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Molecular marker based identification of leaf rust resistance gene Lr25 in selected wheat accessions

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Abstract

Wheat (*Triticum aestivum* L.) rusts are among the most widespread and destructive diseases of wheat crop. Leaf rust, caused by *Puccinia triticina* Eriks is a common and widespread disease of wheat in several countries of the World including Pakistan. Molecular survey was conducted to screen altogether 19 Pakistani wheat germplasm/accessions obtained from National Agriculture Research Council (NARC) Islamabad, Pakistan. For the presence of leaf rust resistance gene Lr25, specific SSR primers linked to Lr25 were utilized. The research studies revealed that out of the 19 accessions grown in the botanical garden at Abdul Wali Khan University Mardan during the month of March and April 2013, 4 accessions were observed having Lr25 gene, which showed high resistance. Two accessions revealed moderate resistance while 13 accessions did not show the presence of Lr25 gene and were regarded as susceptible to leaf rust disease. The identification of Lr25 in Pakistani wheat germplasm will help in accelerating the breeding program in future, including pyramiding of different wheat resistant genes in wheat varieties.

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Introduction

Wheat (*Triticum aestivum* L.) is among the world's most consumed cereal crops and is the most important food grain (Sleper and Poehlman, 2006). Global wheat production was reported as 713.2 million metric tons in 2013-14, thereby ranking it the third cereal crop in production after maize (872.79 million metric tons) and rice (719.74 million metric tons). In Pakistan, 25.3 million tons of wheat was produced from 9.03 million hectares of cultivated area, contributing 10.3% value addition in agriculture and 2.2% GDP of the country (Anonymous, 2014). It is a staple food of more than 35% world's population (Ogbonnaya *et al.,* 2013). Like many other developing countries, agriculture is the main sector of the economy of Pakistan and wheat is the most important agricultural product. Demand of wheat hasbeen increasing with the ever increasing population and is expected that will increase by 40% by the year 2030 (Dixon *et al.,* 2009). Wheat crop is hit by many biotic and abiotic stresses which cause reduction in its production (Jellis, 2009). Among abiotic stresses rusts are very important worldwide due to their ability to mutate and multiply rapidly and air dispersal from one field to another and even over longer distances. Rusts at present are the most important diseases of wheat worldwide, which threaten global food security (Hovmøller *et al*., 2010). Leaf rust, caused by *Puccinia triticina* Eriks., is a severe fungal disease in most of the wheat growing areas (Park *et al*., 2007). Leaf rust has potential to cause losses up to 50%and because of its more frequent and widespread occurrence, leaf rust probably results in greater total annual losses worldwide than stem and stripe rusts (Huerta-Espino *et al*., 2011). It occurs on the leaf blades, leaf sheaths that can also be infected under favorable conditions, high densities of inoculum and very susceptible cultivars (Roelfs *et al.,* 1992).

Genetic resistance is most efficient system of reducing yield losses caused by leaf rust (Kolmer, 1996). The resistance to leaf rust disease in wheat is the most economical and environmental friendly and preferable method to tackle this disease.

Developing and managing durable resistance in cultivars is very difficult. The hidden and the new evolving races of the leaf rust recurrently have sensitized the resistance of newly resistant cultivars. The resistance exploited is centered on genes which are effective during the course of the plant growth cycle (Fahmi *et al*., 2005). To date, at least 73 leaf rust resistance genes derived from *Triticum* and related genera or species have been formally named (McIntosh *et al*., 2012; Park *et al.,* 2014), most of which confer race-specific resistance.

Numerous studies have been conducted on the life cycles of pathogens rust and management. The use of chemicals is neither economical nor feasible on a large scale. The only economical and practical control of rust diseases can be achieved through genetic resistance (McIntosh, 1988; Pathan and Park, 2006). Chemical control against the disease is not economical; therefore, the culture of rust resistant varieties is of paramount importance (Anonymous, 2005). With current emergence of genomics, many race-specific and nonspecific leaf rust resistance genes have been tagged with molecular markers that can be used in marker-assisted breeding (McIntosh *et al*., 2012; Chen, 2013; Rosewarne *et al*., 2013).Successive release of rust resistant varieties in Pakistan has reduced losses caused by rust (Khan, 1987).

The objectives of the current study were to characterize wheat genotypes for the success of their response to leaf rust through PCR to identify potential sources of novel resistance for use in future breeding programs.

Materials and method

Plant materials

During present study 19 wheat accessions (table 1) cultivated in Pakistan were obtained from National Agriculture Research Council (NARC) Islamabad, Pakistan. Research studies were performed at the Department of Botany, Abdul Wali Khan University Mardan. All accessions were sown in pots during the month of March and April.

DNA extraction

The genomic DNA of 19 wheat accessions was extracted from young leaves. Total genomic DNA was extracted from all nineteen wheat accessions according to Doyle and Doyle, (1987) DNA extraction protocol with slight modifications.

Lr25 primer sequence

F20 - CCACCCAGAGTATACCAGAG

R19 - CCACCCAGAGCTCATAGAA.

PCR analysis

The DNA isolated of all the samples having good quality bands were subjected to PCR analysis. The PCR amplification was performed according to Williams *et al.* (1990) with certain modifications. Initial denaturation at 94°C for 4 minutes followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 58°C for 1 minute and extension at 72°C for 1 minute and final extension at 72°C for 7 minutes was given. The amplified products of PCR were run on 2% agarose gel. The gels after running were visualized under UV light for presence and absence of the DNA band for leaf rust resistance gene Lr25 in all accessions. Band size of the required gene was confirmed by running 100bp DNA ladder along the samples.

Results and discussion

Genetic resistance is one of the important methods to control many pathogenic epidemics. With the advancement of genetics, many race-specific and nonspecific leaf rust and yellow rust resistance genes have been tagged with molecular markers that can be used in marker-assisted breeding (McIntosh *et al.*, 2012; Chen, 2013; Rosewarne *et al.*, 2013).

Table 1. List of 19 wheat accessions studied for Leaf Rust resistant using primer Lr25.

S. No	Acc No./Name	S. No	Acc No./Name
1	010746	11	NARC 2009
2	010783	12	Sehar
3	010790	13	Dera-98
4	010762	14	Atta habib
5	010785	15	KT-2000
6	Lasani08	16	Kaghan93
7	Faisalabad08	17	Pak-81
8	Aqab2000	18	PS-80
9	Gomal	19	Tatara
10	Suliman96	-	-

This study was carried out for the molecular screening of the presence of Lr25 gene in the selected wheat varieties using PCR based specific SSR marker. In the current study, 19 bread wheat accessions obtained from NARC, Islamabad were screened against leaf rust. Microsatellites primer pairs were used to evaluate the extent of molecular characterization and genetic diversity among nineteen different accessions of wheat. The results based on rust resistance showed that four accessions including with accession number or name 010746 (1), 010762 (4), 010785 (5) and Dera-98 (13) were found to be more resistant.

Two accessions with accession number or name Gomal (9), and NARC 2009 (11) were found to be moderately resistant and thirteen varieties with names or accession number 010783 (2), 010790 (3), Lasani08 (6), Faisalabad08 (7), Aqab2000 (8), Suliman96 (10), Sehar (12), Atta habib (14), KT-2000 (15), Kaghan93 (16), Pak-81 (17), PS-80 (18) and Tatara (19) showed no resistance to leaf rust thereby revealing no presence of Lr25 gene in these accessions.

Our results agreed with similar results reported by Baber *et al.,* (2010) and Malik *et al.,* (2007) while screening Pakistani wheat germplasm for leaf rust resistance through PCR based molecular markers.

The molecular information derived from the variation among these genotypes explains changes in their character.

In a survey during 2003 and 2004 in Pakistan, the frequency of virulence was detected as 91 and 85 percent, respectively. Rizvi *et al.,* (1984) also reported the earlier virulence of Lr25 to be 4.4% in Pakistan.

This suggests that there is a shift of the virulence gene from near Western countries. The present study may be helpful for future screening to identify the resistant source in wheat germplasm against leaf rust and their utilization in useful breeding program of wheat.

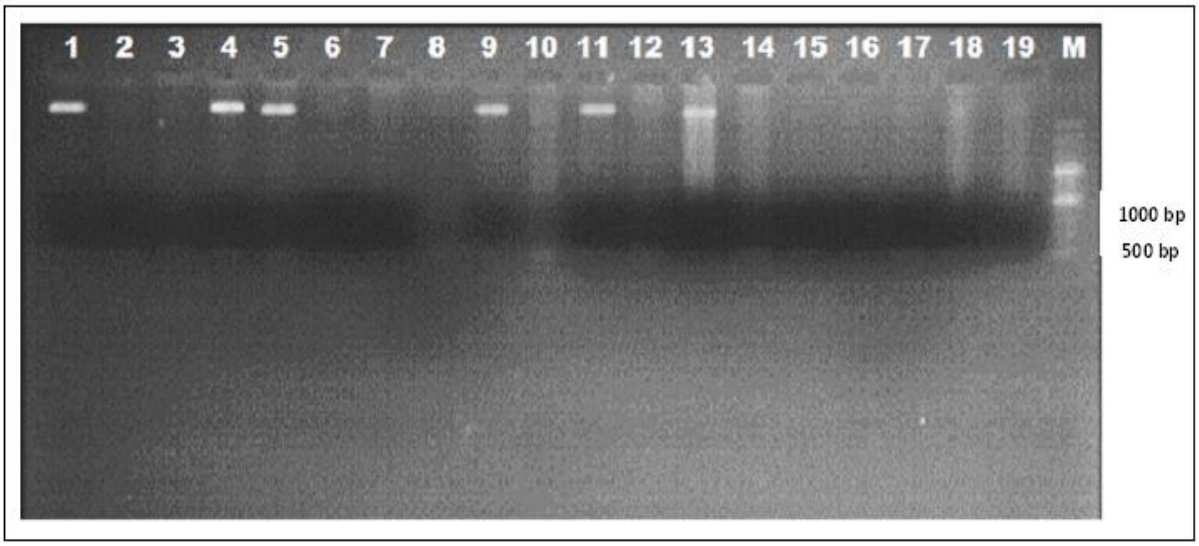

Fig. 1. PCR Amplification Profile of leaf rust resistant primer Lr25 for 19 wheat varieties, M = 100 bp size arker.

Conclusion

Nineteen bread wheat accessions cultivated in Pakistan were screened using SSR marker specific for Lr25 gene presence. The polymorphic survey revealed that out of the 19 varieties, 4 varieties were more resistant, 2 varieties were moderately resistant while 13 varieties were totally non-resistant.

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