MOLECULAR CHARACTERIZATION OF PAKISTANI SPRING WHEAT (*TRITICUM AESTIVUM* L.) LINES FOR RESISTANCE TO YELLOW RUST (*PUCCINIA STRIIFORMIS*), LEAF RUST *(PUCCINIA TRITICINA*), AND STEM RUST *(PUCCINIA GRAMINIS*) UTILIZING MICROSATELLITE MARKERS

BEGUM, S.[1*] – ALI, G. M.[1] – IKRAM, R.[1] – KHATTAK, S. H.[2] – LAW, D.[3] – KHAN, N. U.[4] – SHAKEEL, M.[5] – ZAFAR, M. Z.[1] – ELSADEK, M. F.[6] – AL-NUMAIR, K. S.[7] – TAN, D. K. Y.[8] – YASIN, M.[5*]

[1]*National Institute for Genomics and Advanced Biotechnology, National Agricultural Research Centre, Park Road, Islamabad, Pakistan*

[2]*Oilseeds Research Program, National Agricultural Research Center, Park Road, Islamabad, Pakistan*

[3]*Faculty of Health and Life Sciences, INTI International University Nilai, Negeri Sembilan, Malaysia*

[4]*Department of Plant Breeding and Genetics, Faculty of Agriculture, Gomal University, Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan*

[5]*Gomal Center of Biochemistry and Biotechnology, Gomal University, Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan*

[6]*Department of Biochemistry, College of Sciences, King Saud University, Riyadh, Saudi Arabia*

[7]*Department of Community Health Sciences, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia*

[8]*Plant Breeding Institute, Sydney Institute of Agriculture, School of Life and Environmental Sciences, Faculty of Science, University of Sydney, Sydney, Australia*

**Corresponding authors*
e-mail: drmywazir-biotech@gu.edu.pk
sania.idrees@parc.gov.pk

(Received 27th Dec 2024; accepted 24th Mar 2025)

Abstract. Wheat rust, including leaf rust *(Puccinia triticina f. sp. tritici)*, stripe rust *(Puccinia striiformis f. sp. tritici)*, and stem rust *(Puccinia graminis f. sp. tritici)*, is among the most destructive foliar diseases impacting wheat production in Pakistan. This study aimed to evaluate the status of various resistance genes against leaf, stem, and stripe rust in improved Pakistani wheat breeding lines and to identify the suitable lines for gene pyramiding aimed at rust resistance. Forty advanced wheat lines were selected from the National Wheat Disease Screening Nursery (NWDSN) and National Uniform Wheat Yield Trials (NUWYT) for testing at the seedling stage based on the gene-for-gene resistance concept. These lines were subjected to field experiments employing a Randomized Complete Block Design at the National Agriculture Research Center, Islamabad Pakistan. The *Yr29* gene was indicated through the amplification of SSR markers Xwmc367 and Xgwm44 in 22 to 35 wheat lines. Additionally, *Yr26* and *Yr18* genes were amplified in 39 and 17 lines, respectively. The stem rust resistance gene *Sr25* (marker Xgwm539) was identified in 30 lines, while *Sr9* (marker Xgwm1117) was found in 36 genotypes. Moreover, *Wmc344* amplified a 150 bp fragment in 38 wheat lines which shows the presence of *Lr19/Sr25* gene. *Lr14* and *Lr60* were present in 12 and 37 lines, respectively. The resistance resources identified in this study are expected to assist breeders in creating new rust-resistant wheat varieties through gene pyramiding.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

Keywords: *DNA markers, food production, gene pyramiding, molecular detection, rust resistance genes yellow rust Puccinia striiformis f. sp. tritici (Pst), leaf rust Puccinia triticina f. sp. tritici (Ptt), stem rust Puccinia graminis f. sp. tritici (Pgt)*

Abbreviations: Yr, (*Puccinia, striiformis* f. sp. tritici Erikss. (Pst) causes stripe or yellow) rust on wheat, Lr, leaf rust (*Puccinia triticina f*. sp. tritici (Ptt) causes leaf rust in wheat and Sr stem rust, (*Puccinia graminis* f. sp. tritici (Pgt) causes stem rust in wheat, MAS (Marker Assisted Selection), SSR (Simple Sequence Repeat) and STS (Sequence Tagged Site) marker, PCR (Polymerase-chain-reaction), (CDRP), National Agricultural Research Center (NARC), National Uniform Wheat Yield Trials (NUWYT) and National Wheat Disease Screening Nursery (NWDSN) Islamabad, Pakistan, ((NH4)2SO4) Ammonium sulfate, $Mgcl_2$ (Magnesium chloride) (dNTPs) deoxynucleotide triphosphates, pmol (pico-mole), rpm (revolution per minute), ml (milliliter), mM (mili molar), CTAB, (Cetyl-Tri-methyl-Ammonium-Bromide), µL (micro-liter), PVP (Polyvinyl-Pyrrolidone, Tris-base (hydroxyl-methyl-amino-methane), EDTA, Disodium-Ethylene-di-amine-tetra-acetic acid, SDS (Sodium Dodecyl Sulfate), EB (Ethidium bromide), R (resistance), MR (moderately resistance), IT (infection type), S (susceptible).

Introduction

Wheat is the most important staple food crop in the world including in Pakistan (Alotaibi et al., 2023; Nasir et al., 2019). It is cultivated on a total area of 9.2 million hectares with a projected annual production of 25.5 million tons (Lele and Goswami, 2021). However, its national yield potential is constantly threatened by pests, especially leaf and stripe rusts. Wheat rust (leaf, stripe and stem rust) caused by fungal pathogens; *Puccinia triticina* f.sp. *tritici (Ptt)*, *Puccinia striiformis* f.sp. *tritici* (*Pst*)*, Puccinia graminis* f.sp. *tritici* (*Pgt*), respectively, are the most devastating foliar diseases affecting wheat in Pakistan and other wheat producing countries (Altaf et al., 2024). In favorable environmental conditions, the rust diseases spread fast and can reduce yields by up to 70% (Waqas et al., 2019). Wheat production reduced due to stripe rust ranges from 10%–70% in wheat-growing areas depending on the susceptibility of lines, time of early disease development**,** disease development rate and disease duration (Dedryver et al., 2009). *P. graminis* f. sp. *tritici* is the most destructive of the three rusts. Cultivation of resistant varieties is the most economic, effective, environmentally friendly and practical method to reduce yield losses caused by leaf and stripe rusts (King et al., 2021; Ganal et al., 2018; Draz et al., 2015).

This study was conducted in the Punjab province of Pakistan, focusing on the major wheat growing regions. This area is characterized by a semi-arid climate, with hot summers and mild winters. The average temperature during the growing season (October to April) ranges from 10°C to 25°C, with occasional frost in winter. Rainfall is relatively low, averaging between 250 and 500 mm annually, and is primarily concentrated between December and February, which can influence both wheat growth and susceptibility to rust diseases (Bansal et al., 2020; Qureshi et al. 2018). The Punjab region has experienced varying levels of rust prevalence, particularly leaf rust and yellow rust, which are the most common rust types affecting wheat crops. The presence of suitable climatic conditions, such as moderate temperatures and relative humidity levels during the growing season, often leads to outbreaks of these diseases (Raza and Khan, 2021). Over the past decade, reports indicate increasing occurrences of rust due to the emergence of new virulent strains, emphasizing the critical need for enhanced resistance breeding (Imran and Saleem, 2022). The ecological factors outlined above including climate, altitude, and soil conditions play a significant role in disease development (Ali and Hussain, 2023).

For instance, relative humidity levels that fluctuate during the growing season can lead to a higher incidence of infection, particularly when combined with the aforementioned

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

climatic conditions conducive to rust spread (Kamvar et al., 2014). For this study, site selection was carefully executed to ensure representativeness of the primary wheat-growing regions in Pakistan. The selected fields have a history of rust outbreaks, making them ideal for investigating rust resistance in spring wheat. Natural infections were documented in the selected sites, with thorough monitoring conducted to confirm disease presence and severity. In addition to observing natural infections, we implemented controlled conditions to ensure a consistent disease pressure (Haq and Amanullah, 2021). When needed, artificial inoculation with specific rust spores was performed, employing techniques that replicated natural infection cycles (Kapoor et al., 2018). Environmental conditions such as temperature and relative humidity were meticulously controlled in these instances to maintain an optimal environment for disease expression, thereby enabling us to obtain reliable data on the effectiveness of the rust resistance genes being characterized (Khan et al., 2023).

SSR (Simple Sequence Repeat) and STS (Sequence Tagged Site) markers are significantly associated with increased rust resistance in spring wheat lines. The presence of specific rust resistance genes can be identified and characterized through molecular analysis of these markers, providing predictive indicators for breeding programs. The justification for selecting specific rust resistance genes stems from their significance in combating the most damaging strains of rust, such as stem rust (*Puccinia graminis*), leaf rust (*Puccinia triticina*), and yellow rust (*Puccinia striiformis*) Li, Y. (2020). These pathogens are responsible for substantial yield losses globally, making research into resistance mechanisms vital (Begum et al., 2014). By focusing on certain well documented resistance genes like *Lr34, Sr31*, and others known for their effectiveness against various biotypes of rust, this study aims to leverage existing genomic knowledge to accelerate the breeding of resistant wheat varieties (Malik and Bhat, 2022). Moreover, the choice of SSR and STS markers is strategically significant. SSRs, due to their abundance and high degree of polymorphism, facilitate the identification of genetic diversity and the mapping of traits. STS markers, on the other hand, provide robustness in identifying specific alleles tied to known resistance genes (Kumar et al., 2021). The integration of these molecular markers is essential for precise phenotypic selection and can expedite the breeding cycle necessary for developing rust-resistant spring wheat lines (Juliana et al., 2018).

Molecular markers detection is suitable for screening resistant sources and identifying important genes in wheat varieties. So far, a total of 79 *Lr* genes have been reported and mapped on the wheat chromosomes (Qureshi et al., 2018). However, most of the *Lr* genes have lost their resistance, and those that still show good resistance include *Lr9, Lr19, Lr24, Lr38, Lr47, Lr51,* and *Lr53* (Gao et al., 2019). There are about 12 adult-plant resistance (APR) genes to leaf rust, namely *Lr12, Lr13, Lr22a, Lr22b, Lr34, Lr35, Lr37, Lr46, Lr48, Lr49, Lr67* and *Lr68*. Among these APR genes, only four (*Lr34, Lr46, Lr68* and *Lr67*) are known to be genes for resistance to leaf rust (Patil et al., 2022). In spring wheat grown in USA, *Lr34* has been widely used where pathotypes of leaf rust with virulence to *Lr34* had not been observed (Mago et al., 2002; Frere et al., 2022) *Lr34* is closely Frere linked to powdery mildew resistance, barley yellow dwarf virus and leaf tip necrosis (Singh, 1992). The genetic linkage of *Lr34* is linked to genotype Ardito and Mentana released in Italy during early nineteen century (Nei et al., 1978; Roussel et al., 2022). The *Lr34* was replicated and showed that *Ltn1/Yr18/Lr34* has similar genetic characteristics and are linked to each other (Ali et al., 2018). The leaf and yellow rust resistance genes, *Lr46/Yr29*are also firmly pleiotropic (Begum et al., 2022). (Suenaga et

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

al., 2003) identified that molecular marker Xwmc44 is closely linked to *Lr46/Yr29* which is located on chromosomal arm1BL. Its importance is similar to*Yr18/Lr34* gene which provides partial/non-specific resistance to plants rather than complete immunity. In the absence of *Lr34,* genotypes having *Lr46* demonstrate long lasting resistance over control. Without any necrotic or chlorotic effects, the plants with *Lr34*/*Yr18* also reduce the spore production of the pathogen. Molecular mapping of *Lr46*/*Yr29* were identified on chromosome 1B provides the durable resistance against both leaf and yellow rust (Rasheed et al., 2016).

Bux et al. (2011) monitored the virulence analysis of *Yr* genes in CIMMYT tester lines under field conditions at various regions of Pakistan including Faisalabad, Sakrand, Quaid-i-Azam University and Pirsabak (KP) and indicated that *Yrsp, Yrcv, Yr26, Yr15, Yr10, Yr5* and *Yr3* were most effective while *Yr27, Yr17, Yr9, Yr8, Yr7, Yr6, Yr2, YrA* and association of Super Kauz (*Yr27, Yr18, Yr9*) and Opata (*Yr18/Yr27*) were susceptible. The gene *Yr18* demonstrated low to moderate level of rust resistance. Resistance genes *Yr25, Yr22, Yr21, Yr20, Yr17, Yr9, Yr8, Yr7, Yr6, Yr4, Yr3, Yr2,* and *Y1 are* ineffective against currently prevalent pathotypes (Khan et al., 2004; Li et al., 2023). In several regions of the world including Australia, India, China and Pakistan, virulence to these genes is recognized as race specific, but some genes including *Yr15* and *Yr5* were resistant to all rust races in the USA until now (Khan et al., 2018; Chen, 2007). Different experiments have been conducted to identify stripe rust resistance genes in wheat cultivars using isolates of *P. striiformis*. One such experiment was conducted in China where 52 wheat cultivars were studied using 13 isolates of *P. striiformis* and 19 known Yr genes (Jin et al., 2009). They detected twelve genes, *Yr1, Yr2, Yr6, Yr7, Yr8, Yr9, Yr11, Yr12, Yr17, Yr18, Yr24* and Avocet in 28 wheat cultivars, either alone or in combination. Seventeen cultivars were found to have *Yr11* gene while 15 contained *Yr2, Yr6* and *Yr17*. The timely detection of new variants and the identification of resistant material through strong and vibrant program of monitoring wheat rust virulence have curtailed any major loss to wheat during the last 35 years the effort made by (Singh et al., 2000) research involves only the use of available resistance and its intelligent deployment based on pathotype distribution (Singh et al., 2004). This has diversified the genetic base of rust resistance in wheat through pre-breeding activities which was planned to transfer the new/unutilized rust resistance genes *Yr5, Yr10, Yr15, Yr(CD), Yr(Mega), Yr(Hobbit), Yr(SP), Yr(China-84)* into the future varieties, to reduce the risks of rust epidemics (Singh et al., 2000). Virulence to genes *Yr5* and *Yr15* occurred as a rare phenomenon in wheat growing regions of the globe (Chen, 2005). Based on recent survey in China, genes *Yr41, Yr39, Yr36, Yr24/Yr26, Yr18, Yr15, Yr10* and *Yr5* as well as the advanced lines having *Yr16, Yr14, Yr13* and *Yr12* are still effective and could be used in breeding techniques (William et al., 2003). A set of molecular markers was used for genetic efficacy in breeding programs, two markers pleiotropic to *Sr2* a stem rust resistance gene, several linked with chromosomal loci having *Sr38/Lr37/Yr17* resistance gene, one for *Lr28*, two specified DNA markers for the associated genes *Lr35/Sr39*. Gene *Sr2,* provides durable resistance to stem rust black rust and generally under field conditions (Sharp et al., 2001). The stem and yellow rust resistance genes *Sr2/Yr30* are pleiotropic to each other (Singh et al., 2000). Spielmeyer et al. (2003) has reported that a molecular marker Xgwm-533 (a microsatellite) is strongly linked to particular gene Sr2 that is being utilized in selection of genotypes in breeding technologies. Durable resistance (non-specific-resistance) caused by *Sr2* can be used to combat different stem rust pathogen races (Roussel et al., 2020).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

A simple and effective yet difficult and time-consuming way to control rust fungus is to pyramid genes by incorporating both some major and minor genes into a single cultivar or accession. For gene postulation, traditional techniques are used which are stage and environment-dependent. Many *Lr, Yr* and *Sr* genes in wheat are mapped and identified using differrent DNA markers and marker systems intently linked with *Lr, Yr* and *Sr* genes (Kaur et al., 2021). The DNA-based and PCR-based techniques are handy and successively used to identify the resistance genes of the stripe, stem and leaf rust. Currently, there is deficient information on DNA markers regarding leaf, stem and stripe rust resistance genes in our wheat genotypes (Wani et al 2020) The current study may significantly assist the gene pyramiding of numerous rust resistance genes, which is the ultimate goal of breeders in the future selection and screening of wheat genotypes to improve or incorporate resistance genes against the rust fungus. They can also help to speed up the process in terms of marker-assisted selection (MAS) for rust-resistant breeding.

In our study, we utilized specific rust races, including *Puccinia triticina* (leaf rust) and *Puccinia graminis* (stem rust), for both phenotypic and molecular screening. These races were chosen due to their known prevalence and virulence in Pakistan, where stem rust races such as Ug99 have raised significant concerns for wheat production. However, we did not assess race-specific responses, which is a limitation of the study. Future research should focus on evaluating the durability of the identified resistance genes against evolving rust races through comprehensive race-specific screening. We selected SSR (Simple Sequence Repeat) and STS (Sequence Tagged Site) markers for rust resistance screening due to their demonstrated reliability, high polymorphism, and ability to provide robust genetic linkage information. SSR markers are co-dominant and highly reproducible, making them suitable for distinguishing between homozygous and heterozygous states (Murray and Thompson, 1980). STS markers, being sequence-specific, enhance the precision of genetic mapping and marker-assisted selection (Hulbert et al., 2001). The combination of these markers allows for a comprehensive evaluation of rust resistance genes. In articulating a clear research question and hypotheses in the introduction, along with a strong rationale for selecting specific rust resistance genes, sets the stage for a focused and impactful investigation (Begum et al., 2022). It allows researchers to target critical genetic traits that can significantly contribute to the development of resilient wheat varieties, thereby addressing an urgent need in global agriculture. The outcomes of such research not only advance scientific understanding but also pave the way for practical applications that enhance food production systems worldwide.

The current study aims to assist in the gene pyramiding of multiple rust resistance genes in wheat, which is a key objective for breeders seeking to develop new rust-resistant varieties. Specifically, the study focuses on identifying wheat genotypes carrying effective Lr, Sr, and Yr resistance genes through marker-assisted selection (MAS).

Materials and methods

Wheat genotype

Seeds were sown in the last week of November during the growing season in the field area of Crop Disease Research Program (CDRP), National Agricultural Research Center (NARC) Islamabad, Pakistan. Seedlings of 40 accessions were collected from National Uniform Wheat Yield Trials (NUWYT) and National Wheat Disease Screening Nursery

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

(NWDSN), National Agricultural Research Center (NARC) Islamabad, Pakistan. Accessions and their details regarding parentage are shown in *Table 1*.

Table 1. *Pakistani advanced wheat lines for stripe, leaf and stem rust characterization*

S No.	Code	Insert name
1	HWT15101	99172
2	WHT15104	99346
3	WHT15109	112802
4	WHT15111	DN-102
5	WHT15115	CT-09137
6	WHT15117	SRN-09111
7	WHT15119	NRL-1123
8	WHT15122	NRL-1130
9	WHT15126	V-12001
10	WHT15130	V-10110
11	WHT15151	V-11160
12	WHT15133	12266
13	WHT15135	11098
14	WHT15137	11138
15	WHT15139	12304
16	WHT15140	11C022
17	WHT15147	11C023
18	WHT15144	AUR-08010
19	WHT15149	TW-11510
20	WHT15154	TW-96098
21	WHT15159	ESW-9525
22	WHT15162	DANI-1313
23	WHT15164	NIA-CIM-04-10
24	WHT15167	PR-106
25	WHT15169	PR-110
26	WHT15171	PR-111
27	WHT15174	PR-112
28	WHT15176	PR-113
29	WHT15178	NR-423
30	WHT15180	NR-429
31	WHT15181	NR-436
32	WHT15184	NR-449
33	WHT15188	9459-1
34	WHT15190	KT-338
35	WHT15191	09-FJ-34
36	WHT15193	SKD-II
37	WHT15195	V-11005-2013
38	CHECK	LOCAL CHECK
39	WHT15197	FSD-08
40	WHT15199	PAK-2013

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

Experimental management and inoculation

Each advanced Breeding line from Pakistan was sown in a single row by maintaining 30 cm row to row distance. A single line of susceptible cultivar, Morocco was sown repeatedly after every 10 lines of experimental material. Fertilizer NPK was applied as a basal dose at the rate of 120, 75 and 60 kg/ha. Three irrigations were applied at different growth stages i.e. first at tillering stage, second at booting stage and third at grain formation stage. Weeds were controlled by using sprays.

Rust tests in the field

Rust severity was recorded as percent of rust infection on the plants according to the modified Cobb's scale ranging from 0 to 100 (Peterson et al., 1948). As severity was determined by visual observation, readings cannot be absolutely correct. In a segregating population consisting of 40 different wheat lines, evaluations for stripe, stem, and leaf rust were conducted at weekly intervals. The assessment of disease began at the flag leaf stage of wheat and concluded before the leaves displayed signs of yellowing or browning.

The Area under the Disease Progress Curve (AUDPC) was employed, utilizing a computer-based program developed at CIMMYT. To provide a relative measure, the Relative Area Under the Disease Progress Curve (RAUDPC) was calculated by normalizing the AUDPC of Morocco to 100%. Data were recorded after visually observing rust postules on check variety, Morocco. The rusts data were noted by type of infection (IT) using a scale of 0-9 (Afridi et al., 2019). Lines were recorded 0, where there was no evident infection. Material will be classified Resistant (R) when no necrotic region was observed. The lines were rated Moderately Resistant (MR) when little uredia were surrounded by necrotic area with a minute chlorosis (*Table 2*). Moderately susceptible when medium level of uredia was observed by chlorosis and susceptible when considerable uredinium was detected with maximum chlorosis. The severity of the disease was determined by the percentage of diseased leaf area on the plants.

Table 2. *Types of rust infection*

Symbol	Types of rust infection
O	No visible infection
R	Resistant, necrotic areas may be with or without minute uredia
MR	Moderately resistant
MS	Moderately susceptible
S	Susceptible large uredia, little or no chlorosis

Molecular marker detection

Genomic DNA extraction

Genomic DNA was extracted from fresh leaf tissues of wheat lines according to the protocol modified by Begum et al. (2022) with minor modifications. Approximately 200 mg of fresh leaf tissue from each line was homogenized using a mortar and pestle in the presence of 2-3 mL of 2% CTAB solution (*Table 3*). An aliquot of 750 µL of the resulting emulsion was transferred to a 1.5 mL Eppendorf tube and incubated in a water bath at 65°C for 30 min. Following incubation, 750 µL of Chloroform: Isoamyl Alcohol (24:1) was added to the tube, and it was centrifuged at 12,000 rpm for 10 min at 4°C. The supernatant was carefully transferred to a new tube, and approximately 0.8 volumes of

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

isopropanol were added to facilitate DNA precipitation. The sample was then incubated at 4°C for 10 mins, followed by centrifugation at 12,000 rpm for 10 min at 4°C, after which the supernatant was discarded. The DNA pellet was washed by adding 200 µL of 70% ethanol and subjected to centrifugation at 12,000 rpm at room temperature for 10 min. Following the ethanol wash, the DNA pellet was air-dried for 30-40 min. Finally, the DNA pellet was re-suspended in 100 µL of TE buffer, and 1 µL of RNase A (10 mg/mL) was added to remove RNA content through incubation at 37°C for 30 min.

Eighteen molecular markers (SSR and STS) linked to known 6*Lr* genes (*Lr19, Lr24*, Lr26, *Lr34*, *Lr46*, *Lr60*), 6 *Yr* genes (*Yr5, Yr9, Yr10, Yr17, Yr26, Yr29*) and 5 Sr genes (*Sr4, Sr6, Sr9, Sr25, Sr31*) were used to assess all germplasm (*Table 3*). Morocco was used as a susceptible control, whereas the differential lines with their respective *Lr, Yr* and *Sr* genes were used as a resistance control during molecular marker detection. The amplified products of Simple Sequence Repeats (SSR) and Sequence Tagged Site (STS) markers were resolved on 2% agarose gel and were stained with EB (Ethidium bromide). The fragments were imagined below ultra violet light in the gel documentation system at NIGAB to identify *Sr, Lr* and *Yr* genes.

Table 3. *Composition of 2% CTAB*

Chemical	Mol. Wt.	Final conc.	100 mL
Tris Base[a] (pH 8.0)	121.14	100 mM	10 mL
0.5 MNa2 EDTA[b] (pH 8.0)	372.24	20 mM	4 mL
CTAB[d]	364.4	2%	2 g
NaCl	58.44	1.4 M	8.18 g
PVP[e]	40,000	1%	1 g
ddH2O	Final volume 100 mL		

a: Tris-base (hydroxymethylaminomethane), b: Disodium Ethylenediaminetetraacetic acid, c: Sodium Dodecyl Sulfate, d: Cetyltrimethylammonium Bromide, e: Polyvinyl-Pyrrolidone

Polymerase chain reaction

The polymerase chain reaction (PCR) was set up in a total volume of 20 µL. The reaction mixture included 1× Taq buffer containing ammonium sulfate ($(NH^4)_2SO_4$), 3 mM magnesium chloride ($MgCl_2$), a mix of 0.2 mM Deoxy-nucleotide tri-phosphates (dNTPs), 10 pmol of both forward and reverse primers, one unit of Taq DNA polymerase (Fermentas, Life Sciences), and 25 ng of DNA template. Amplification was conducted using an Applied Biosystems Thermal Cycler (Veriti 96-well) with an initial denaturation step at 94°C for 5 min, followed by 35 cycles each consisting of three phases: denaturation at 94°C for 40 s, annealing of primers for 30 s, and extension at 72°C for 40 s. A final extension was performed at 72°C for 10 min. The optimized PCR profiles for all primer pairs utilized are summarized in *Table 4*.

Gel electrophoresis

The PCR-amplified products were subjected to electrophoresis on a 1.5%-3% agarose gel stained with Ethidium Bromide. Visualization of the bands was achieved using a UV trans illuminator, and images were captured as outlined in the DNA quantification section. The presence or absence of bands corresponding to the *Yr* genes was evaluated by comparing the results against a DNA ladder with predetermined band sizes.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

Table 4. *DNA markers for detection of stripe, leaf and stem rust resistance genes*

S.No.	Primer name	Linkage	References	PCR Profile
1	Xgwm3-F Xgwm3-R	SSR for Lr24/Sr4 -		94°C: 4 min 1 cycle 94°C: 40 s, 60°C: 40 s, 72°C: 1 min 35 cycles 72°C: 7 min 1 cycle
2	Xgwm120-F Xgwm120-R	SSR for Sr2 -	Smith et al. (2007)	94°C: 4 min 1 cycle 94°C: 40 s, 58°C: 40 s, 72°C: 1 min 35 cycles 72°C: 7 min 1 cycle 94°C: 4 min 1 cycle 94°C: 40 s, 60°C: 40 s, 72°C: 60 s 35 cycles 72°C: 7 min 1 cycle
3	S19M93-100-F S19M93-100-R	STS for Yr5 -	Smith et al. (2007)	94°C: 4 min 1 cycle 94°C: 40 s, 60°C: 40 s, 72°C: 1 min 35 cycles 72°C: 7 min 1 cycle 94°C: 4 min 1 cycle 94°C: 40 s, 55°C: 40 s, 72°C: 1 min 35 cycles 72°C: 7 min 1 cycle
4	S23M41-275-F S23M41-275-R	STS for Yr5 -	Smith et al. (2007)	94°C: 4 min 1 cycle 94°C: 40 s, 60°C: 40 s, 72°C: 1 min 35 cycles 72°C: 7 min 1 cycle 94°C: 4 min 1 cycle 94°C: 40 s, 58°C: 40 s, 72°C: 1 min 35 cycles 72°C: 7 min 1 cycle
5	Iag 95-F Iag 95-R	Sr31/Lr26/Yr9 -		94°C: 4 min 1 cycle 94°C: 40 s, 58°C: 40 s, 72°C: 1 min 35 cycles 72°C: 7 min 1 cycle
6	XPsp 3000-F XPsp 3000-R	Yr10 -		94°C: 4 min 1 cycle 94°C: 40 s, 58°C: 40 s, 72°C: 1 min 35 cycles 72°C: 7 min 1 cycle
7	VENTRIUP-F LN2	Yr17 -	Helguera et al. (2003)	94°C: 4 min 1 cycle 94°C: 40 s, 58°C: 40 s, 72°C: 1 min 35 cycles 72°C: 7 min 1 cycle
8	Cslv34-F Cslv34-R	*Lr34*/Yr18 -	Lagudah et al. (2006)	94°C: 4 min 1 cycle 94°C: 40 s, 58°C: 40 s, 72°C: 1 min 35 cycles 72°C: 7 min 1 cycle
9	Xgwm3832-F Xgwm3832-RRRRR RRRRR	*Lr34* *Lr34*		94°C: 4 min 1 cycle 94°C: 40 s, 60°C: 40 s, 72°C: 1 min 35 cycles 72°C: 7 min 1 cycle
10	Xwmc 367-F Xwmc 367-R	*Lr46*/Yr29 -		94°C: 4 min 1 cycle 94°C: 40 s, 58°C: 40 s, 72°C: 1 min 35 cycles 72°C: 7 min 1 cycle
11	Xwmc 44-1B-F Xwmc 44-1B-R	*Lr46*/Yr29 -	Somers et al. (2004)	94°C: 4 min 1 cycle 94°C: 40 s, 58°C: 40 s, 72°C: 1 min 35 cycles 72°C: 7 min 1 cycle
12	Xgwm159 Xgwm159	Lr60 -ee		94°C: 4 min 1 cycle 94°C: 40 s, 60°C: 40 s, 72°C: 1 min 35 cycles 72°C: 7 min 1 cycle
13	Xgwm1117-F Xgwm1117-R	Sr9 -		94°C: 4 min 1 cycle 94°C: 40 s, 58°C: 40 s, 72°C: 1 min 35 cycles 72°C: 7 min 1 cycle
14	Xgwm174-F Xgwm174-R	*Lr34* -		94°C: 4 min 1 cycle 94°C: 40 s, 58°C: 40 s, 72°C: 1 min 35 cycles 72°C: 7 min 1 cycle
15	Xgwm539-F Xgwm539-R	Sr25 -		94°C: 4 min 1 cycle 94°C: 40 s, 58°C: 40 s, 72°C: 1 min 35 cycles 72°C: 7 min 1 cycle
16	Xgwm314 Xgwm314	Yr26 -		94°C: 4 min 1 cycle 94°C: 40 s, 58°C: 40 s, 72°C: 1 min 35 cycles 72°C: 7 min 1 cycle
17	Xgwm169 Xgwm169	*Lr34* -	Tsilo et al. (2009)	94°C: 4 min 1 cycle 94°C: 40 s, 63°C: 40 s, 72°C: 1 min 35 cycles 72°C: 7 min 1 cycle
18	Xcfd43-2d-F Xcfd43-2d-R	Sr6 -		94°C: 4 min 1 cycle 94°C: 40 s, 60°C: 40 s, 72°C: 1 min 35 cycles 72°C: 7 min 1 cycle

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

Statistical analysis

Analyses of variance (ANOVA) was performed by using Minitab 16 statistical software program and used poppr program (version 2.5.0).

Results

To study variations at *Yr, Lr* and *Sr* loci in 40 Pakistani wheat lines, 18 STS and SSR markers were utilized. A total of 11 DNA markers *Xwmc367 (Yr29), Xwmc44 (Lr46/Yr29), Xgwm-3(Lr24/Sr4), Xgwm314, Xgwm539, (Yr26) Xgwm1117 (Sr9)* and *Xgwm3832, Xgwm174, Xgwm169, Xgwm111, (Lr34)*, *Xgwm159 (Lr60) wmc444 (Lr19/Sr25)* produced reproducible PCR products which previously found an association with *Yr, Sr, Lr* genes (*Figs. 1–6*). Whereas *S19M93-100, S23M41-275, Xgwm120 (Yr5), Iag95 (Yr9), Xpsp3000 (Yr10), VENTRIUP-F/LN2, (Yr17)*, *Xwmc453 (Sr6)* did not amplify the expected band.

Marker for the Yr29 gene

The *Xwmc-367* SSR marker (*Fig. 1*) was used to identify the presence/absence of the *Yr29* gene. Out of 40 lines, 34 wheat lines showed a 200-bp band, whereas 6 wheat lines did not show any fragments, specifying the absence of the *Yr29* gene. Furthermore, a distinct microsatellite marker, *wcm-344* (*Fig. 1*), was utilized to distinguish the presence of the*Yr29* gene and this marker also linked with *Lr19* and *Sr25* genes. The marker produced a 200-bp fragment in 22 wheat lines, indicating the likely presence of the *Yr29* gene. Eighteen wheat lines did not amplify any band, signifying the probable absence of the *Yr29* gene.

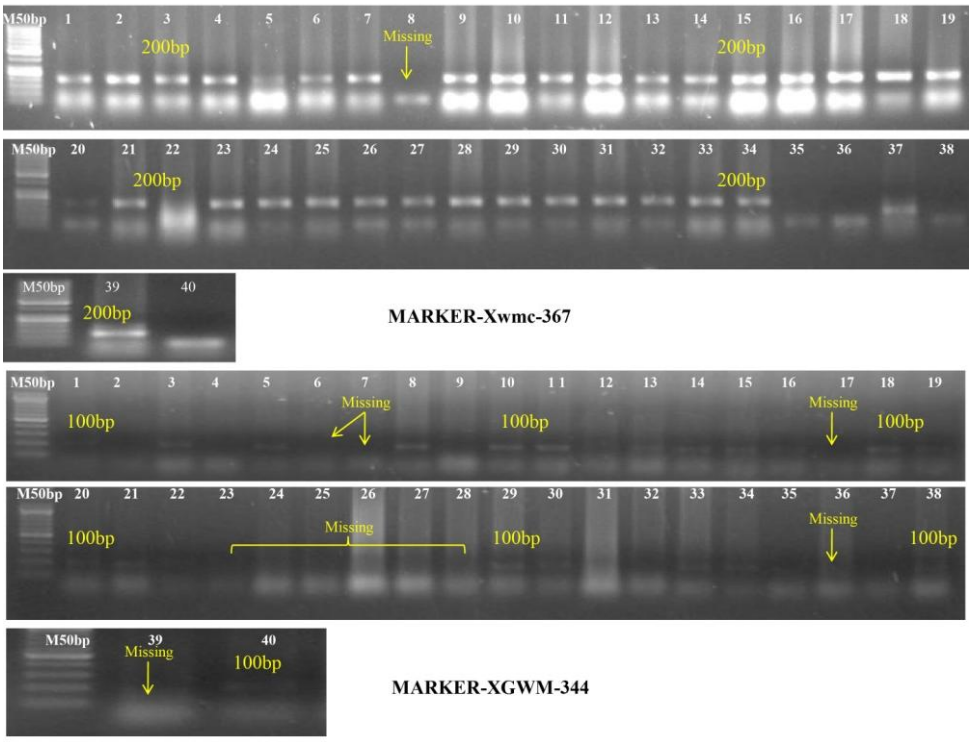

Figure 1. *Microsatellite marker Xwmc367 amplified 200 bp fragment and XGWM-344 amplified 100 bands with 50 bp ladder*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

Markers for the Yr26 and Lr34 gene

The SSR marker, *Xgwm314* (*Fig. 2*), was employed to uncover the presence/absence of the *Yr26* gene, which produced a 150 and 130-bp band in 34, showing the *Yr26* gene. The remaining 5 lines did not amplify the fragment. Microsatellite markers, *Xgwm3832* (*Fig. 2*), were utilized to perceive the *Lr34* gene, which produced a 185-bp band in 36 lines, displaying the presence of the *Lr34* gene. The remaining 4 lines did not amplify the 185-bp band.

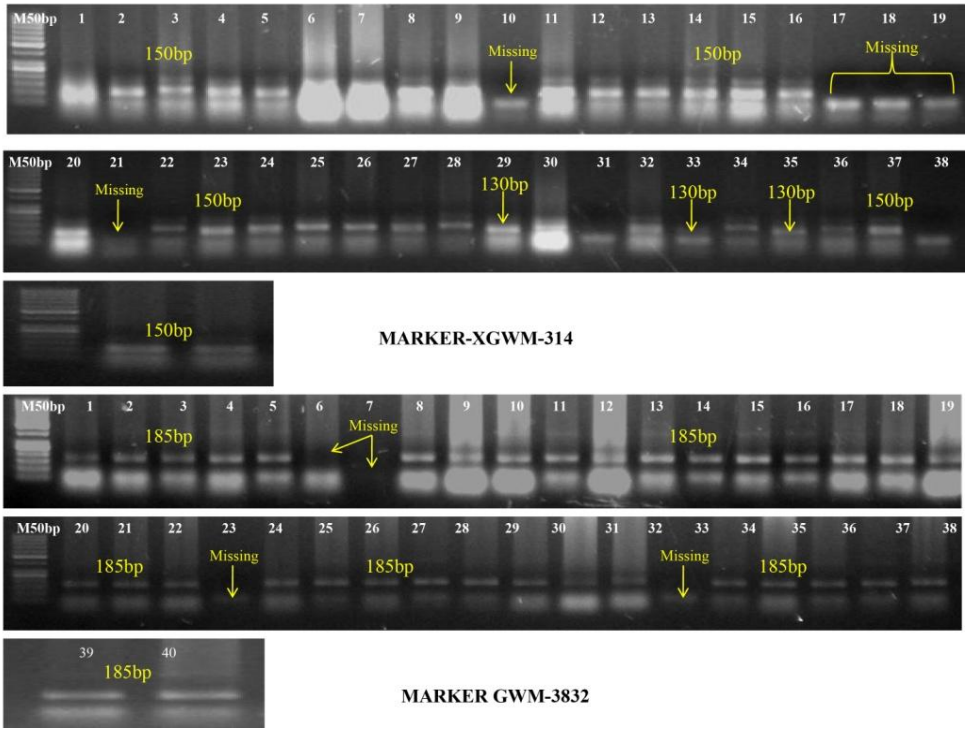

Figure 2. *Microsatellite markers XGWM-314 amplified 150 and 130 bp fragment and GWM-3832 amplified 185 bp bands with 50 bp ladder*

Markers for Lr24/Sr4, Lr34, Lr60 and Sr25 genes

Xgwm174 (*Fig. 3*) was also used to amplify the *Lr34* gene, which exhibited 200 bp bands in 6 wheat lines indicating the presence of the *Lr34* gene, while 34 wheat lines did not produce any expected bands indicating the absence of the *Lr34* gene. Marker *Xgwm169* (*Fig. 3*) linked to the *Lr34* gene amplified 195 bp bands in 19 lines, whereas 21 wheat lines did not intensify any fragments. Marker *Xgwm111* (*Fig. 4*) was also used to amplify the 200 bp fragment in 18 wheat lines, which indicated the presence of the *Lr34* gene and the remaining 22 wheat lines did not produce any bands. Marker *Xgwm159* (*Fig. 4*) was used to distinguish the *Lr60* gene, which produced a 200 bp band in 38 lines, whereas 2 lines did not show any band which depicted the absence of the *Lr60* gene. *Xgwm-3* (*Fig. 5*) was used to detect the *Lr24/Sr4* gene which amplified a 200 bp and 550 bp fragment in 17 wheat lines whereas 23 lines did not amplify any fragment and exhibited absence of the *Lr24/Sr4* gene. Marker *Xgwm539* (*Fig. 5*) was used to identify *Sr25* gene. The marker showed 120 bp in 15 wheat lines and residual 25 lines did not generate any fragment that indicated the absence of the *Sr25* gene.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

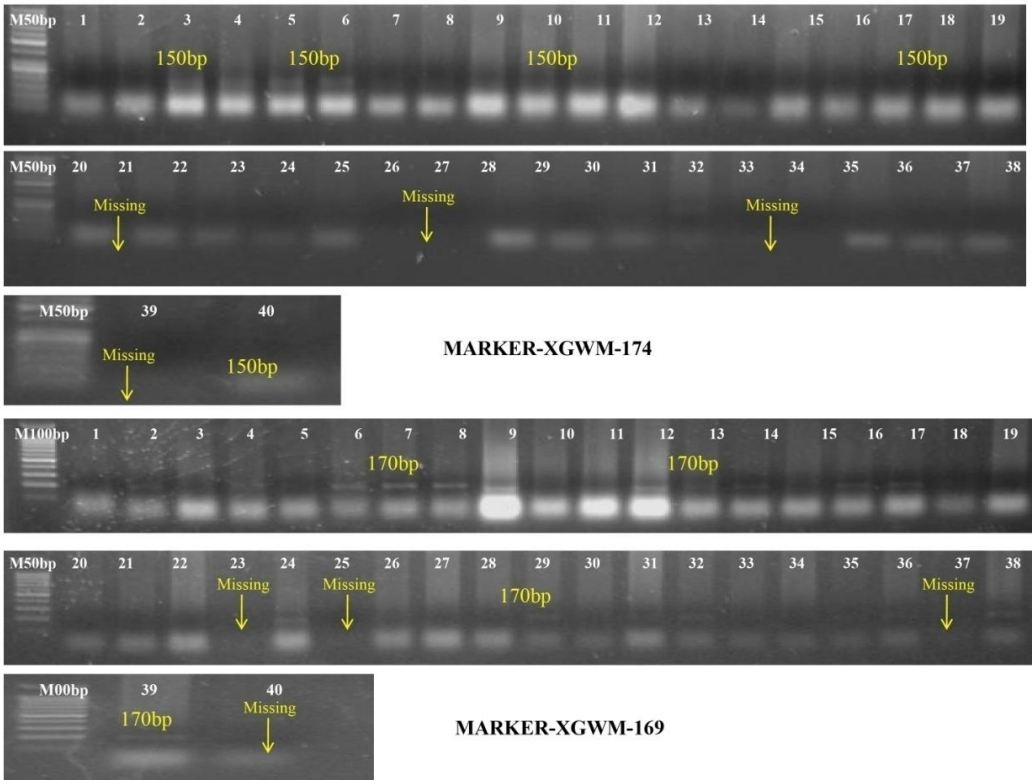

Figure 3. *Microsatellite markers XGWM-174 amplified 150 bp fragment and XGWM-169 amplified the 170 bp fragment with 100 bp ladder*

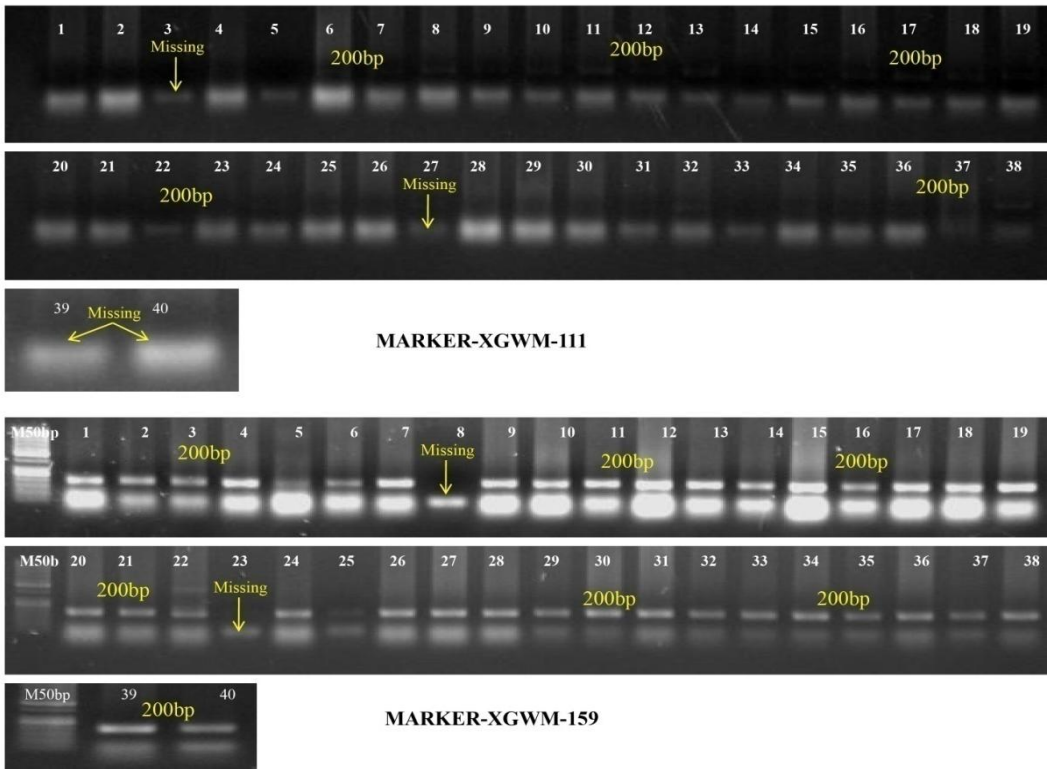

Figure 4. *Microsatellite markers XGWM-111 and XGWM-159 amplified 200 bp fragments in both markers with 50 bp ladder*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

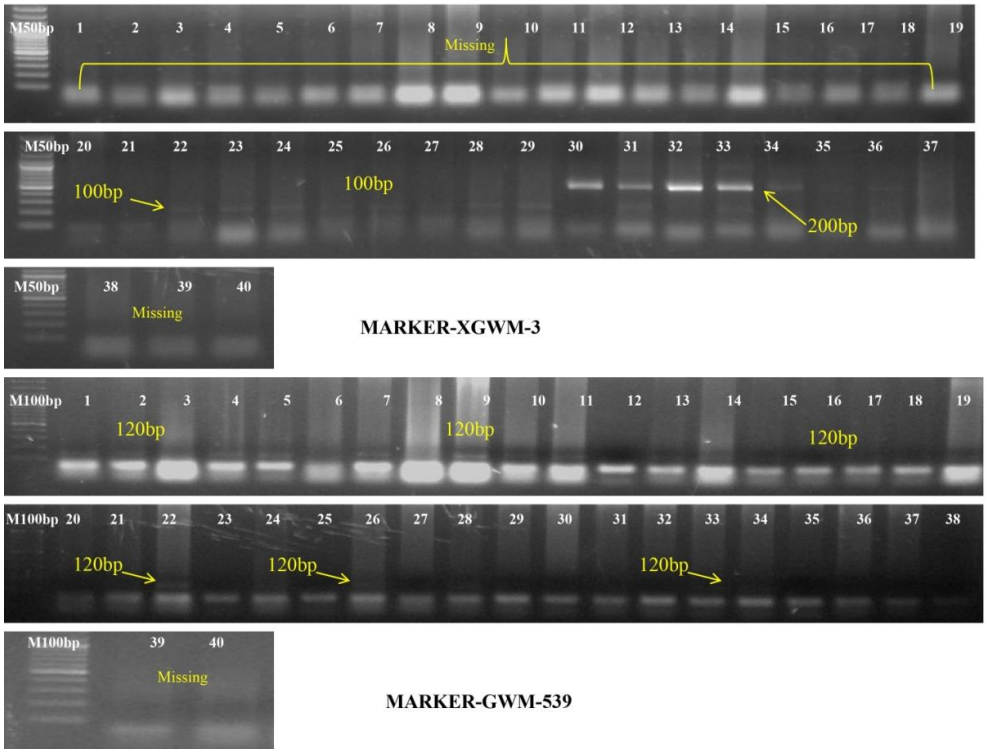

Figure 5. *Microsatellite markers XGWM-3 amplified 100 and 200 bp fragments with 50 bp Ladder and XGWM-539 amplified 120 bp with 100 bp ladder*

Marker for Sr9 genes

Marker *Xgwm1117* (*Fig. 6*) was also used to detect the *Sr9* gene. The marker Amplified170 and 150 bp fragments on 36 lines, and the remaining 4 lines did not amplify any band.

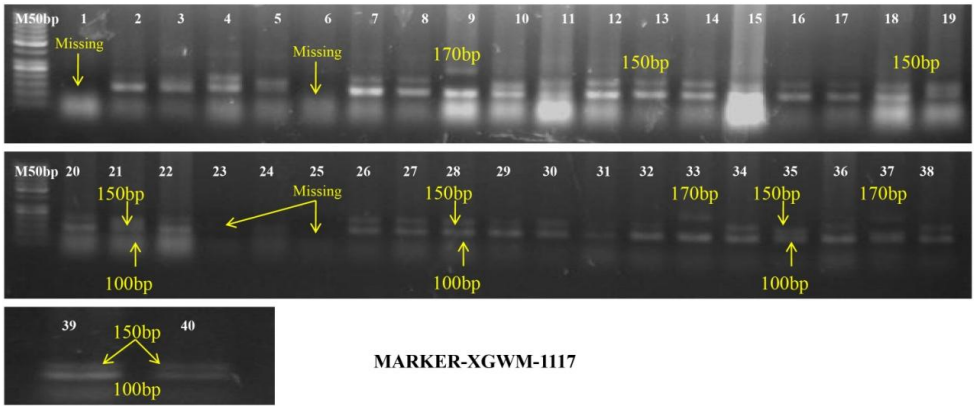

Figure 6. *Microsatellite markers XGWM-1117 amplified 150 bp and 170 bp fragments with 50 bp ladder*

Rust disease screening in the field

Out of 40 wheat breeding lines of a segregating population, 33% lines showed their resistance against yellow rust whereas 60% lines were recorded to behave moderately to

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

yellow rust pressure whereas, 7% lines of wheat showed their susceptibility to yellow rust disease. This segregating wheat population showed high level of resistant varieties in consecutive two years that comprised of 80% resistant varieties to yellow rust disease while 10% were moderately resistant lines and only 5% were susceptible to yellow rust disease and 55% wheat lines showed resistance to leaf rust and the others showed susceptibility to leaf rust resistance. Whereas 29% of stem rust lines showed resistance and other lines recorded moderate resistance to stem rust i.e., 11% and 60% lines showed susceptible to stem rust disease (*Table 5*).

Table 5. *Rust disease scoring of 40 wheat breeding lines/Accessions from Pakistan*

S/No	Accessions	Yellow rust	Leaf rust	Stem rust
1	11123	50 MR	30MS	20MS
2	11126	40MS	70S	0
3	11144	0	20S	20MS
4	11145	0	20S	20MS
5	11152	80S	30MSS	0
6	11154	50MR	30MS	30MS
7	11155	20MR	70S	30MS
8	11160	70S	70S	0
9	11161	80S	70S	0
10	11162	80S	10MS	20MS
11	11163	80S	70S	0
12	11164	80S	70S	0
13	11166	70S	20MS	5MS
14	11168	0	40MS	0
15	11170	0	70MS	5MS
16	11171	0	30S	5MS
17	11173	80S	10MS	20MS
18	11174	80S	30MS	0
19	11177	80S	70S	0
20	11178	80S	0	10MS
21	11179	0	10MS	0
22	11181	70S	30MS	10MSS
23	11183	70MRMS	40S	0
24	11184	80S	70S	0
25	11185	80S	30MS	5MS
26	11186	70MS	40MS	0
27	11187	70S	30MS	0
28	11188	0	20MS	TMS
29	11189	80S	TMS	0
30	11190	80S	20MS	0
31	11192	0	5MS	10MS
32	11193	0	0	0
33	11194	80S	30MS	0
34	11195	60S	20MS	0
35	11197	0	20MS	0
36	11198	90S	20MS	0
37	11200	80S	40MS	0
38	11202	80S	TMS	0
39	11205	20RMR	30MS	0
40	11207	80S	40S	10MS

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

Genetic diversity in NUWYT advanced breeding population

Statistical analyses

The poppr program (version 2.5.0) was used to estimate the likelihood of each genotype belonging to the NUWYT population. A model-based classification approach (Bayes classification) was performed. The NUWYT population assumption was defined and 100 iterations were performed using the Euclidean distance calculation model. A distance-based MSN analysis was performed using the method outlined in the method described in Kamvar et al. (2014) (*Fig. 1A*). For this purpose, the number of seeds with the distance gradients and the limits was kept from 15 and 0.2 to 500, respectively. The reduced marker group consisted of 11 markers and was used for structural analysis of the NUWYT population of 40 samples. The simulation was performed for the poppr package and the genotype of the multi-locus was 31, while the standard error of scarcity analysis was zero for the total wheat population. The Shannon-Weiner H Diversity Index of 3.33 has now been observed in the population. Overall, 24.2 Stoddard and Taylor index levels were observed in wheat individuals. Similarly, the Simpson index was 0.959, Evenness 0.863, Nei gene diversity (expected heterozygosity) was 0.292 calculated by the following model:

$$Hexp = \left(\frac{n}{n-1}\right) \times \left(1 - \sum P^2\right)$$

where P represents the allelic frequencies of a given locus and *n* the number of alleles observed (Nei, 1978). The value of the association index for the population factor was 0.143, and the standardized association index for each population factor was 0.0137, as shown in *Table 6*. The results of these analyzes provide information on the presence of the genetic diversity of the population. Elite germplasm has evolved over time for better survival against rust, tolerance to drought and salinity.

An entire vector corresponding to the number of genotypes in the NUWYT, MLG population. An entire vector indicating the number of multilocus genotypes was found in the specified population, eMLG. The expected MLG number in the smallest common sample size (defined by the minsamp parameter), SE Typical error for the rarefaction analysis, H Shannon-Weiner diversity index, Stoddard and Taylor G index, Simpson lambda index, equity E, hexagon, Nei gene diversity (expected heterozygosity), numerical vector that indicates the association value for the population factor, rbarD A numerical vector that provides the value of the standardized association index for each population factor.

Table 6. *Tentative identification of statistical summary parameters for genetic diversity in Pakistani advanced breeding lines*

Population	N	MLG	eMLG	SE	H	G	Lambda	E.5	Hexp	Ia	rbarD
NUWYT	40	31	31	0	3.33	24.2	0.959	0.863	0.292	0.143	0.0137

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

Nei's coefficient

As Nei's distance is the negative log of 0:1, this means that it is possible to obtain distances of infinity that may be corrected to be:

$$10 \times max(M)$$

where M is the distance matrix computed on NUWYT wheat population consisting 40 individuals without infinite values.

	NUWYT-1	NUWYT-2	NUWYT-3	NUWYT-4	NUWYT-5	NUWYT-6	NUWYT-7	NUWYT-8	NUWYT-9	NUWYT-10	NUWYT-11	NUWYT-12	NUWYT-13	NUWYT-14	NUWYT-15	NUWYT-16	NUWYT-17	NUWYT-18	NUWYT-19	NUWYT-20	NUWYT-21	NUWYT-22	NUWYT-23	NUWYT-24	NUWYT-25	NUWYT-26	NUWYT-27	NUWYT-28	NUWYT-29	NUWYT-30	NUWYT-31	NUWYT-32	NUWYT-33	NUWYT-34	NUWYT-35	NUWYT-36	NUWYT-37	NUWYT-38	NUWYT-39
NUWYT-2	0.221																																						
NUWYT-3	0.126	0.192																																					
NUWYT-4	0.403	0.288	0.192																																				
NUWYT-5	0.126	0.192	0.000	0.192																																			
NUWYT-6	0.560	0.403	0.462	0.403	0.462																																		
NUWYT-7	0.403	0.134	0.192	0.288	0.192	0.221																																	
NUWYT-8	0.403	0.288	0.347	0.693	0.347	0.626	0.470																																
NUWYT-9	0.280	0.059	0.251	0.347	0.251	0.280	0.192	0.192																															
NUWYT-10	0.126	0.192	0.118	0.347	0.118	0.462	0.347	0.192	0.118																														
NUWYT-11	0.126	0.192	0.118	0.347	0.118	0.462	0.347	0.192	0.118	0.000																													
NUWYT-12	0.280	0.192	0.251	0.347	0.251	0.280	0.347	0.192	0.118	0.118	0.118																												
NUWYT-13	0.178	0.112	0.170	0.399	0.170	0.332	0.245	0.112	0.053	0.053	0.053	0.053																											
NUWYT-14	0.178	0.112	0.170	0.399	0.170	0.332	0.245	0.112	0.053	0.053	0.053	0.053	0.000																										
NUWYT-15	0.067	0.134	0.059	0.288	0.059	0.626	0.288	0.288	0.192	0.059	0.059	0.192	0.112	0.112																									
NUWYT-16	0.178	0.112	0.170	0.399	0.170	0.332	0.245	0.112	0.053	0.053	0.053	0.053	0.000	0.000	0.112																								
NUWYT-17	0.280	0.059	0.251	0.347	0.251	0.280	0.192	0.192	0.000	0.118	0.118	0.118	0.053	0.053	0.192	0.053																							
NUWYT-18	0.178	0.245	0.170	0.245	0.170	0.515	0.399	0.245	0.170	0.053	0.053	0.170	0.105	0.105	0.112	0.105	0.170																						
NUWYT-19	0.221	0.288	0.192	0.134	0.192	0.626	0.470	0.470	0.347	0.192	0.192	0.192	0.245	0.245	0.134	0.245	0.347	0.112																					
NUWYT-20	0.154	0.221	0.126	0.221	0.126	0.560	0.403	0.403	0.280	0.126	0.126	0.126	0.178	0.178	0.067	0.178	0.280	0.178	0.067																				
NUWYT-21	0.077	0.326	0.203	0.549	0.203	0.770	0.549	0.549	0.385	0.203	0.203	0.385	0.255	0.255	0.144	0.255	0.385	0.255	0.326	0.259																			
NUWYT-22	0.221	0.134	0.192	0.134	0.192	0.626	0.288	0.470	0.192	0.192	0.192	0.347	0.245	0.245	0.134	0.245	0.192	0.112	0.134	0.221	0.326																		
NUWYT-23	0.391	0.458	0.517	0.458	0.517	0.679	0.458	0.746	0.517	0.517	0.517	0.805	0.570	0.570	0.458	0.570	0.517	0.347	0.458	0.679	0.602	0.235																	
NUWYT-24	0.280	0.059	0.251	0.192	0.251	0.462	0.192	0.347	0.118	0.251	0.251	0.251	0.170	0.170	0.192	0.170	0.118	0.170	0.192	0.280	0.385	0.059	0.294																
NUWYT-25	0.259	0.326	0.385	0.326	0.385	0.770	0.549	0.549	0.385	0.385	0.385	0.608	0.488	0.488	0.326	0.488	0.385	0.255	0.326	0.483	0.405	0.144	0.091	0.203															
NUWYT-26	0.221	0.134	0.192	0.134	0.192	0.626	0.288	0.470	0.192	0.192	0.192	0.347	0.245	0.245	0.134	0.245	0.192	0.112	0.134	0.221	0.326	0.000	0.235	0.059	0.144														
NUWYT-27	0.221	0.134	0.192	0.134	0.192	0.626	0.288	0.470	0.192	0.192	0.192	0.347	0.245	0.245	0.134	0.245	0.192	0.112	0.134	0.221	0.326	0.000	0.235	0.059	0.144	0.000													
NUWYT-28	0.221	0.134	0.192	0.134	0.192	0.626	0.288	0.470	0.192	0.192	0.192	0.347	0.245	0.245	0.134	0.245	0.192	0.112	0.134	0.221	0.326	0.000	0.235	0.059	0.144	0.000	0.000												
NUWYT-29	0.178	0.112	0.170	0.245	0.170	0.515	0.245	0.245	0.170	0.170	0.170	0.170	0.105	0.105	0.112	0.105	0.170	0.105	0.112	0.178	0.255	0.112	0.347	0.053	0.255	0.112	0.112	0.112											
NUWYT-30	0.126	0.192	0.118	0.192	0.118	0.685	0.347	0.347	0.251	0.118	0.118	0.251	0.170	0.170	0.059	0.170	0.251	0.053	0.059	0.126	0.203	0.059	0.294	0.118	0.203	0.059	0.059	0.059	0.053										
NUWYT-31	0.336	0.221	0.280	0.067	0.280	0.560	0.403	0.626	0.280	0.280	0.280	0.280	0.332	0.332	0.221	0.332	0.280	0.178	0.067	0.154	0.489	0.067	0.391	0.126	0.259	0.067	0.067	0.067	0.178	0.136									
NUWYT-32	0.515	0.245	0.304	0.112	0.304	0.178	0.245	0.399	0.170	0.304	0.304	0.170	0.223	0.223	0.399	0.223	0.170	0.223	0.245	0.332	0.661	0.245	0.570	0.170	0.438	0.245	0.245	0.245	0.223	0.304	0.178								
NUWYT-33	0.221	0.288	0.192	0.288	0.192	0.626	0.288	0.470	0.347	0.192	0.192	0.347	0.245	0.245	0.134	0.245	0.347	0.112	0.134	0.221	0.326	0.134	0.235	0.192	0.326	0.134	0.134	0.134	0.112	0.059	0.221	0.399							
NUWYT-34	0.221	0.288	0.192	0.134	0.192	0.626	0.470	0.470	0.347	0.192	0.192	0.192	0.245	0.245	0.134	0.245	0.347	0.112	0.000	0.067	0.326	0.134	0.458	0.192	0.326	0.134	0.134	0.134	0.112	0.059	0.067	0.245	0.134						
NUWYT-35	0.259	0.326	0.203	0.326	0.203	0.770	0.549	0.326	0.385	0.203	0.203	0.203	0.255	0.255	0.144	0.255	0.385	0.255	0.144	0.077	0.405	0.326	1.007	0.385	0.693	0.326	0.326	0.326	0.255	0.203	0.259	0.438	0.326	0.144					
NUWYT-36	0.403	0.134	0.347	0.288	0.347	0.626	0.288	0.288	0.192	0.347	0.347	0.347	0.245	0.245	0.288	0.245	0.192	0.245	0.288	0.403	0.549	0.134	0.458	0.059	0.326	0.134	0.134	0.134	0.112	0.192	0.221	0.245	0.288	0.288	0.326				
NUWYT-37	0.259	0.144	0.203	0.144	0.203	0.483	0.326	0.549	0.203	0.203	0.203	0.203	0.255	0.255	0.144	0.255	0.203	0.255	0.144	0.077	0.405	0.144	0.602	0.203	0.405	0.144	0.144	0.144	0.255	0.203	0.077	0.255	0.326	0.144	0.182	0.326			
NUWYT-38	0.221	0.134	0.192	0.470	0.192	0.626	0.288	0.134	0.192	0.192	0.192	0.112	0.112	0.134	0.112	0.192	0.245	0.288	0.221	0.326	0.288	0.746	0.192	0.549	0.288	0.288	0.288	0.112	0.192	0.403	0.399	0.288	0.288	0.144	0.134	0.326			
NUWYT-39	0.336	0.067	0.280	0.403	0.280	0.560	0.221	0.403	0.126	0.280	0.280	0.280	0.178	0.178	0.221	0.178	0.126	0.332	0.403	0.336	0.489	0.221	0.679	0.126	0.483	0.221	0.221	0.221	0.178	0.280	0.336	0.332	0.403	0.403	0.403	0.221	0.259	0.221	
NUWYT-40	0.259	0.326	0.203	0.549	0.203	1.176	0.549	0.326	0.385	0.203	0.203	0.385	0.255	0.255	0.144	0.255	0.385	0.255	0.326	0.259	0.405	0.326	1.007	0.385	0.693	0.326	0.326	0.326	0.255	0.203	0.483	0.661	0.326	0.326	0.182	0.326	0.405	0.144	0.259

In this section, the sizes of the systematic biases involved in the heterozygosity of the sample and the genetic distances of the samples were evaluated. The formulas are used here to obtain undistorted estimates of average heterozygosity and genetic elimination of the wheat population. The number of NUWYT wheat individuals that will be used to estimate the average heterozygosity was very low, since the number of loci examined 11 and the average heterozygosity are also low. The number of individuals that would be used to estimate the genetic distance was forty, since the genetic distance was large.

Minimum spanning tree

Minimal extension network MSN using Hamming distance in a simulated wheat data set of 40 individuals genotyped on 11 SSR markers and STS loci were created with the poppr.msn software (version 2.5.0). Each node represents a unique multilocus genotype. The shading of the nodes (colors) represents the membership of the population, while the widths of the edges and the shading represent a relationship. The length of the edge is arbitrary (*Fig. 7*).

UPGMA tree was produced from Euclidean's distance with bootstrap replicates. Data from population of the NUWYT data set can be interpreted as subdivision into three clusters (*Fig. 8*).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

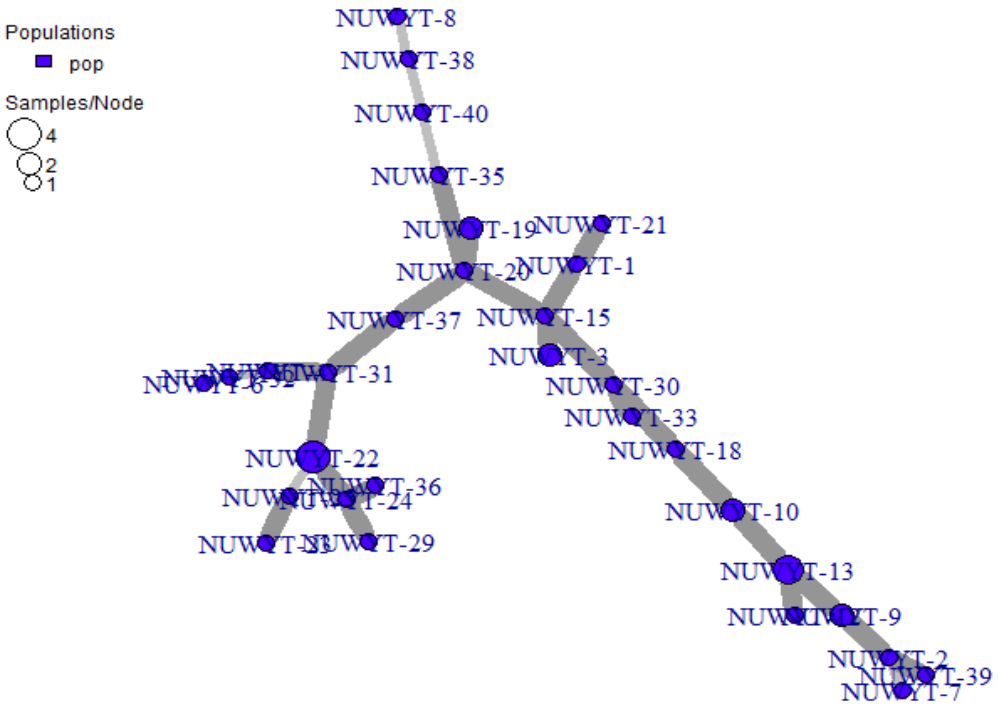

Figure 7. *Dendrogram*

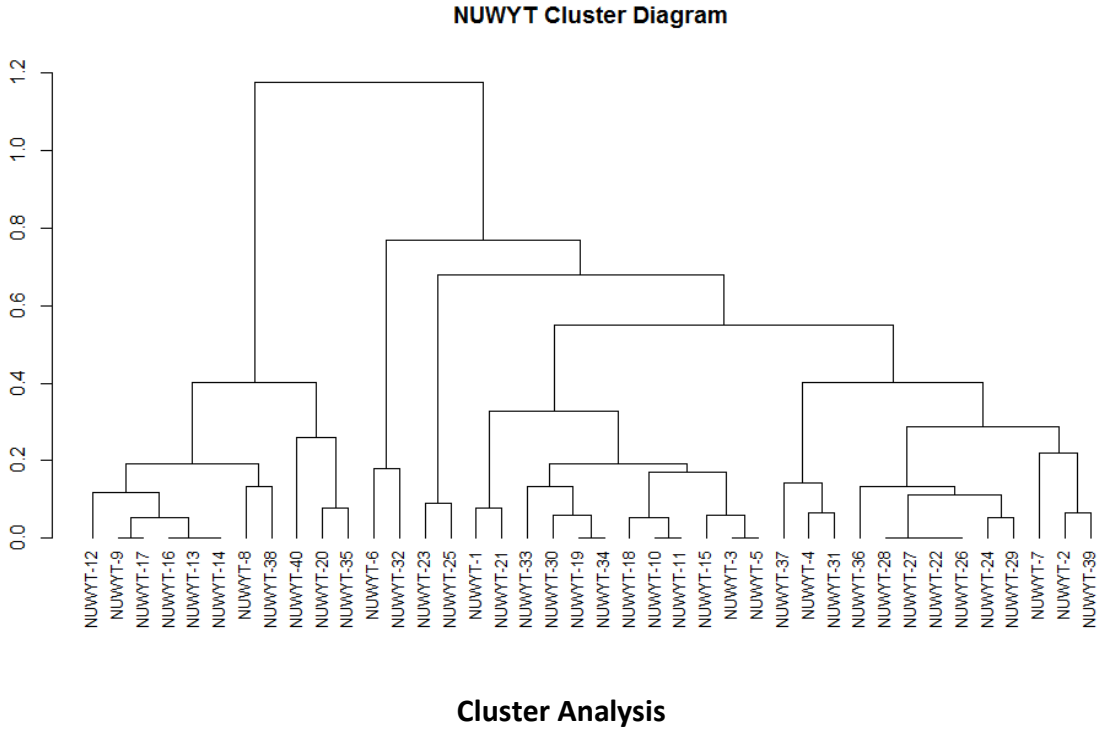

Figure 8. *Dendrogram based on genetic distance*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

Discussion

Marker Assisted Selection (MAS) has the potential to give long-term rust resistance and prevent future rust epidemics. From the 2NS chromosome of *Triticum ventricosum*, an alien chromosomal section containing three rust resistance genes, *Lr37*, *Sr38*, and *Yr17* was translocated into bread wheat (Juliana et al., 2018). Only seven of the wheat types investigated possessed the *Yr17-Lr37-Sr38* gene combination, even though the virulence of *Yr17* has been identified in several locations throughout the globe (Robert et al., 2000). This marker was effective in detecting the stem rust resistance gene *Sr31* in African wheat germplasm by Pretoriuset et al. (2010).

The SSR marker Xwmc-367 has emerged as a pivotal genetic tool in the study of wheat, particularly for its association with important agronomic traits, including disease resistance and yield improvement. Its utility in genetic mapping and marker-assisted selection (MAS) underscores its significance in wheat breeding programs. The Xwmc-367 marker has shown a strong correlation with key phenotypic traits, including resistance to various diseases such as leaf rust and stripe rust. This correlation allows breeders to utilize Xwmc-367 as a reliable indicator for selecting resistant genotypes in breeding populations. As a simple sequence repeat (SSR) marker, Xwmc-367 is advantageous due to its high level of polymorphism, ease of scoring, and ability to provide fine mapping of quantitative trait loci (QTL). In our analysis, the effectiveness of Xwmc-367 in distinguishing between resistant and susceptible genotypes was evident. When assessed in conjunction with other markers, it contributed valuable information that facilitated the identification of superior lines that carry beneficial traits. The presence of the Xwmc-367 allele in resistant lines highlights its potential as a genetic marker for enhancing disease resistance in wheat breeding. Moreover, the integration of the Xwmc-367 marker into routine screening protocols can significantly expedite the selection process for desirable traits in breeding programs. This marker facilitates early-stage selection by allowing breeders to screen progeny for desired alleles prior to the onset of phenotypic expression, thereby reducing the time and resources spent on field experiments. The Xwmc367 marker previously used by other findings showed similar results with these primers (Begum et al., 2014)

The SSR marker Wcm-344 has garnered attention in wheat research due to its potential association with key agronomic traits, including disease resistance and yield-related characteristics. Its polymorphic nature makes it a useful tool for genetic mapping and marker-assisted selection (MAS) in wheat breeding programs. Studies have indicated that Wcm-344 may be linked to important resistance genes, which could enhance the ability to select for disease resistance traits in breeding populations. The utilization of Wcm-344 in conjunction with other molecular markers can provide a more comprehensive understanding of the genetic architecture underpinning complex traits in wheat. One of the primary advantages of employing Wcm-344 is its ability to facilitate early selection within breeding programs. By allowing breeders to genotype progeny for the presence of favorable alleles at an early stage, Wcm-344 contributes to more efficient selection processes, ultimately reducing the time required to develop new varieties. Understanding the stability of Wcm-344's associations with desired traits in different contexts will be crucial for its practical application in breeding efforts.

The SSR markers Xgwm314 and Xgwm3832 have gained significance in wheat genetics due to their associations with critical traits such as disease resistance and yield enhancement. Both markers are valuable tools for molecular breeding, enabling researchers and breeders to implement marker-assisted selection (MAS) strategies

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

effectively. Xgwm314 can map closely to loci associated with resistance to various pathogens, including leaf rust and Fusarium head blight (Rasheed et al., 2016; Singh et al., 2019). Its polymorphic nature allows for clear differentiation between resistant and susceptible genotypes, facilitating the selection of superior breeding lines. The consistent performance of Xgwm314 across multiple genetic backgrounds highlights its reliability as a marker in breeding programs focused on improving disease resistance (Sandhu et al., 2021).

Xgwm3832, on the other hand, has shown promise in association studies related to yield traits and drought tolerance. Its linkage to QTLs that govern grain yield under abiotic stress conditions provides an opportunity for breeders to enhance the resilience of wheat varieties to climate variability (Ganal et al., 2018). The identification of favorable alleles at Xgwm3832 has the potential to support the development of high-yielding and drought-resistant cultivars, addressing pressing challenges in global food security. Both markers, when used in combination, can offer a robust approach to breeding programs aimed at enhancing not only disease resistance but also overall agronomic performance. Implementing these markers in genomic selection frameworks can lead to substantial improvements in breeding efficiency and effectiveness. Xgwm314 and Xgwm3832 represent important genetic tools in wheat breeding, contributing to the advancement of resilient and high-performing varieties. Continued research and validation of their associations with desirable traits will be crucial for maximizing their potential in breeding applications.

Xgwm174 has been associated with resistance to several fungal diseases, including leaf rust and powdery mildew, which are significant threats to wheat production worldwide. Studies have shown that Xgwm174 is linked to quantitative trait loci (QTLs) that can provide insights into the genetic mechanisms underlying disease resistance (Khan et al., 2018). The identification of favorable alleles at this marker allows breeders to select for plants that may exhibit improved resilience to these pathogens, thus potentially enhancing yield stability in the field. Its robustness across diverse genetic backgrounds adds to its appeal as a reliable marker for disease resistance (Bansal et al., 2020).

Xgwm169, while similarly employed in the context of disease resistance, has been studied for its connections to traits like grain quality and yield. This marker is linked to important agronomic traits, such as kernel weight and size, which are critical in determining overall yield and marketability (Kumar et al., 2021). The presence of Xgwm169 in breeding programs can facilitate the selection of high-quality grains along with disease resistance, thus providing a more comprehensive approach to improving wheat cultivars. Both markers can be employed synergistically in breeding programs. Their simultaneous use could enable the development of wheat varieties that not only resist common pathogens but also deliver higher yield and improved grain quality. Additionally, as climate change increasingly affects agriculture, the implementation of these markers in genomic selection can lead to the development of varieties that thrive under variable environmental conditions.

Xgwm111 has been linked to resistance against multiple diseases, including *Fusarium* head blight and leaf rust. Its consistent performance across different wheat genetic backgrounds makes it a reliable tool for breeders aimed at enhancing disease resistance in new cultivars (Rasheed et al., 2016). By integrating Xgwm111 into breeding programs, researchers can effectively select for lines that are likely to perform better under pathogen pressure. On the other hand, Xgwm3 is noted for its association

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

with yield-related traits, particularly in drought-prone environments. The marker's connection to quantitative trait loci (QTLs) for grain weight and stress resilience is crucial for developing varieties that can maintain productivity under adverse conditions (Zhang et al., 2020). This makes Xgwm3 a vital component in efforts to improve overall yield stability in wheat. Both markers Xgwm111 and Xgwm3 hold promise for enhancing wheat breeding programs by providing tools to select for disease resistance and improved yield traits, catering to the demands of sustainable agriculture.

Xgwm1117 has been associated with resistance to several important wheat pathogens, including various races of leaf rust (*Puccinia triticina*) and *Fusarium* head blight (*Fusarium graminearum*). The identification of favorable alleles at this marker allows breeders to select for plants that demonstrate robust resistance mechanisms, ultimately contributing to higher yields and better quality grains in environments prone to these diseases (Uauy et al., 2005). Moreover, the presence of Xgwm1117 has been shown to interact beneficially with other resistance genes, enhancing the overall efficacy of disease management strategies in wheat production. Marker Xgwm539 is primarily recognized for its association with yield-related traits, particularly under stress conditions such as drought and heat. Research indicates that Xgwm539 is linked to QTLs responsible for grain weight, size, and overall yield stability, making it essential for breeders focused on developing high-yielding varieties that can withstand environmental stressors (Liu et al., 2018). The integration of this marker into breeding programs can facilitate the selection of genotypes that not only maintain high productivity but also possess desirable agronomic characteristics.

Ejaz et al. (2012) previously utilized this marker to detect the stem rust resistance gene *Sr31* in Pakistani wheat varieties. The primers *VENTRIUP* and *LN2* amplified a 259-bp fragment in 34 wheat lines and the positive control, showing the existence of the *Yr17* gene, whereas the 259 bp band was not seen in 66 wheat lines and the negative control, showing the likely absence of the *Yr17* gene. Using race analysis of 27 Australian spring wheat cultivars, Qamar et al. (2008) proposed *Yr17* and confirmed it in global wheat germplasm.

Xgwm159 is its association with resistance to leaf rust (*Puccinia triticina*) and other foliar pathogens. The ability to incorporate Xgwm159 into breeding strategies enables the selection of genotypes that are more resilient to these diseases, which is crucial for maintaining wheat production in various environments. This marker assists breeders in identifying plants that carry effective resistance genes, thus reducing the reliance on chemical fungicides and promoting sustainable agricultural practices. Furthermore, Xgwm159 is also associated with yield-related traits under both optimal and stress conditions. Studies have shown that lines carrying favorable alleles of this marker tend to exhibit improved traits such as grain weight and overall yield potential, making it a valuable asset in the development of high-performing wheat varieties (Cavanagh et al., 2013). The use of Xgwm159 in marker-assisted selection (MAS) allows for more precise breeding strategies, enabling breeders to efficiently combine multiple desirable traits within a single genotype. As climate change continues to impact agricultural productivity, leveraging markers like Xgwm159 will be crucial in developing wheat varieties that can thrive under diverse environmental conditions while meeting the global demand for food.

Additionally, the AFLP-converted STS marker iag95 has been identified as diagnostic for the detection of the 1BL.1RS translocated segment, which harbors the resistance genes *Sr31, Yr9, Lr26*, and Pm8 (Mago et al., 2002). Although the marker

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

iag95 is effective in detecting *Yr9*, its dominant inheritance complicates the differentiation between heterozygous and homozygous dominant varieties, thereby limiting its applicability in the selection of *Yr9* in segregating populations. This marker has previously been utilized for the detection of stripe rust resistance gene *Yr9* in Pakistani wheat varieties (Begum et al., 2014).

Likewise, Begum et al. (2014) used this marker Xwmc419 to amplify a 141-bp fragment in 95 wheat lines, showing the presence of the *Yr26* gene in these lines (data not provided). The 141-bp fragment was not produced by this marker in the remaining five lines, indicating the absence of *Yr26*. The fragment with all three markers was created by the negative control "Avocet S." The data of 86 genotypes for the presence/absence of *Yr26* were comparable to those of the Xgwm11 and Xgwm18 markers, while fourteen lines exhibited differences, eleven lines positive, but negative, with the Xgwm11 marked. Detection of the *Yr26* gene has been reported for markers Xbarc181, Xwmc419, and CYS-5. However, CYS-5 would be better used as this indicator is the closest to Yr26 (0.5 cM) (Wen et al., 2008). In virulent testing, wheat varieties containing the *Yr26* gene were resistant to most *P. striiformis* races (Wang et al., 2002). However, pathogenicity was recently found in this gene. The goal of this study was to establish the status of several leaf, stem and stripe rust resistance genes in Pakistani improved wheat lines and to identify lines that could be employed as parents in rust resistance gene pyramiding. It is also suggested that advanced wheat lines be evaluated for the presence/absence of rust resistance genes using DNA markers so that only desirable gene combinations are carried forward in future wheat varieties.

Conclusions

The molecular characterization of spring wheat lines for rust resistance utilizing microsatellite markers has revealed significant insights into the genetic diversity of Pakistani advanced breeding lines. The study confirmed the presence of important resistance genes, specifically *Sr*, *Lr*, and *Yr*, which play crucial roles in providing resistance against stem, leaf, and stripe rust diseases. This genetic variability demonstrates the potential of these advanced wheat lines to be harnessed in breeding programs aimed at developing wheat varieties that can withstand the increasing threat of rust pathogens. The findings underscore the importance of maintaining and utilizing diverse germplasm to enhance the durability of rust resistance in wheat crops.

Recommendations

Routine screening: It is recommended that future breeding programs routinely screen the identified advanced wheat lines for the presence of desired rust resistance genes (Sr, Lr, Yr) to monitor genetic progress and effectiveness in rust resistance.

Gene pyramiding: To broaden the genetic base of future wheat varieties, it is essential to pyramid multiple resistance genes using marker-assisted selection (MAS). This strategy will enhance the overall resistance to a range of rust pathogens and help mitigate the risk of disease outbreaks due to potential virulence shifts.

Integration into breeding programs: The advanced wheat lines characterized in this study should be integrated into existing wheat breeding programs focused on improving disease resistance. Incorporating these lines can accelerate the development of resilient

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

wheat varieties that are more adaptable to the climatic and biotic stresses faced in Pakistan.

Field validation: It is vital to conduct field experiments to validate the laboratory findings of resistance. This will provide a comprehensive understanding of how these lines perform under natural conditions and different environmental factors.

Collaboration for broader genetic resources: Collaborating with international research institutions can facilitate access to a broader range of genetic resources and resistance traits, further enhancing the breeding efforts against wheat rust diseases in Pakistan.

Acknowledgements. The authors extend their appreciation to the Researchers Supporting Project number (RSP2025R349) King Saud University, Riyadh, Saudi Arabia. We are thankful to the National Institute for Genomics and Advanced Biotechnology (NIGAB), National Agricultural Research Centre, Islamabad for providing the research facilities. We are also thankful to the Crop Diseases Research Programme, Islamabad, Pakistan, for providing the seed materials.

REFERENCES

[1] Afridi, K., Khan, N. U., Gul, S., Bibi, Z., Ali, S., Ali, N., Khan, A. (2019): Genetic characterization of stripe rust and yield traits in bread wheat. – International Journal of Agriculture and Biology 21(3): 621-629.

[2] Ali, Q., Hussain, A. (2023): Emergence of new strains of wheat rust: implications for disease management in Punjab, Pakistan. – Plant Disease 107(3): 713-718. DOI: 10.1094/PDIS-09-22-2112-PDN.

[3] Ali, Y., Khan, M. A., Atiq, M., Hussain, M. (2018): Novel gene pyramiding to combat rusts in global wheat varieties against prevalent virulence: a review. – Sarhad Journal of Agriculture 34(4): 797-810.

[4] Alotaibi, M. O., Alotaibi, N. M., Ghoneim, A. M., ulAin, N., Irshad, M. A., Nawaz, R., Abbas, A., Ali, S. (2023): Effect of green synthesized cerium oxide nanoparticles on fungal disease of wheat plants: a field study. – Chemosphere 339: 139731.

[5] Altaf, M. T., Tatar, M., Ali, A., Liaqat, W., Mortazvi, P., Kayihan, C., Baloch, F. S. (2024): Advancements in QTL mapping and GWAS application in plant improvement. – Turkish Journal of Botany 48(7): 376-426.

[6] Bansal, P., Grewatsch, S., Sharma, G. (2021): How COVID-19 informs business sustainability research: It's time for a systems perspective. – Journal of Management Studies 58(2): 602-606.

[7] 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.

[8] Begum, S., Khan, M. R., Jan, A., Ur-Rahman, H., Khattak, S. H., Saeed, S., Ahmed, A., Ali, G. M. (2022): Genetic transformation of the EPSPS herbicide resistance gene in Agrobacterium mediated peanut (Arachishypogaea L.) and effective revival of transgenic plants. – Applied Ecology and Environmental Research 20(2): 1335-1349.

[9] Bux, H., Ashraf, M., Chen, X., Mumtaz, S. (2011): Effective genes for resistance to stripe rust and virulence of Puccinia striiformis f. sp. tritici in Pakistan. – African Journal of Biotechnology 10(28): 5489-5495.

[10] Cavanagh, K., Strauss, C., Cicconi, F., Griffiths, N., Wyper, A., Jones, F. (2013): A randomised controlled trial of a brief online mindfulness-based intervention. – Behaviour Research and Therapy 51(9): 573-578.

[11] Chen, X. M. (2005): Epidemiology and control of stripe rust (Puccinia striiformis f. sp. tritici) on wheat. – Canadian Journal of Plant Pathology 27: 314-337.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

[12] Chen, X. M. (2007): Challenges and solution for stripe rust control in the United States. – Australian Journal of Agricultural Research 58: 648-655. https://doi.org/10.1071/AR07045.
[13] Dedryver, F., Paillard, S., Mallard, S., Robert, O., Trottet, M., Nègre, S., Verplancke, G., Jahier, J. (2009): Characterization of genetic components involved in durable resistance to stripe rust in the bread wheat 'Renan'. – Phytopathology 99: 968-973.
[14] Draz, I. S., Abou-Elseoud, M. S., Kamara, A. E., Alaa-Eldein, O. A., El-Bebany, A. F. (2015): Screening of wheat genotypes for leaf rust resistance along with grain yield. – Annals of Agricultural Sciences 60(1): 29-39.
[15] Ejaz, M., Iqbal, M., Shahzad, A., Atiq-ur-Rehman, Ahmed, I., Ali. G. (2012): Genetic variation for markers linked to stem rust resistance genes in Pakistani wheat varieties. – Crop Science 52: 2638-2648.
[16] FAOSTAT (2016): Food and Agriculture Organization of the United Nations. – FAOSTAT, Rome.
[17] Food and Agriculture Organization of the United Nations. (2016). FAOSTAT statistical database. Rome, Italy. Retrieved from http://faostat.fao.org/default.aspx
[18] Ganal, N. N., Navarez, J. C. (2018). Meaning in life and happiness among tertiary education students. – AsTEN Journal of Teacher Education 3(1): 1-11.
[19] Gao, P., Zhou, Y., Gebrewahid, T. W., Zhang, P., Yan, X., Li, X., Yao, Z., Li, Z., Liu, D. (2019): Identification of known leaf rust resistance genes in common wheat cultivars from Sichuan province in China. – Crop Protection 115: 122-129.
[20] Haq, M. A., Amanullah, S. (2021): Influence of climatic factors on wheat leaf and yellow rust prevalence in Pakistan. – Journal of Phytopathology 169(4): 234-245. DOI: 10.1111/jph.13012.
[21] Helguera, M., Khan, I. A., Kolmer, J., Lijavetzky, D., Zhong-qi, L., Dubcovsky, J. (2003): PCR Assays for the Lr37-Yr17-Sr38 cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. – Crop Science 43: 1839-1847.
[22] Imran, S., Saleem, A. (2022): Climate change and its impact on wheat production in Punjab, Pakistan: a review. – Agricultural Sciences 13(1): 45-57. DOI: 10.4236/as.2022.131005.
[23] Jin, Y., Szabo, L., Rouse, M., Fetch, T., Pretorius, Z. A., Wanyera, R., Njau, P. (2009): Detection of virulence to resistance gene Sr36 within race TTKS lineage of Puccinia graminis f. sp. tritici. – Plant Disease 93: 367-370.
[24] Juliana, P., Singh, R. P., Singh, P. K., Poland, J. A., Bergstrom, G. C., Huerta-Espino, J., Sorrells, M. E. (2018): Genome-wide association mapping for resistance to leaf rust, stripe rust and tan spot in wheat reveals potential candidate genes. – Theoretical and Applied Genetics 131: 1405-1422.
[25] Kamvar, Z. N., Tabima, J. F., Grünwald, N. J. (2014): Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. – PeerJ 2: e281.
[26] Khan, M. M., Iqbal, M. J., Abbas, M., Raza, H., Waseem, R., Ali, A. R. S. H. A. D. (2004): Loss of vigour and viability in aged onion (Allium cepa L.) seeds. – Int. J. Agric. Biol. 6(4): 701-711.
[27] Kapoor, K. K., Tamilmani, K., Rana, N. P., Patil, P., Dwivedi, Y. K., Nerur, S. (2018): Advances in social media research: Past, present and future. – Information Systems Frontiers 20: 531-558.
[28] Kaur, G., Pathak, M., Singla, D., Sharma, A., Chhuneja, P., Sarao, N. K. (2021): High-density GBS-based genetic linkage map construction and QTL identification associated with yellow mosaic disease resistance in bitter gourd (Momordica charantia L.). – Frontiers in Plant Science 12: 671620.
[29] Khan, A., Fornes, O., Stigliani, A., Gheorghe, M., Castro-Mondragon, J. A., Van Der Lee, R., Bessy, A., Cheneby, J., Kulkarni, S. R., Tan, G., Baranasic, D., Arenillas, D. J., Sandelin, A., Vandepoele, K., Lenhard, B., Ballester, B., Wasserman, W. W., Parcy, F.,

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

Mathelier, A. (2018): JASPAR 2018: update of the open-access database of transcription factor binding profiles and its web framework. – Nucleic acids research 46(D1): D260-D266.

[30] King, W. C., Rubinstein, M., Reinhart, A., Mejia, R. (2021): Time trends, factors associated with, and reasons for COVID-19 vaccine hesitancy: A massive online survey of US adults from January-May 2021. – PloS one 16(12): e0260731.

[31] Khan, M. I., Khattak, R. A. (2023): Evaluation of wheat rust resistance in major wheat growing regions of Punjab, Pakistan. – Pakistan Journal of Botany 55(2): 345-360. DOI: 10.30848/PJB2023-2(9).

[32] Khan, M. A., Mumtaz, H. (2004): Combining yellow rust resistance with high yield in grain wheat. – In: Second Regional Yellow Rust Conference for Central and West Asia and North Africa, March 22-26, 2004, Islamabad, Pakistan.

[33] King, K. M., Bucur, D. E., Ritchie, F., Hawkins, N. J., Kaczmarek, A. M., Duan, Y., Fraaije, B. A. (2021): Fungicide resistance status and chemical control options for the brassica pathogen Pyrenopeziza brassicae. – Plant Pathology 70(9): 2086-2103.

[34] Kumar, S., Kumar, M., Mir, R. R., Kumar, R., Kumar, S. (2021): Advances in molecular markers and their use in genetic improvement of wheat. – In: Wani, S. H., Mohan, A. & Singh, G. P. (eds.): Physiological, molecular, and genetic perspectives of wheat improvement. Springer Nature, pp. 139-174.

[35] Lagudah, E. S., McFadden, H., Singh, R. P., Huerta-Espino, J., Bariana, H. S., Spielmeyer, W. (2006): Molecular genetics characterization of the Lr34/Yr18 slow rusting resistance gene region in wheat. – Theoretical and Applied Genetics 114: 21-30.

[36] Lele, U., Goswami, S. (2021): Financing for Sustainable Structural Transformation. – In: Lele, U. et al. (eds.): Food for All: International Organizations and the Transformation of Agriculture. Oxford University Press, Oxford.

[37] Li, Y. (2020). Development of Host and Pathogen Mutants for Characterization of Wheat-Puccinia striiformis F. Sp. tritici Interactions Using Ethyl Methanesulfonate. Washington State University.

[38] Li, Y. (2020): Development of Host and Pathogen Mutants for Characterization of Wheat-Puccinia striiformis F. Sp. tritici Interactions Using Ethyl Methanesulfonate. Washington State University.

[39] Li, Z., Lan, C., He, Z., Singh, R. P., Rosewarne, G. M., Chen, X., Xia, X. (2014): Overview and application of QTL for adult plant resistance to leaf rust and powdery mildew in wheat. – Crop Science 54(5): 1907-1925.

[40] Lui, T. W., Bartosiak, M., Piccoli, G., Sadhya, V. (2018): Online review response strategy and its effects on competitive performance. – Tourism Management 67: 180-190.

[41] Mahmood, N., Arshad, M., Kächele, H., Ma, H., Ullah, A., Müller, K. (2019): Wheat yield response to input and socioeconomic factors under changing climate: evidence from rainfed environments of Pakistan. – Science of the Total Environment 688: 1275-1285.

[42] Maia, N. (1967): Obtention de blés tendres résistants au piétin-verse par croisements interspécifiques blés x Aegilops. – CR Hebd Séances Acad. Agric. Fr 53: 149-154.

[43] Malik, T. A., Bhat, S. A. (2022): Resistance breeding in wheat: strategies for managing rust diseases in Pakistan. – International Journal of Agriculture and Biology 24(5): 1401-1410. DOI: 10.17957/IJAB/15.2165.

[44] Mago, R., Spielmeyer, W., Lawrence, G., Lagudah, E., Ellis, J., Pryor, A. (2002): Identification and mapping of molecular markers linked to rust resistance genes located on chromosome 1RS of rye using wheat-rye translocation lines. – Theoretical and Applied Genetics 104(8): 1317-1324.

[45] Nasir, M. A., Huynh, T. L. D., Tram, H. T. X. (2019): Role of financial development, economic growth and foreign direct investment in driving climate change: a case of emerging ASEAN. – Journal of Environmental Management 242: 131-141.

[46] Nei, M. (1978): Estimation of average heterozygosity and genetic distance from a small number of individuals. – Genetics 89(3): 583-590.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

[47] Peterson, R. F., Campbell, A. B., Hannah, A. E. (1948): A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. – Canadian Journal of Research 26(5): 496-500.
[48] Pretorius, Z. A., Bender, C. M., Visser, B. (2010): First report of a Puccinia graminis f. sp. tritici race virulent to the Sr24 and Sr31 wheat stem rust resistance genes in South Africa. – Plant Disease 94: 784-784.
[49] Qamar, M., Ahmad, S. D., Shah, A. H., Wellings, C., Batool, F. (2008): Postulation of stripe rust resistant genes in some Australian bread wheat cultivars and their response to temperature. – Pakistan Journal of Botany 40: 2573-2585.
[50] Qureshi, N., Bariana, H., Kumran, V. V., Muruga, S., Forrest, K. L., Hayden, M. J., Bansal, U. (2018): A new leaf rust resistance gene Lr79 mapped in chromosome 3BL from the durum wheat landrace Aus 26582. – Theoretical and Applied Genetics 131: 1091-1098.
[51] Raza, W., Ghazanfar, M. U., Rehman, A. U., Fayyaz, M. (2018): Screening of wheat germplasm against stripe rust disease under field conditions in Pakistan. – Plant Protection 2(3): 87-92.
[52] Rasheed, A., Wen, W., Gao, F., Zhai, S., Jin, H., Liu, J., Guo, Q., Zhang, Y., Dreisigacker, S., Xia, X., He, Z. (2016): Development and validation of KASP assays for genes underpinning key economic traits in bread wheat. – Theoretical and Applied Genetics 129(10): 1843-1860.
[53] Robert, O., Dedryver, F., Rolland, B., Abelard, C., Jaudeau, B. (2000): Relationships between molecular identification of the gene Yr17 and adult plant resistance against stripe and leaf rusts in bread wheat varieties. – Acta Phytopathologica et Entomologica Hungarica 35: 59-63.
[54] Roussel, M., Merlini, G., Chevret, S., Arnulf., Stoppa, A. M., Perrot A., Jaccard, A. (2020): A prospective phase 2 trial of daratumumab in patient with previously treated systematic light-chain amyodiosis. – Blood, The Journal of American Society of Hematology 135(18): 153-1540.
[55] Sandhu, N., Sethi, M., Kumar, A., Dang, D., Singh, J., Chhuneja, P. (2021). Biochemical and genetic approaches improving nitrogen use efficiency in cereal crops: a review. Frontiers in plant science, 12, 657629.
[56] Sharp, P. J., Johnston, S., Brown, A. G., McIntosh, A. M., Palloota, M., Carter, H. S., Bariana, S., Khatkar, E. E., Lagudah, S., Sing, R. P., Khairallah, M., Potter, R., Jones, M. J. K. (2001): Validation of molecular markers for wheat breeding. – Australian Journal of Agricultural Research 52: 1357-1366. https://doi.org/10.1071/AR01052.
[57] Singh, J. A., Guyatt, G., Ogdie, A., Gladman, D. D., Deal, C., Deodhar, A., Reston, J. (2019): 2018 American College of Rheumatology/National Psoriasis Foundation guideline for the treatment of Psoriatic Arthritis. – Journal of Psoriasis and Psoriatic Arthritis 4(1): 31-58.
[58] Singh, R. P. (1992): Association between gene Lr34 for leaf rust resistance and leaf tip necrosis in wheat. – Crop Science 32(4): 874-878.
[59] Singh, R. P., Nelson, J. C., Sorrells, M. E. (2000): Mapping Yr28 and other genes for resistance to stripe rust in wheat. – Crop Science 40: 1148-1155. https://doi.org/10.2135/cropsci2000.4041148x.
[60] Singh, R. P., William, H. M., Huerta-Espino, J., Rosewarne, G. (2004): Wheat Rust in Asia: Meeting the Challenges with Old and New Technologies. – In: Proceedings of the 4th International Crop Science Congress. Brisbane, Australia, 26 Sep – 1 Oct 2004.
[61] Smith, P. H., Hadfield, J., Hart, N. J., Koebner, R. M. D., Boyd, L. A. (2007): STS markers for the wheat yellow rust resistance gene Yr5 suggest an NBS–LRR-type resistance gene cluster. – Genome 50: 259-265.
[62] Sommers, D. J., Isaac, P., Edwards, K. (2004): A high density microsatellite consensus map for bread wheat (Triticum aestivum L). – Theoretical and Applied Genetics 109: 1105-1114.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554

[63] Spielmeyer, W., Sharp, P. J., Lagudah, E. S. (2003): Identification and validation of markers linked to broad spectrum stem rust resistance gene Sr2 in wheat (Triticum aestivum L.). – Crop Science 43: 333-336. https://doi.org/10.2135/cropsci2003.0333.
[64] Suenaga, K., Singh, R. P., Huerta-Espino, J., William, M. (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. https://doi.org/10.1094/PHYTO.2003.93.7.881.
[65] Tsilo, T. J., Chao, S., Jin, Y., Anderson, J. A. (2009): Identification and validation of SSR markers linked to the stem rust resistance gene Sr6 on the short arm of chromosome 2D in wheat. – Theoretical and Applied Genetics 118: 515-524.
[66] Uauy, C., Brevis, J. C., Chen, X., Khan, I., Jackson, L., Chicaiza, O., Dubcovsky, J. (2005): High-temperature adult-plant (HTAP) stripe rust resistance gene Yr36 from Triticum turgidum ssp. dicoccoides is closely linked to the grain protein content locus Gpc-B1. – Theoretical and Applied Genetics 112: 97-105.
[67] Wang, A. M., Chen, X. N., He, Z. H. (2007): Wheat stripe rust in China. – Australian Journal of Agricultural Research 58: 605-619.
[68] Wang, L., Ma, J., Zhou, R., Wang, X., Jia, J. (2002): Molecular tagging of the yellow rust resistance gene Yr10 in common wheat, P.I.178383 (Triticum aestivum L.). – Euphytica 124: 71-73.
[69] Wani, S. H., Mohan, A., Singh, G. P. (Eds.) (2020): Physiological, molecular, and genetic perspectives of wheat improvement. Springer Nature.
[70] Waqas, M. A., Kaya, C., Riaz, A., Farooq, M., Nawaz, I., Wilkes, A., Li, Y. (2019): Potential mechanisms of abiotic stress tolerance in crop plants induced by thiourea. – Frontiers in Plant Science 10: 1336.
[71] Wen, W., Li, G., He, Z., Yang, W., Xu, M., Xia, X. (2008): Development of an STS marker tightly linked to Yr26 against wheat stripe rust using the resistance gene-analog polymorphism (RGAP) technique. – Molecular Breeding 22: 507-515.
[72] William, H. M., Singh, R. P., Huerta-Espino, J., Ortiz-Islas, S., Hoisington, D. H. (2003): Molecular marker mapping of leaf rust resistance gene Lr46 and its association with stripe rust gene Yr29 in wheat. – Phytopathology 93: 153-159. https://doi.org/10.1094/PHYTO.2003.93.2.153.
[73] Zhang, Y., Chen, C., Zhu, S., Shu, C., Wang, D., Song, J., Xu, W. (2020): Isolation of 2019-ncov from a stool specimen of a laboratory – confirmed case of the coronavirus disease 2019 b (COVID-19). – China CDC Weekly 2(8): 123.

ELECTRONIC APPENDIX

This paper has an electronic appendix about Nei's coefficients.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_95299554