1
65	0	0	0	0	0	0	0	0	1	1	0	0
66	1	0	1	0	1	0	0	0	1	0	0	1
67	1	0	0	0	1	0	0	0	1	1	0	1
68	1	0	1	0	1	0	1	0	1	0	0	1
69	1	0	1	0	1	1	0	0	1	1	0	1
70	0	0	1	0	0	0	1	0	0	1	0	1
71	0	0	1	0	0	0	1	0	1	0	0	1
72	1	0	0	1	1	0	0	1	1	1	0	0
73	1	0	0	1	1	0	0	1	1	1	0	1
74	1	0	1	0	1	0	0	1	1	0	0	1
75	1	0	1	0	0	0	0	1	1	1	0	0
76	1	0	1	0	0	0	0	1	1	0	0	0
77	1	0	1	0	1	0	0	0	1	0	0	0
78	1	0	1	0	0	0	0	1	1	0	0	0
79	1	0	1	0	0	0	0	1	1	0	0	0
80	1	0	1	0	1	0	0	1	1	0	0	0
81	1	0	1	0	1	0	0	1	1	0	0	0
82	1	0	1	0	1	0	0	1	1	0	0	1
83	1	0	0	0	0	0	0	1	1	0	0	1
84	1	0	1	0	1	0	0	1	1	0	0	1
85	0	0	0	0	1	0	0	0	0	0	0	0
86	1	0	1	0	1	0	0	0	0	0	0	0
87	1	0	1	0	1	0	0	0	0	0	0	0

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118

88	1	0	0	0	1	0	0	0	0	0	0	0
89	1	0	0	0	0	0	0	0	1	0	0	0
90	0	0	1	0	0	0	0	0	1	0	0	0
91	1	0	0	0	0	0	0	0	1	0	0	0
92	1	0	0	0	1	0	0	0	1	0	0	0
93	0	0	0	0	1	0	0	0	1	0	0	1
94	0	0	1	0	1	0	0	0	1	0	0	1
95	1	0	1	0	1	0	0	0	1	0	0	1
96	1	0	1	0	1	0	0	0	1	1	0	1
97	1	0	1	0	1	0	0	0	1	1	0	1
98	1	0	1	0	1	0	0	0	1	1	0	1
99	1	0	1	0	1	0	0	0	1	0	0	0
100	1	0	1	0	1	0	0	0	1	1	0	1

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_80918118