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Original Research

Diversity Analysis for Wheat Genotypes Against Rust Resistant Genes

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Abstract:

The study was designed to evaluate the genetic background of indigenous germplasm by using simple sequence repeats (SSRs) gene markers for the selection of elite lines with multiple gene combinations that can be used in breeding programs for wheat improvement against rust disease. The principal component analysis (PCA) was constructed to estimate the genetic difference between the accessions and revealed an 85.3% variation. A high level of association was found between markers *XWMC170 /XGWM608*, *XGWM44*[a]/*XGWM44*[b], *XGDM111*[a]/*XGWM129*, *XWMC765*[a]/*XWMC765*[b], and *GWM148*[a]/*XWMC765*[b] while low level of correlation was observed between the markers CSLV34b and *CSLV34*[a] i.e -0.688 followed by *GWM148*[a] and *XGWM129* (-0.633). Cluster analysis revealed three

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main groups but group A was totally different from B and C with the genetic distance of 18.5 revealing that these accessions were having totally different genetic bases and are more diversified than the others. A total of 40 reported genetic markers (SSRs) were utilized to amplify the required bands for identifying the genes and their genetic distance in terms of variations between the wheat germplasm. The highest amplification was revealed by *XGDM19* (130 bp), *WMC773* (298 bp), *XWMC331*[a] (210 bp), *BARC114*[a] (105 bp), *barc86*[a] (200 bp), and *XBARC4*[a] (90 bp) i.e. 50, 48, 47, 46, 45, and 42 respectively. The study was useful as disease-related SSRs markers were used for general diversity, making the single SSRs marker used for multiple purposes including disease resistance, genetic diversity, and mineral or ion identification. It will ultimately help in saving the cost, time, and effort rather than using individual/or specific markers for evaluating the genetic distance or base for the diversity analysis.

Keywords: association, cluster analysis, durable resistance, multi-purpose SSRs markers, rust disease

Introduction

Among the cereals used as a source of energy in human nutrition, wheat (*Triticum* L.) ranks first in the world with 215.9 million ha of cultivated area and 766 million tons of production due to its great adaptability [1]. *Triticum durum* Desf., *Triticum compactum* Host (known as club wheat) and *T. aestivum* (known as common or bread wheat) together account for nearly 90 percent of overall world wheat production [2]. Common wheat/bread wheat (*T. aestivum*) is considered an important crop (cereal) and is a major component of bread [3]. Wheat is used as a staple food worldwide and is an important source of dietary fiber and B vitamins due to its favorable nutritional value and ease of storage and processing. Wheat is an important crop for the economies of many countries and provides 20% of the vital calories and 20% of the protein source for more than 4.5 billion people in 94 developing countries [4]. Overall, 95% of people in developing countries rely on flour from wheat or maize as their primary source. A variety of foods, including bread, chapattis, cakes, cookies, and confectionery are made from it. In recent years, it is grown on the largest cultivated area in Pakistan [5]. During the last few decades, better progress has been made in increasing the unit area yield of wheat in the country [6]. In 2018, it was grown on an area of 8.7 million ha in Pakistan with a production of 250 million tonnes in Pakistan [7]. However, among the biotic factors, rust diseases caused by *Puccinia* species belonging to the order Uredinales in the Basidiomycetes class threaten wheat production around the world. One of the most important species causing rust diseases in wheat is stripe (yellow) rust, caused by *Puccinia striiformis* West., and leaf (brown) rust, caused by *Puccinia triticina*, which are biotrophic fungi and cause significant crop losses and quality degradation depending on the growing area [8-10]. It causes yield and quality losses by suppressing photosynthesis in the infected leaves and especially in the flag leaf [11]. It is estimated that 5 million tons of product are lost annually and the market value of this lost product is $1 trillion [12, 13]. Chemical sprays used to control these diseases affect the biological environment, disturb the balance, and may even enter the food chain and have toxic effects on humans. Considering all these negative aspects, genetic resistance of crops in disease control is an alternative approach preferred by producers as a convenient, cost-effective and practical control method. Therefore, it is of great importance for breeding to determine the presence of resistant and susceptible wheat varieties to rust diseases and evaluate them in production [14].

It is noteworthy that, if conventional methods, technologies or practices are applied to wheat cultivation in the South Asian zone, there is a high probability that yields will decrease by almost 30% [15]. The most important goal of bioscientists is to develop new varieties that have all three qualities, i.e. high productivity, stability, and good nutritional quality, and also to avoid yield loss in the near future, as mentioned above [16]. To achieve this, we need to vary and broaden the genetic base of our germplasm to include them in breeding programs, because genetic differentiation/variation of plants determines their ability to potentially increase efficiency and thus their use in different breeding programs, ultimately leading to an increase in food production by plants [17]. Furthermore, in plant breeding programs, direct and morphological selection of crops for grain or leaf yield can be misleading because of the factors mentioned above. Thus, the most successful and accurate method of selection depends on background information about crop stability, morpho-agronomic traits, and genetic variation [18, 19]. Improving germplasm by using molecular markers such as SSRs for genetic diversity is considered critical for sustainable, reliable, and efficient food crop production [20-23]. Measuring the extent of accessible genetic diversity is critical for efficient evaluation and utilization of germplasm. An essential tactic for classifying the germplasm and analyzing the genetic links among the breeding material is the use of multivariate statistical algorithms [5]. Some other suitable methods are stability analysis, cluster analysis, PCA and correlation analysis for identifying genetic diversity, parental selection, tracing the evolutionary path of crops, center of origin and diversity and studying the interaction between environments are currently available [24, 25].

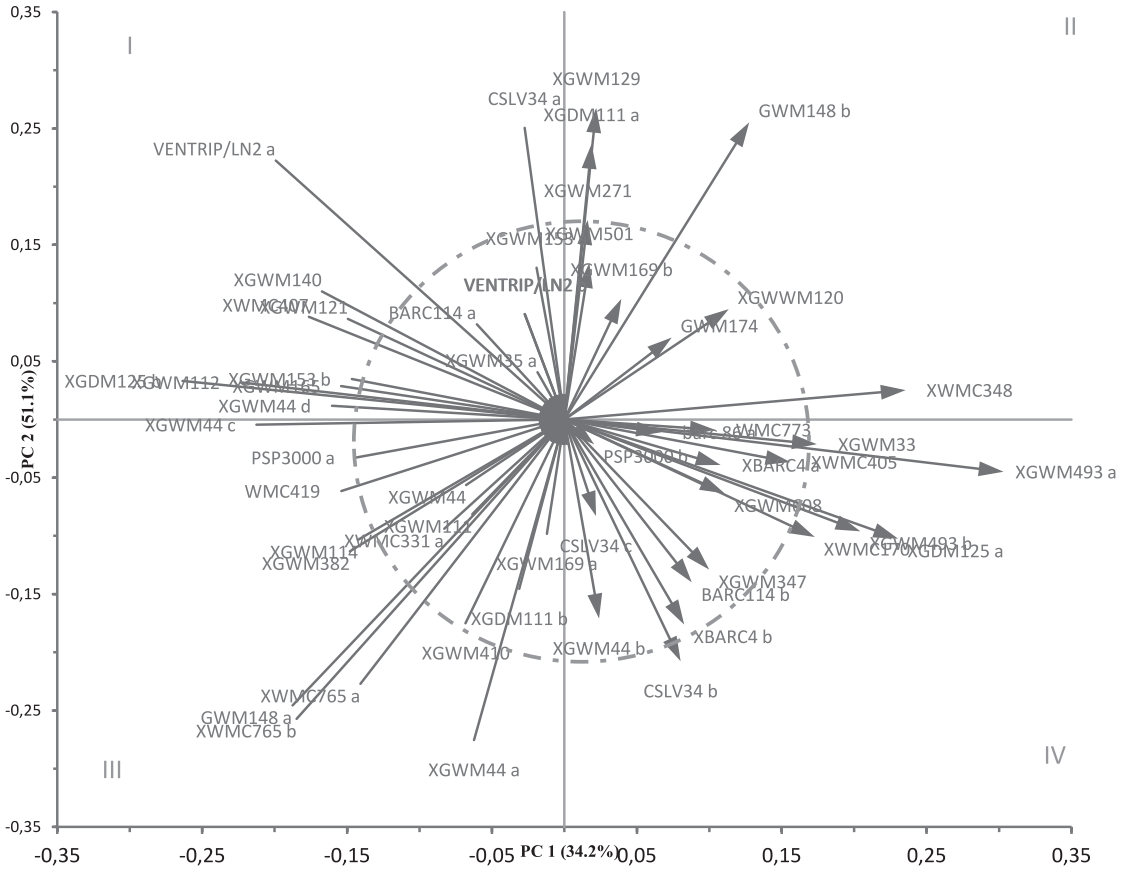

Fig. 1. Principle component analysis for 40 SSRs gene markers used for genetic variations for wheat germplasm.

Simple sequence repeats (SSRs) or microsatellites, i.e. PCR-based molecular markers, have gained importance as genetic markers in a variety of crop species, including wheat. SSRs markers are more numerous, evenly distributed across the genome, and more polymorphic than other genetic markers. These characteristics and their ease of recognition make them most suitable for detecting and distinguishing between accessions that are genetically very similar. SSRs markers have been used in several studies to investigate the genetic diversity of cultivated wheat genotypes of *T. aestivum* (hexaploid). [26]. The use of molecular markers to evaluate genetic variation has received considerable attention because they are not affected by environmental variation. Many scientists have studied the genetic diversity of common wheat using SSRs markers because of their high potential for use in evolutionary studies, genetic diversity, genetic relatedness, and genetic distances [27]. In the present study, genetic markers (SSRs) should be used to identify the genetic basis of wheat germplasm. In addition, the most suitable marker that might be able to identify rare alleles or detect the narrow or broad genetic base will also be reported by using cluster analysis, PCA, correlation and SSR gene marker comparison.

Materials and Methods

Bio-Resources Conservation Institute (BCI), National Agriculture Research Center (NARC), Islamabad, Pakistan provided the breeding material (official germplasm of Pakistan). The germination test (15 seeds of each accession) was done at lab for the selection of 50 best performing land races using Filter paper (Whatman filter paper No. 1) for experimental study and screening of huge breeding material [28]. Accessions with a higher germination rate, greater than 70%, were selected for further testing.

DNA Extraction and Molecular Marker Analysis

A total of 50 genotypes were grown in a tray at room temperature with an equal mixture of sand, farmyard manure, and clay when they were seven days old (Fig. 1). After one week, plant (seedlings) emerged, and they were utilized to isolate DNA. DNA was extracted using the procedure with some modification described by Doyle and Doyle [29] and Begum et al. [30]. Fresh and young leaves of all the accessions were finely ground in 3 mL of 2XCTAB buffer to extract DNA using a modified version of the CTAB technique methodology.

Table 1. List of SSRs markers used for identifying genetic variation among 50 wheat lines.

S. No	Marker	Band Size (bp)	S. No	Marker	Band Size (bp)
1	*barc 86* [a]	200	20	*XBARC4* [a]	90
2	WMC773	298		*XBARC4* [b]	200
3	*BARC114* [a]	105	21	XGWM112	180
	BARC114 [b]	200	22	*XGDM111* [a]	100
4	*CSLV34* [a]	215		*XGDM111* [b]	190
	CSLV34 [b]	190	23	*XWMC331* [a]	210
	CSLV34 [c]	150	24	*XGDM125* [a]	150
5	*PSP3000* [a]	350		*XGDM125* [b]	190
	PSP3000 [b]	300	25	*XGWM169* [a]	200
6	*XGWM35* [a]	225		*XGWM169* [b]	210
7	WMC419	200	26	XGWM501	180
8	XGWWM120	150	27	XGWM347	190
9	GWM174	220	28	*VENTRIP/LN2* [a]	259
10	XGWM140	120		*VENTRIP/LN2* [b]	300
11	XWMC170	200		*VENTRIP/LN2* [c]	1000
12	XWMC405	220	29	XGWM33	200
13	XWMC348	130	30	XGWM165	200
14	XWMC407	120	31	XGWM271	170
15	*GWM148* [a]	190	32	XGWM382	180
	GWM148 [b]	200	33	XGWM608	210
16	*XGWM493* [a]	150	34	XGWM129	220
	XGWM493 [b]	300	35	XGWM121	110
17	*XGWM153* [a]	100	36	XGWM410	140
	XGWM153 [b]	300	37	XGWM114	150
18	XGWM111	185	38	XGDM19	130
19	*XGWM44* [a]	120	39	XGWM44	185
	XGWM44 [b]	285	40	*XWMC765* [a]	110
	XGWM44 [c]	500		*XWMC765* [b]	210
	XGWM44 [d]	700			

Note: a, b, c, and d denotes various bands size amplified by the single primer.

The RNAse (2-3 µL) were added to the DNA pellet and incubated at 37ºC for one h to digest the RNA from the sample. Then centrifugation was performed at 12000 rpm for 12 min. Supernatant was then transferred to new tube by adding chloroform: isoamyl alcohol and inverted gently 4-5 times followed by centrifugation. Chilled iso-propanol was added afterwards and again incubated for 10 min at 4ºC. After centrifugation pellet was removed and washed with 75% ethanol and oven dried for 30 min. The pellet was resuspended in 100 µl of nuclease free water.

Quality and Quantification of DNA

50 g of DNA in 0.8% agarose gel was used to examine the integrity and quality of the DNA during gel electrophoresis. The DNA isolation process was repeated on the samples with identifiable smear in the gels. For DNA quantification (50 accessions) of wheat samples (in 5 replications) subjected to nano-drop for quantification by observing the OD values. The samples with OD value above 0.7 were selected for PCR analysis.

Table 2. Principle component variances and cumulative proportion.

Components	Variance	Proportion	Cumulative proportion
1	9.957	0.142	0.142
2	8.213	0.111	0.253
3	3.902	0.070	0.323
4	2.121	0.056	0.378
5	2.659	0.047	0.426
6	2.534	0.045	0.471
7	2.404	0.043	0.514
8	2.176	0.039	0.553
9	1.970	0.035	0.588
10	1.874	0.033	0.622

PCR Amplification of SSRs Markers

In the current investigation, 40 SSRs markers, including 12 polymorphic and 28 monomorphic markers, were employed (Table 1). For the purpose of determining the molecular basis of genetic diversity, SSRs markers (Primer Invitrogen) were employed. A commercially available kit (Thermo Fisher Scientific, Waltham, MA, Fermentas) was used to extract the DNA, and the procedure described by Begum et al. [31] was followed to perform the PCR (96 well-Applied Biosystems, Model-990, USA). The PCR was performed in 2 µL of 10 X-PCR buffer solutions, 4 µL of 2.5 mM dNTPs, 1.6 µL of 25 mM $MgCl_2$, 1.5 µL of forward and reverse primers, 2.0 µL of genomic DNA, and 7.15 µL of double distilled water, for 20 µL of each reaction. The PCR was performed on an Advanced BioRAD-25 PCR machine.

Gel Electrophoresis/Band Visualization

TBE buffer was used to prepare the agarose gel, which was then stained with Ethidium bromide (EtBr) and visualized under UV light. For photographing, a UV-tec documentation system (made in the UK) was used. PCR products were resolved on a 4% agarose gel in a gel tank with TBE buffer (0.5X) with EtBr staining for visualization. For identification, the fragments were visualized under UV light in IBGE's gel documentation system (BioRad).

Data Analysis

All data were gathered using suggested procedures for statistical analysis to ensure accurate data evaluation. Furthermore, web-based statistical packages "R" version 4.0.2 were used to analyze the data (R Core Team, 2014).

Results

PCA Analysis

The PCA was constructed to find out the genetic difference between the accessions and the SSRs markers

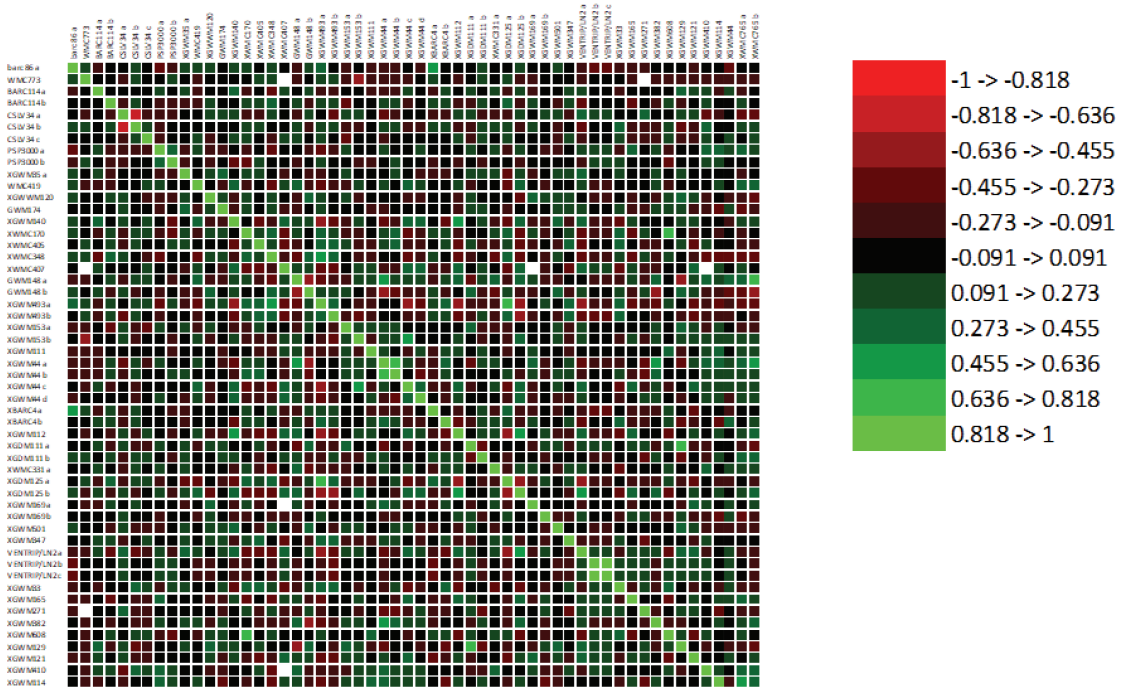

Fig. 2. Heat-map for correlation matrix for 40 SSRs markers used to identify variations among wheat population. The red gauge indicates negative correlation while green gauge shows positive correlation.

Table 3. Cluster analysis based on 40 SSRs markers to group 50 wheat accessions.

S. No	Groups	Cluster	Accessions	Number of accessions
1	A	I	11123, 11126, 11145, 11161, 11160, 11163	6
		II	11154, 11155, 11162, 11164, 11177, 11168, 11170, 11171	8
		III	11144, 11152, 11166, 11173, 11174	5
2	B	IV	11178, 11192, 11181, 11195, 11193, 11200, 11202, 11187, 11188, 11189	10
		V	11179, 11183, 11186, 11190,	4
		VI	11184, 11194, 11197, 11198, 11185	5
3	C	VII	11205, 11210, 11211, 11207, 11214, 11209, 11208, 11215, 11216, 11218,	10
		VIII	11217, 11221	2

used for the study. Overall the PCA analysis revealed 85.3% variation among the germplasm based on the SSRs marker used. The PCA1 and PCA2 contributed 34.2% and 51.1% to the total variation, respectively (Fig. 1). The variance for both these components was also more than the rest of the components 9.957 and 8.213, respectively revealing the major contribution of first two components (Table 2). The SSRs markers (*XGWM169*[b], *GWM174*, *barc86*[a], *PSP3000*[b], *WMC773*, *XBARC4*[a], *XGWM608*, *XGWM347*, *BARC114*[b], *XBARC4*[b], *CSLV34*[c], *XGWM44*[b], *XGWM169*[a], *XGDM111*[b], *XGWM410*, *XGWM44*, *XGWM111*, *XWMC331*[a], *XGWM114*, *XGWM382*, *WMC419*, *PSP3000*[a], *XGWM165*, *XGWM153*[b], *BARC114*[a], *XGWM35*[a], *VENTRIP/LN2*[b], and *VENTRIP/LN2*[c]) in the blue circle were more reliable in finding out the diversity based on the alleles/band size in the study, as they lie close to the origin. Moreover, these markers are contributing to the maximum proportion in finding out the genetic variations among the population.

The markers and accessions were equally distributed among four quadrants i.e. I, II, III, and IV. All the quadrants had negative value as indicated by the measuring scale as shown in the figure except II. The SSRs markers in quadrant II are both reliable and authentic in differentiation of both similar and diversified germplasm. The SSRs marker lying in quadrant II i.e. *XGWM169*[a], *GWM174*, and *barc86*[a] proved to the best suited markers among 40 selected markers for accurately identifying the genetic variation among wheat population.

Correlation Analysis

Association between the parameter was also done in order to check and recommend highest associations of parameters for the studied germplasm. Results indicated the highest association was observed between bands of *VENTRIP/LN2*[b] and *VENTRIP/LN2*[c] i.e. is "1" in accessions 39 (Fig. 2). This complete correlation may be

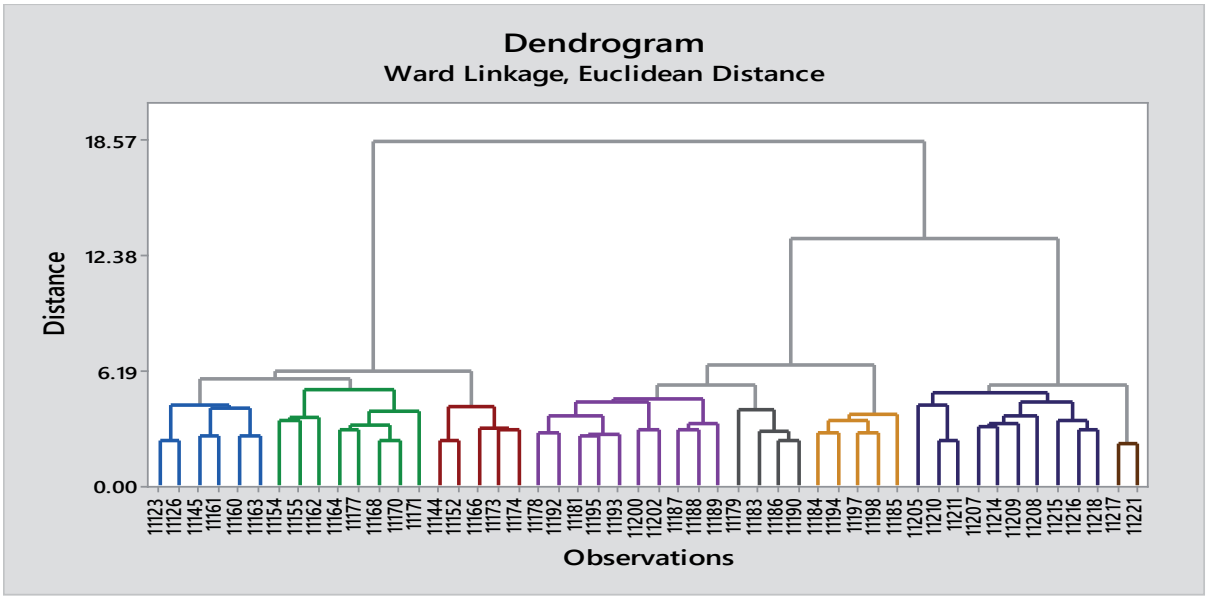

Fig. 3. Cluster analysis for grouping of wheat accession in the different groups based on genetic variations.

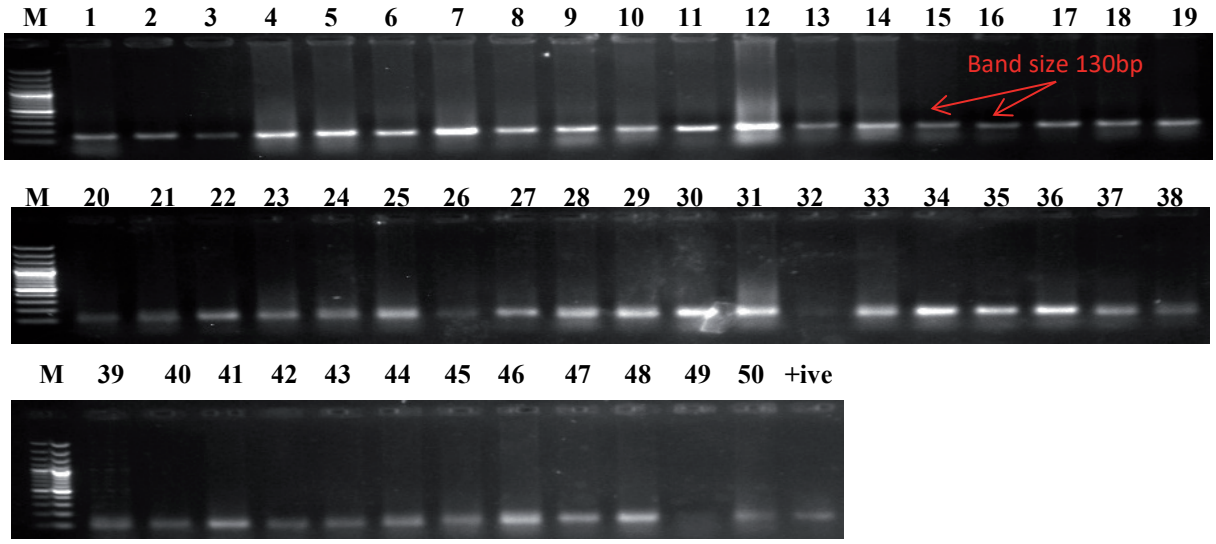

Fig. 4. Amplification of 130 bp bands by XGDM19 gene marker for genetic variation among wheat germplasm. M is 100 bp standard/ ladder, +ive is positive control and 1-50 are wheat accessions.

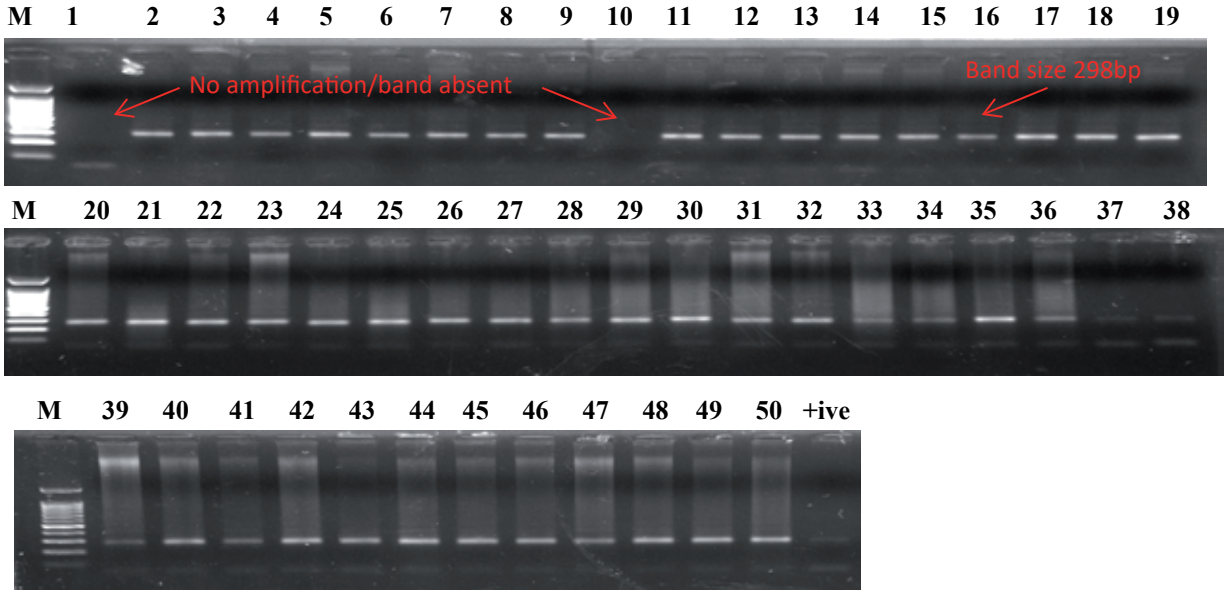

Fig. 5. Amplification of 130 bp bands by WMC773 gene marker for genetic variation among wheat germplasm. M is 100 bp standard/ ladder, +ive is positive control and 1-50 are wheat accessions.

due to the detection of the bands by same SSRs marker *VENTRIP/LN2* or could have the same priming DNA region on different alleles. The association between markers *XWMC170*/*XGWM608*, *XGWM44*[a]/ *XGWM44*[b], *XGDM111*[a]/*XGWM*129, *XWMC765*[a]/*XWMC765*[b], and *GWM148*[a]/ *XWMC765*[b] were also high with the correlation values of 0.368, 0.678, 0.655, 0.653, 0.653 and 0.643, respectively. The positive association between the parameters shows that both will equally detect the genetic variation in the wheat germplasm, which will help in confirmation of accurate detection of genetic variations.

Similarly the lowest correlation was observed between the markers CSLV34b and CSLV34a i.e -0.688 followed by *GWM148*[a] and *XGWM129* i.e -0.633. The other lowest or negative association was observed between the genetic markers *XGWM493*[a]/ *VENTRIP/LN2*[a], *GWM148*[a]/ *GWM148*[b], *XWMC765*[b]/ *GWM148*[b], *XGDM125*[a]/ *VENTRIP/LN2*[a] and *XGWM493*[a]/ *XGDM125*[b] with correlation values of -0.613, -0.608, -0.608, -0.600 and -0.569, respectively. The negative correlation shows that both the marker must not be used for confirmatory as they have the least chance to detect the same genetic variation among the wheat germplasm, though they can be used for collection and identifying different genetic variations but that will need the confirmations of other SSRs markers for accurate detection of genetic diversity.

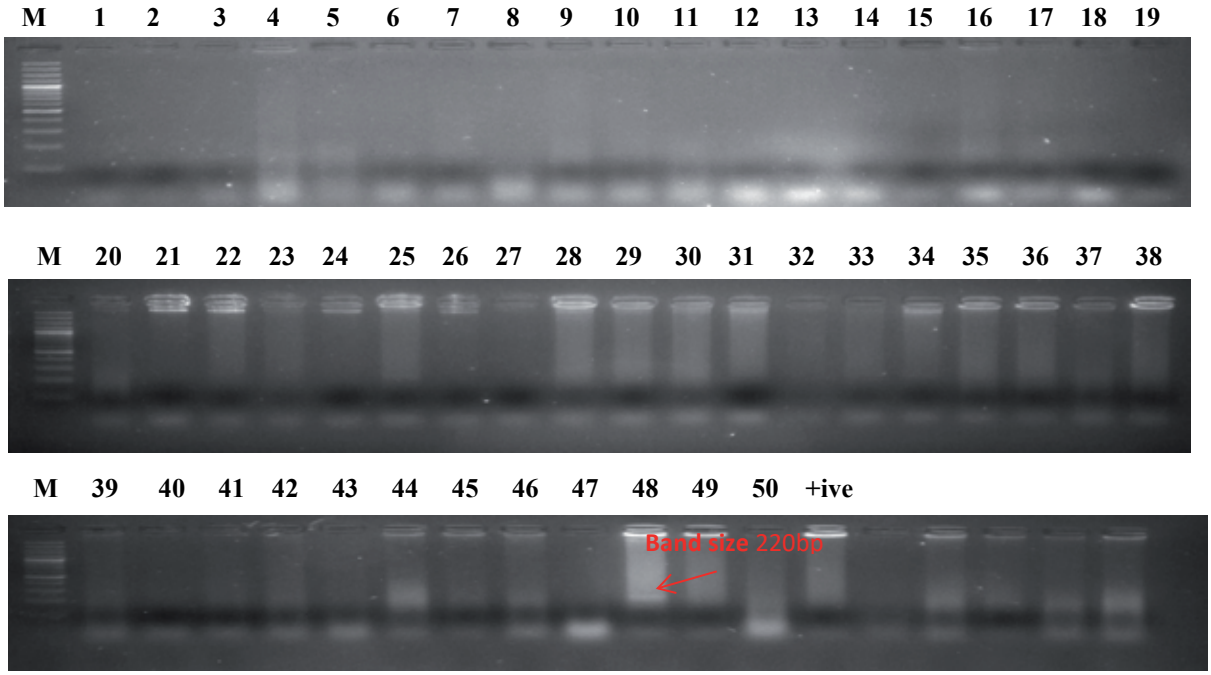

Fig. 6. Amplification of 130 bp bands by gwm174 gene marker for genetic variation among wheat germplasm. M is 100 bp standard/ladder, +ive is positive control and 1-50 are wheat accessions.

Cluster Analysis

The entire 40 SSRs marker were applied on the studied germplasm (50 selected accessions) to group the accessions on the basis of genetic differentiation. Cluster analysis revealed three main groups A, B, and C. Group A and B had three cluster each while group C has only two cluster and least number of germplasm percentage i.e only 12 accessions. Cluster I, II and III were separated at genetic distance of 6.19 (Euclidian distance), while Cluster IV, V and VI were differentiated form each other with 6.21 Euclidian distances. Both cluster of group C i.e VII and VIII were separated from each other with a genetic distance of 5.7 (Euclidian distance). Group A and B had total of 19 accessions each that were distributed between three cluster, respectively (Table 3 and Fig. 3).

The groups in the present study revealed that the studied germplasm of wheat were having broad genetic base as the accessions were not in same group and were distributed in various cluster. The cluster of group B and C were separated from each other with a Euclidean genetic distance of 14.2. While the group A was totally different from B and C with the genetic distance of 18.5 revealing that these accessions were having totally different genetic base and are more diversified than the others.

Wheat Germplasm Genetic Variation

A total of 40 reported SSRs markers were used in the present study. These markers were able to amplify the required bands (Table 1, Figs 4-6) for identifying the genetic distance in term of variations between the wheat germplasm used in the present study. The highest amplification was revealed by *XGDM19* (130 bp), *WMC773* (298 bp), *XWMC331*[a] (210 bp), *BARC114*[a] (105 bp), *barc86*[a] (200 bp), and *XBARC4*[a] (90 bp) i.e. 50, 48, 47, 46, 45, and 42 respectively. While the gene markers i.e. *CSLV34*[c], *GWM174*, *XGWM169*[b], *VENTRIP/LN2*[b] (300 bp), and *VENTRIP/LN2*[c] (1000 bp) were only able to amplify one wheat accessions out of 50. The SSRs markers *XGWM153*[b] (300 bp), *BARC114*[b] (200 bp), and *XGWM44*[d] (700 bp) also amplified bands in very less accessions i.e. 2, 3, and 4 accessions.

Discussion

Most agronomic traits are influenced by environmental factors and heritability, making genetic basis dissection difficult if molecular techniques are not used [32, 33]. Breeding nowadays only concentrates on yield-related characteristics, which results in a decrease in genetic base and variation of the wheat crop [34, 35], this is cause for serious concern because narrowing wheat's genetic base could be devastating in combating climate/weather change by only disease resistance-related parameters or yield-related issues [36]. Non-conventional approaches including high throughput techniques like transformation, Marker assisted selection, gene sequencing etc., must be employed to solve the issues [37, 39].

Cluster analysis can identify similarities and differences among germplasm based on genetic variations or distance between two different/distinct groups or clusters [40, 42]. The accessions in a single

cluster/group are genetically related or similar, while all of the accessions in the other cluster are genetically dissimilar. Our findings are consistent with previous research that found different clusters and subgroups of ninety wheat accessions based on geographical zone [15, 43, 44]. The PCA can be utilized effectively in trials that may involve multiple location for evaluation of genetic variability [15-35, 45, 46]. The present study PCA results are similar to previous findings as their results showed above 80% variation by PC compartments [35, 36, 45]. The PC plot showed high genetic diversity that may be that these landraces consisted of exceedingly admixed lineages. The findings of other studies are dissimilar to present study as their PC1-PC7 contributed approximately 80% of the total variability/variation while in the present study PC1 and PC2 contributed almost 80% to the total variations. Though PCA explained the source of the dataset used in the investigation, observed different variations in germplasm that were discovered in the two studies. In the present era one of the prime ways to improve the crop yield is to reduce the loss mainly because of the outbreak of lethal diseases. The best and suitable way for genetic diversity or broadening the genetic base is the used of disease related SSRs markers, as they will serve dual purpose i.e. is genetic diversity among the germplasm as well as identification of important disease resistant genes [15]. In the current study, accessions that were having more combinations of resistance gene were investigated further in breeding program with available rust markers for broadening the genetic base that is specific to disease resistance, as newly developed cultivars may have a broad genetic background but still be susceptible to lethal diseases because they were developed primarily to improve yield-related parameters [15]. By locating accessions with rust-resistant genes, the current study sought to extend the genetic base of our existing wheat varieties and increase the genetic variety of Pakistan germplasm. Similar study was done by other researcher as they used SSRs marker linked to rust disease and found genetic diversity [15].

The SSRs marker *Xgwm120*, that is presents 12 cM distance from *Yr5* Begum et al. [31], showed a 150-bp fragment 42% wheat land races. The marker *psp3000* was used for the detection of *Yr10* gene. Similarly the *Xgwm44* revealed polymorphic nature by amplifying various bands with sizes 120, 185, 500, and 700 bp. The band size 185 bp is closely linked to *Lr* 19 gene [47] and is generally used for its identification. It is also used for mapping of gene as well. Previous studies findings reported to screen the population with this marker for *Lr 19* gene [48]. It has also been linked to the *Yr18* gene. The *csLV34* marker successfully amplified 150 bp as well as 190 bp fragments in 26% of accessions demonstrating the presence of the *Yr18* gene, on the other hand a marker with 215 bp size was associated with the absence of *Yr18* and was able to amply in 60% of the total population. Because this marker is co-dominant, it is appropriate for use in early segregating generations [49]. As previously reported [15], the occurrence of this gene in the Pakistan wheat population is quite low, as a result, there is an urgent need to extend the race-nonspecific yellow rust resistance. Previously, Begum et al. [50] all advance wheat lines were tested for the occurrence of *Lr*34/*Yr*18 using CSLV34, which yielded a 150 bp PCR product that was amplified in almost 43% accessions. *Yr26* gene was detected using two markers i.e. *barc 86* (200 bp) and *Wmc773* (298 bp). The former was found in 90% of the accessions, while the latter was found in 96% of the accessions. In virulent tests, accessions with the presence of *Yr26* gene were having resistant for the majority of *Pst* races [15, 33]. The virulence, however, has lately been identified in Australian wheat population. Three additional markers (SSRs) were utilized for the identification/presence of the *Yr29* gene. Similarly the *Wmc* 419 (with a band size of 200 bp) was found in 54% of the population, whereas *Xgwm* 140 (band size of 120 bp) and xgwm 410 (with band size of 140 bp) were found in 48% and 56% of the landraces, respectively. Similar findings were also reported by many other studies as well [15, 31] for *Yr26* and *Yr 29* genes. The resistance for *Yr* with *Xbarc4* (90-100 bp) is located on arm 5BS of the chromosome was amplified in 84% accessions. This gene has been linked to yellow rust resistance [51] but some study revealed a significant QTL for Powdery mildew resistance on 5BS as well [52]. The presence of *Lr49* in this population was confirmed by screening microsatellite marker (SSRs *Xwmc348* in 42% of the land races. Similar results were reported by previous studies [53] as well. The SSRsmarker *Xgwm153* was identified in almost 82% (100 bp) and 2% (300 bp) of the population, respectively. Its location has reported at 8.2 cM from the *YrP138* resistance gene and is distinct from other known resistance in that it exhibits resistance to the dominant Chinese *Pst raceCYR32* at the seedling stage [54].

The marker *Xwmc*407 was also applied to identify *Lr17* in 50% of the studied population, which is present on short-arm of wheat 2A chromosome [33]. However, it is also commonly used to test for the presence of the *Yr17* gene [15]. *Xgwm140* was also used in previous studies for genetic diversity to identify heat tolerant lines in wheat of guinea savanna [55]. They have also reported low allele frequencies (25 %) for random DNA marker allele while in present study it differentiated nearly 48% population from the rest. Sardouie-Nasab et al. [56] used *XWMC170* i.e. is located on 2A and is for stripe rust but it was used for Haplotype analysis of QTLs attributed to salinity tolerance in wheat. He also further reported that this is also linked to Na^+ ions. In present study it was only used for identifying rust resistance genes rather than ions demarcation.

Conclusion

Further studies on genetic diversity profiling may lead to the development of efficient and economical

new breeding lines that are resistant or tolerant, have higher genetic characteristics, are environment-friendly, human-friendly and meet the needs of societies. The study was useful as disease related SSRs markers were used for general diversity, making the single SSRs marker used for multiple purposes including disease resistance, genetic diversity, mineral or ion identification. Furthermore the dual nature of marker can be fruitful as a single marker may be linked to multiple traits which will ultimately help in saving the cost, time and efforts rather using individual/ or specific markers for evaluating the genetic distance or base for the diversity analysis. Moreover, the combination of recent studies on the breeding traits of the same/similar genotypes existing in the gene pool and genetic diversity analyses can lead breeders to predict further selection of varieties to develop elite breeding lines resistant to fungal outbreaks from the existing gene pool.

Author Contributions

Conceptualization: S.H.K., S.A.K.B., I.K., & S.B., Data curation: S.H.K., M.N.K & S.N., Formal analysis: G.M.A., A.H & A.Z., Investigation: S.H.K & I.K., Methodology: B.A., A.K & M.I., Software: A.K & S.A.K.B., Writing-original draft: S.H.K., S.B & S.N., Writing - review & editing: B.A., F.A.S & R.U., Funding acquisition: R.U

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. FAO.2020. https://www.fao.org/3/cb1447en/cb1447en.pdf (acssesed on Sep 19, 2023).
2. ADNAN M., FAHAD S., SALEEM M.H., ALI B., MUSSART M., ULLAH R., ARIF M., AHMAD M., SHAH W.A., ROMMAN M. Comparative efficacy of phosphorous supplements with phosphate solubilizing bacteria for optimizing wheat yield in calcareous soils. Scientific Report. **12**, 11997, **2022**.
3. MWALE V.M., TANG X., CHILEMBWE E. Assessment of genetic diversity among sixty bread wheat (*Triticum aestivum*) cultivars using microsatellite markers. African Journal of Biotechnology. **15** (21), **2016**.
4. BRAUN H.J., SAARI E.E. An assessment of the potential of Puccinia striiformis f. sp. tritici to cause yield losses in wheat on the Anatolian plateau of Turkey. Vorträge für Pflanzenzüchtung. 24, 121-123, 1992.
5. AJMAL S.U., MINHAS N.M., HAMDANI A., SHAKIR A., ZUBAIR M., AHMAD Z. Multivariate analysis of genetic divergence in wheat (*Triticum aestivum*) germplasm. Pakistan Journal of Botany. **45** (5), **2013**.
6. AHMAD M., ISHAQ M., SHAH W.A., ADNAN M., Fahad S., SALEEM M.H., KHAN F.U., MUSSARAT M., KHAN S., ALI B., MOSTAFA Y.S., ALAMRI S., HASHEM M. Managing Phosphorus Availability from Organic and Inorganic Sources for Optimum Wheat Production in Calcareous Soils. Sustainability. **14** (3), **2022**.
7. TRIBUNE T.E. Wheat production estimated at 25, 16 million tons Available online: https://tribune.com.pk/story/1953202/wheat-production-estimated-25-16-million-tons (accessed on Dec 3, 2022).
8. CHEN X., PENMAN L., WAN A., CHENG P. Virulence races of *Puccinia striiformis* f. sp. *tritici* in 2006 and 2007 and development of wheat stripe rust and distributions, dynamics, and evolutionary relationships of races from 2000 to 2007 in the United States. Canadian Journal of Plant Pathology. **32**, 315-333, **2010**.
9. CARLETON M.A. A serious new wheat rust in this country. Science. **42**, 58-59, **1915**.
10. WILLIAM M., SINGH R.P., HUERTA-ESPINO J., ORTIZ ISLAS S., HOISINGTON D. Molecular Marker Mapping of Leaf Rust Resistance Gene Lr46 and Its Association with Stripe Rust Resistance Gene Yr29 in Wheat. Phytopathology. **93**, 153-159, **2003**.
11. CHEN X.M. Epidemiology and control of stripe rust (*Puccinia striiformis* f. sp. *tritici*) on wheat. Canadian Journal of Plant Pathology. **27**, 314-337, **2005**.
12. BEDDOW J.M., PARDEY P.G., CHAI Y., HURLEY T.M., KRITICOS D.J., BRAUN H.J., PARK R.F., CUDDY W.S., YONOW T. Research investment implications of shifts in the global geography of wheat stripe rust. Nature Plants. **1**, 15132, **2015**.
13. WELLINGS CR. Global status of stripe rust: a review of historical and current threats. Euphytica. **179**, 129-141, **2011**.
14. TOK D. Sarı Pas Hastalığına Dayanıklı Ve Duyarlı Ekmeklik Buğday (Triticum aestivum L.) Çeşitlerinde Dna Metilasyon Polimorfizminin İncelenmesi. Yüksek Lisans Tezi, Marmara Üniversitesi, Fen Bilimleri Enstitüsü, syf. 17, **2010**.
15. KHATTAK S.H., BEGUM S., AQEEL M., FAYYAZ M., BANGASH S.A.K., RIAZ M.N., SAEED S., AHMED A., Ali G.M. Investigating the allelic variation of loci controlling rust resistance genes in wheat (*Triticum aestivum* L.) land races by SSR marker. Applied Ecology & Environmental Research. **18** (6), **2020**.
16. FASOULA V.A. Two novel whole-plant field phenotyping equations maximize selection efficiency. In Proceedings of the Modern variety breeding for present and future needs. Proceedings of the 18th EUCARPIA general congress, Valencia, Spain, 9-12 September, 2008; Editorial Universidad Politécnica de Valencia, 2008; pp. 361-365.
17. POUDEL D.D., DUEX T.W. Vanishing springs in Nepalese mountains: Assessment of water sources, farmers' perceptions, and climate change adaptation. Mountain Research and Development. **37** (1), **2017**.
18. KALIMULLAH S., KHAN J., IRFAQ M., RAHMAN H.U. Genetic variability, correlation and diversity studies in bread wheat (*Triticum aestivum* L.) germplasm. The Journal of Animal & Plant Sciences. **22** (2), **2012**.
19. SINGH D., DHILLON T.S., JAVED T., SINGH R., DOBARIA J., DHANKHAR S.K. KIANERSI F., ALI

B., POCZAI P., KUMAR U. Exploring the Genetic Diversity of Carrot Genotypes through Phenotypically and Genetically Detailed Germplasm Collection. Agronomy. **12** (8), **2022**.

20. ALI S., ULLAH S., KHAN M.N., KHAN W.M., RAZAK S.A., WAHAB S., HAFEEZ A., BANGASH S.A.K., POCZAI P. The Effects of Osmosis and Thermo-Priming on Salinity Stress Tolerance in *Vigna radiata* L. Sustainability. **14** (19), **2022**.
21. ALI B., HAFEEZ A., JAVED M.A., AFRIDI M.S., ABBASI H.A., QAYYUM A., BATOOL T., ULLAH A., MARC R.A., JAOUNI S.K.Al.; ALKHALIFAH, D.H.M., SELIM S. Role of endophytic bacteria in salinity stress amelioration by physiological and molecular mechanisms of defense: A comprehensive review. South African Journal of Botany. **151**, 33-46, **2022**.
22. ALI B., WANG X., SALEEM M.H., AZEEM M.A., AFRIDI M.S., NADEEM M., GHAZAL M., BATOOL T., QAYYUM A., ALATAWI A. *Bacillus mycoides* PM35 Reinforces Photosynthetic Efficiency, Antioxidant Defense, Expression of Stress-Responsive Genes, and Ameliorates the Effects of Salinity Stress in Maize. Life. **12** (2), **2022**.
23. ALI B., WANG X., SALEEM M.H., HAFEEZ A., AFRIDI M.S., KHAN S., ULLAH I., AMARAL JÚNIOR A.T. do., ALATAWI A., ALI, S. PGPR-Mediated Salt Tolerance in Maize by Modulating Plant Physiology, Antioxidant Defense, Compatible Solutes Accumulation and Bio-Surfactant Producing Genes. Plants. **11** (3), **2022**.
24. BEGUM S., KHAN M.R., JAN A., UR-RAHMAN H., KHATTA K.H., SAEED S., AHMED A., ALI G.M. Genetic Transformation of the Epsps Herbicide Resistance Gene in Agrobacterium Mediated Peanut (*Arachis hypogaea* L.) and Effective Revival of Transgenic Plants. Applied Ecology and Environmental Research. **20** (2), **2022**.
25. KHATTAK S.H., KALEEM I., FARRUKH A., NOOR S., JAMIL K., KAMAL T., SIDDIQUI N.R., ALI G.M. Fruit Ripening Characterization and Amylase Mystery in Bananas. Global Journal of Nutrition & Food Science. **4** (1) , **2022**.
26. SENTURK AKFIRAT F., UNCUOGLU A.A. Genetic diversity of winter wheat (*Triticum aestivum* L.) revealed by SSR markers. Biochemical Genetics. **51**, 223-229, **2013**.
27. SALEM K.F.M., EL-ZANATY A.M., ESMAIL R.M. Assessing wheat (*Triticum aestivum* L.) genetic diversity using morphological characters and microsatellite markers. World Journal of Agricultural Sciences. **4** (5)**, 2008**.
28. PANJABI B., BASU R.N. Testing germination and seedling growth by an inclined glass plate blotter method. Indian Journal of Plant Physiology. **25**, 289-295. **1982**.
29. DOYLE A,J., DOYLE L.J. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin. **19**, 11-15, **1987**.
30. BEGUM S., KHATTAK S.H., SHAHZAD A., NOOR S., ZAHEER S. Molecular characterization of Pakistani wheat genotypes for leaf rust resistance. Journal of Pure and Applied Agriculture. **4** (1), **2019**.
31. BEGUM S., IQBAL M., AHMED I., FAYYAZ M., SHAHZAD A., ALI G.M. Allelic variation at loci controlling stripe rust resistance in spring wheat. Journal of Genetics. **93** (2), **2014**.
32. UR REHMAN M.A., KHATTAK S.H., INAM S., REHMAN N., HUSSAIN M., ALI, S., SHAHID M.M., KHAN M.R. Unveiling of genetic variability in garlic (*Allium sativum* L.) genotypes grown in Pakistan. Journal of Pure and Applied Agriculture. **5** (3), **2020**,
33. WANG X., LUO G., YANG W., LI Y., SUN J., ZHAN K., LIU D., ZHANG A. Genetic diversity, population structure and marker-trait associations for agronomic and grain traits in wild diploid wheat *Triticum urartu*. BMC Plant Biology. **17** (112), **2017**.
34. REN J., SUN D., CHEN L., YOU F.M., WANG J., PENG Y., NEVO E., SUN D., LUO M.C., PENG J. Genetic diversity revealed by single nucleotide polymorphism markers in a worldwide germplasm collection of durum wheat. International Journal of Molecular Sciences. **14**, 7061-7088, **2013**.
35. MENGISTU D.K., KIDANE Y.G., CATELLANI M., FRASCAROLI E., FADDA C., PÈ M.E., DELL'ACQUA M. High-density molecular characterization and association mapping in Ethiopian durum wheat landraces reveals high diversity and potential for wheat breeding. Plant Biotechnology Journal. **14** (9), **2016**.
36. BANGASH S.A.K., KHAN M.S., AMBREEN., KHATTAK S.H., SIDDIQUE A.N. Genetic transformation of *Brassica juncea* with antimicrobial *Wasabi defensin* gene. Pakistan Journal of Botany. **45** (3), **2013**.
37. JAMIL K., KHATTAK S.H., FARRUKH A., BEGUM S., RIAZ M.N., MUHAMMAD A., KAMAL T., TAJ T., KHAN I., RIAZ S. Biogenic Synthesis of Silver Nanoparticles Using Catharanthus roseus and Its Cytotoxicity Effect on Vero Cell Lines. Molecules. 27 (19), 2022.
38. FARRUKH A., KHATTAK S.H., KALEEM I., BEGUM S., JAMIL K., KAMAL T., RIAZ M.N., SIDDIQUI N.R., IKRAM R., NOOR S. Plant Based Nanotechnology–A New Trend in Therapeutic Approaches of Diabetes. Endocrinology & Diabetes: Open Access Journal. **1** (1), **2022**.
39. QAISER R., AKRAM Z., ASAD S., INAM-UL-HAQ., MALIK S.I., FAYYAZ M., SUFIYAN M., KHATTAK S.H., SANDHU K.S., SIDHU G.S. Genome-Wide Association Mapping and Population Structure for Stripe Rust in Pakistani Wheat Germplasm. Pakistan Journal of Botany. **54** (4), **2022**.
40. SHAHEEN A., SHAHZAD A., SHAH S.H., KHATTAK S.H., IDREES S., KHAN S.U. Molecular and morphological evaluation of salt tolerance genes in advanced wheat lines (Part 1). Pakistan Journal of Botany. **55** (3), **2023**.
41. ZAHID G., BEGUM S., ALMANI S., KHATTAK S.H., SOOTHAR R.K., SOOMRO S.A. Utilization of SSR markers to identify slow rusting genes in spring wheat (*Triticum aestivum* L.). Pakistan Journal of Agricultural Research. **35** (1), **2022**.
42. GUL R., KHAN H., SATTAR S., FARHATULLAH M.F., SHADMAN K.B.S.A., KHATTAK S.H., ARIF M., ALI, A. Comparison among nodulated and non nodulated chickpea genotypes. Sarhad Journal of Agriculture. **27** (4), **2011**.
43. ISLAM S., HAQUE M.S., EMON R.M., ISLAM M.M., BEGUM S.N. Molecular characterization of wheat (*Triticum aestivum* L.) genotypes through SSR markers. Bangladesh Journal of Agricultural Research. **37** (3), **2012**.
44. CHEN X., MIN D., YASIR T.A., HU Y.G. Genetic Diversity, Population Structure and Linkage Disequilibrium in Elite Chinese Winter Wheat Investigated with SSR Markers. PLoS One, **7** (9) , **2012**.
45. DEVESH P., MOITRA P.K., SHUKLA R.S., PANDEY S. Genetic diversity and principal component analyses for

yield, yield components and quality traits of advanced lines of wheat. Journal of Pharmacognosy and Phytochemistry. **8** (3), **2019**.
46. BHANUPRIYA B., SATYANARAYANA N., MUKHERJEE S., SARKAR K. Genetic diversity of wheat genotypes based on principal component analysis in Gangetic alluvial soil of West Bengal. Journal of Crop and Weed. **10** (2), **2014**,
47. WAQAR A., KHATTAK S.H., BEGUM S., REHMAN T., SHEHZAD A., AJMAL W., ZIA S.S., SIDDIQI I., ALI, G.M. Stripe Rust: A Review of the Disease, Yr Genes and its Molecular Markers. Sarhad Journal of Agriculture. **34** (1), **2018**
48. XING L.I., YANG W., LI Y., LIU D., YAN H., MENG Q., ZHANG T.A. SSR marker for leaf rust resistance gene Lr19 in wheat. Agricultural Sciences in China. **5** (2), **2006**.
49. LAGUDAH E.S., MCFADDEN H., SINGH R.P., HUERTA-ESPINO J., BARIANA H.S., SPIELMEYER W. Molecular genetic characterization of the Lr34/Yr18 slow rusting resistance gene region in wheat. Theoretical and Applied Genetics. **114** (1), **2006**.
50. BEGUM S., KHATTAK S.H., SHAHZAD A., NOOR S., ZAHEER S. Molecular characterization of Pakistani wheat genotypes for leaf rust resistance. Journal of Pure and Applied Agriculture. **4** (1), **2019**.
51. MACCAFERRI M., ZHANG J., BULLI P., ABATE Z., CHAO S., CANTU D., BOSSOLINI E., CHEN X., PUMPHREY M., DUBCOVSKY J. 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** (3), **2015**.
52. LILLEMO M., ASALF B., SINGH R.P., HUERTA-ESPINO J., CHEN X.M., HE Z.H., BJØRNSTAD Å. 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** (8), **2008**.
53. BANSAL U.K., HAYDEN M.J., VENKATA B.P., KHANNA R., SAINI R.G., BARIANA H.S. Genetic mapping of adult plant leaf rust resistance genes Lr48 and Lr49 in common wheat. Theoretical and Applied Genetics. **117** (3), **2008.**
54. YUE Y.L., YAO Z.J., REN X.X., Li, W. Molecular mapping of a gene for resistance to stripe rust in wheat variety PIW138. Agricultural Sciences in China. **9** (9), **2010**.
55. MALA K.K., AMINU D. Identification of heat tolerant lines and genetic diversity in wheat (*Triticum aestivum* L.) using molecular markers in guinea savanna, Nigeria. FUW Trends in Science & Technology Journal. **2** (1B), **2017**.
56. SARDOUIE-NASAB S., MOHAMMADI-NEJAD G., ZEBARJADI A. Haplotype analysis of QTLs attributed to salinity tolerance in wheat (*Triticum aestivum*). Molecular Biology Reports. **40** (7), **2013**.