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IDENTIFICATION AND VALIDATION OF LEAF RUST RESISTANCE GENES IN SPRING WHEAT (*TRITICUM AESTIVUM* L. EM. THELL) GENOTYPES USING MOLECULAR MARKERS

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ABSTRACT

The study was aimed to identify leaf rust resistance gene(s) (Lr) with the help of molecular markers that condition leaf rust resistance in selected wheat cultivars from India and CIMMYT origin. For this purpose leaf rust resistance (Lr) gene(s) has been determined in 10 wheat genotypes using linked molecular markers. The presence of different resistance genes like Lr13, Lr34, Lr35, Lr37, Lr39, Lr46, Lr47, Lr50, Lr67 and Lr68 were validated through PCR-based molecular markers such as SSR, STS and CAPS. The presence of Lr13, Lr34, Lr35, Lr37, Lr39, Lr46, Lr47, Lr50, Lr67 and Lr68 in the tested genotypes was confirmed by a unique amplification of molecular markers Xgwm630, csLV34-7DS, Sr39/Lr35, VENTRIUP-LN2, Xgwm210, Xgwm259,PS10, Xgwm382, cfd71 and cs7BLNLR. Of 10 genotypes, the presence of Lr13 gene was present in all wheat genotypes except HUW468. Genotypes, HUW234, HUW468 and Chirya3 showed the presence of 150 bp fragment specific to LR34gene. A single fragment of 512 bp (SCS265) specific to Lr35, Lr37, Lr39, Lr47 and Lr67 were not present in all the 10 genotypes. Lr46 gene is present in all the genotypes except HUW234, Fret2/Tukru/Fret2. Lr50 is present in all the genotypes while Lr68 is only present in three genotypes like HUW510, Waxwing2*/Kiritati, Kiritati/Seri/Rayon.

INTRODUCTION

Leaf rust is one of the most harmful diseases of wheat globally. *Puccinia triticina* is a fungal pathogen and causal agent of leaf rust in wheat. According to the UN Food and Agriculture Organization (FAO, 2012), may result in a loss of 10% of the yield (in some years, as much as 30%). In addition to yield reduction, it also affects grain quality. The transfer of leaf rust resistance genes (*Lr*. genes)into wheat cultivars is the finest method of their protection in terms of environmental safety. Identification and validation of leaf rust resistance genes may allow efficient introgression into popular cultivar, thus helping to the release of cultivars that are genetically uniform with improved resistance. Till date, 71 *Lr* genes have been identified on different chromosomes of wheat (McIntosh *et al.*, 2013.According to McIntosh *et al.* (2013), these resistance genes have been found in the *Triticum aestivum* and its related wild species. Correspondingly, wheat cultivars vary in disease resistance depending on which *Lr* genes present. Traditional methods for identifying *Lr* genes are labour expensive and time consuming process. While, in the comparison of traditional methods molecular markers, result may obtain in very short time. At present, individual *Lr* gene(s) can be identified with the use of molecular markers, such as single nucleotide polymorphisms (SNPs), cleaved amplified polymorphic sequences (CAPSs), sequence-tagged sites (STSs), sequence-characterized amplified regions (SCARs) and simple sequence repeats (SSRs) (Helguera *et al.*, 2000; Mateos-Hernandez *et al.*, 2006; Williams *et al.*, 2007, Kandan *et al.*, 2013, Lillemo *et al.*, 2013 and Zala *et al.*, 2014). We were identified several genes from available literature on leaf rust resistance gene(s) *i.e., Lr13, Lr34, Lr35, Lr37, Lr39, Lr45, Lr46, Lr50, Lr67*and *Lr68* in wheat using PCR analysis in the some Indian and CIMMYT originated varieties (Lagudah *et al.*, 2006; Mateos-Hernandez *et al.*, 2006; Lagudah *et al.*, 2009; Herrera-Foessel *et al.*, 2011, 2012 and Lillemo *et al.*, 2013). Some more robust markers have been validated in parental genotypes and used for either incorporation of resistance genes or pyramiding of resistance genes (Slikova *et al.*, 2004; Datta *et al.*, 2006, 2007, 2011). Similar finding have been done by several workers (Urbanovich *et al.*, 2006; Gurjar *et al.*, 2012, 2014). For this, it is necessary to validate molecular markers linked with resistance genes of interest into the wheat genotypes individually as well as in combination that assist marker assisted selection (MAS).

Therefore, paper deals with the objective of, validation of molecular markers linked with leaf rust resistance gene(s) in some Indian and CIMMYT origin wheat genotype and to determine the effect of genes in combinations in each genotype under study.

MATERIALS AND METHODS

Plant material

Most popular Eastern Indian cultivars *i.e.* HUW234, HUW468 and HUW510 and CIMMYT originated wheat genotypes (Waxwing2*/Kiritati, Kiritati/Seri/Rayon, Ning8119, Waxwing2*/ Kukuna, Kauz/ Pastor// PBW343 and Chirya3, Fret2/ Tukru/ Fret2) were served as the object of the study. Cultivars bred in India (Indigenous) were taken from Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University (BHU), Varanasi. However, other breeding genotypes were provided by International Centre for the Improvement of Maize and Wheat (CIMMYT), Mexico through Banaras Hindu University, Varanasi.

DNA extractions and PCR Protocol

DNA isolation for PCR analysis of the parents and backcross progenies for foreground and background selection was carried out from 30 day old seedlings following Saghai-Maroof *et al.* (1984) with minor modification. The extraction buffer used was 1 M Tris pH 8.0, 0.5 M EDTA, 5 M NaCl, 1.7 g/L PVP 40 (0.3mL per mg lyophilized leaf), and the resulting DNA was dissolved in 0.2 mL HPLC water per mg lyophilized leaf. Each 15 μL PCR comprised 40 ng template DNA, 0.2 μM of each primer, 150 μM dNTP, 1x PCR buffer (10 mMTris pH 8.4, 50 mMKCl, 1.8 mM MgCl2, 0.01 mg/mL gelatin) and 0.25 U Taq DNA polymerase. The cycling regime was initiated by a denaturation (94ºC/4 min), followed by 40 cycles of 94ºC / 45s, 65.3ºC / 30 to 65 / 60 s and 72ºC / 60 s and completed with a final extension (72ºC / 10 min). Amplicons were separated via agarose gel (2.5%) electrophoresis and visualized by EtBr staining. The leaf rust resistance (*Lr)* genes were identified with the use of PCR with primers marking individual genes. The primers (linked gene related markers and their nucleotide sequences) were selected on the basis of published literature by several workers that done his work on spring wheat genotypes for leaf rust resistance (*Lr)* genes (Table 1). PCR was performed as recommended by the several scientists, with slight modifications in annealing temperature. The amplification products were separated and visualized by electrophoresis in 1.6% agarose gel in Tris-acetate buffer solution. The gels were documented by staining with ethidium bromide (EtBr). The separated bands were visualized under UV transilluminator and photographed using BIORAD Gel Documentation system. For references in gene size, Gene Ruler 100 bp DNA Ladder Plus (Fermentas) was used as a molecular weight marker.

Selection of genes and their molecular markers for validation

The leaf rust resistance genes like *Lr13, Lr34, Lr35, Lr37, Lr39, Lr46, Lr47, Lr50, Lr67* and *Lr68* were validated through PCR-based molecular markers such as SSR, STS and CAPS. All the genes was visualized by a unique amplification of molecular markers *Xgwm630, csLV34-7DS, Sr39/Lr35, VENTRIUP-LN2, Xgwm210, Xgwm259,PS10, Xgwm382, cfd71* and cs7BLNLR (Table 1).

RESULTS AND DISCUSSION

For this study, we have used 10 cultivars bred in Indian and CIMMYT origin. The origin of *Lr34, Lr46, Lr67*and Lr68 genes are*Triticum aestivum* (Lagudah *et al.,* 2006,William *et al.,* 2003 and Herrera-Foessel *et al.,* 2012). Thus, the designated source of *Lr39 is Ae. Tauschii* and the *Lr37* is designated by *Ae. Ventricosa* Tausch, *Lr35*, *Lr47* and *Lr51* from *Ae. Speltoides* and *T. Ameniacum* (Jakubz.) is a designated source of *Lr50* from (MacKey, 1963). Approximately 38 leaf rust resistance genes have been characterized on the basis of molecular markers (McIntosh *et al.,* 2013).

The type of resistance and monitoring of the virulence spectrum in the pathogen play a crucial role in the management of disease through breeding techniques. Leaf rust resistance genes *Lr13* is most widely distributed worldwide (Mcintosh *et al.,* 1995) and it is usually present in wheat varieties of CIMMYT and providing resistant (Rajaram *et al.,* 1988). Under normal

Table 1: The primer sequences of molecular markers utilized in this studied

S.N.	Name of genes/ qtls	Linked Marker	Temp Marker°C	Nucleotide sequence	Reference
1	*Lr13,*	*Xgwm630*	*30*	5'- GTG CCT GTG CCA TCG TC-3 '5'- CGA AAG TAA CAG CGC AGT GA-3'	Seyfarth *et al.,* 2000
2	*Lr34*	*csLV34-7DS*	*60*	5'- GTT GGT TAA GAC TGG TGA TGG-3 '5'- TGC TTG CTA TTG CTG AAT AGT	Lagudah *et al.,* 2006
3	*Lr35*	*Sr39/Lr35*	*60*	5'- AGA GAG AGT AGA AGA GCT GC -3 '5'- AGA GAG AGA GCA TCC ACC -3'	Gold *et al.,* 1999
4	*Lr37*	*VENTRIUP-LN2*	*65*	5'- AGGGGCTACTGACCAAGGCT-3 '5'- TGCAGCTACAGCAGTATGTACACAAAA-3'	Helguera *et al.,* 2003
5	*Lr39*	*Xgwm210*	*60*	5'- TGCATCAAGAATAGTGTGGAAG-3 '5'- TGAGAGGAAGGCTCACACCT-3'	Raupp *et al.,* 2001
6	*Lr46*	*Xgwm259*	*57.5*	5'- AGG GAA AAG ACA TCT TTT TTT TC-3 '5'- CGA CCG ACT TCG GGT TC-3'	William *et al.,* 2003
7	*Lr47*	*PS10*	*65*	5' - GCT GAT GAC CCT GAC CGG T 3 '5'- TCT TCA TGC CCG GTC GGG T 3'	Helguera *et al.,* 2000
8	*Lr50*	*Xgwm382*	*60*	5'- GTC AGA TAA CGC CGT CCA AT-3 '5'- CTA CGT GCA CCA CCA TTT TG-3'	Brown-Guedira, Singh, 2004
9	*Lr67*	*cfd71*	*60*	5'- CAA TAA GTA GGC CGG GAC AA -3 '5'- TGT GCC AGT TGA GTT TGC TC -3'	Hiebert *et al.,* 2010
10	*Lr68*	*cs7BLNLRR*	*60*	5'- GAA GGA GTG CTT CCT CCA CTG -3 '5'- CTT GGT TCT CCT GTT CTT CCC -3'	Herrera-Foessel *et al.,* 2012

conditions, this resistance gene only acts at the adult plant stage which is characteristic for the so called adult-plant resistance (APR) or partial resistance and as a single gene *Lr13* is no longer effective in most wheat-growing areas (Mcintosh *et al.,* 1995). The location of *Lr13* has been identified by Singh et al. (1991). This study confirms the presence of linked marker (*Xgwm630* with 120bp) of *Lr13* in all genotypes except one with appropriate band size (Table 2, Figure 1a). However, *Lr13* in combination with *Lr34* gives more durable resistance against leaf rust (Ezzahiri and Roelfs, 1989). The *Lr34* is the APR leaf rust resistance gene and it is an important part of the Hexaploid *T. aestivum* L. genome. Dyck (1987) was first described *Lr34*. Cytogenetic analysis was earlier used to locate the *Lr34* gene locate on the arm of chromosome 7DS (Dyck, 1994). The marker (*csLv34*) has been developed and used to characterization *Lr34* gene. There are many markers available like SSR, RFLP and recently RFLP was converted to the sequence tagged site (STS) (Lagudah *et al.,* 2006).*Lr34* has been recently cloned and it codes a protein that resembles an Adenosine Triphosphate (ATP) binding transporter of the pleiotropic drug resistance subfamily (Krattinger *et al.,* 2009). The product size of *Lr34* gene is 150 bp (Figure 1b). This gene has supported resistance to leaf rust in wheat for more than fifty years and is extensively used in breeding programs worldwide (McIntosh *et al.,* 1995; Krattinger *et al.,* 2009). However, this marker was found in three cultivars such as HUW234, HUW468 and Chirya3 (Table 2).

The partial resistance genes *Lr34* and *Lr46* are considered to be durable (Singh *et al.,* 1998; Singh *et al.,* 2001). Co-segregating genes *Lr34* and *Yr18* (leaf rust and stripe rust resistance) have remained effective for more than 50 years and also give to the protection against spot blotch (William *et al.,* 2003, Lillemo *et al.*, 2013). Cultivars with *Lr34* and two to three additional genes have shown a stable environmental response and final disease ratings lower than five present under heavy disease pressure (Singh *et al.,* 2001). Yield losses of around 7-10% for such cultivars are comparable to 6-10% yield loss in cultivars carrying race-specific types of resistance under high disease pressure (Sayre *et al.,* 1998).

The resistance gene *Lr35* was transferred by Karber and Dyck (1990) from chromosome 2S of the diploid wild relative *Triticum speltoides* to chromosome 2B of hexaploid wheat. *Lr35* confers a hypersensitive reaction upon infection by an avirulent race and give adult plant resistance (Kolmer, 1996). But the linked molecular marker is not amplified in all 10 genotypes. At present, no virulent leaf rust race for *Lr35* have been found (Kerber and Dyck, 1990). The *Lr35* gene has not yet been used in modern varieties (McIntosh *et al.,* 1995).

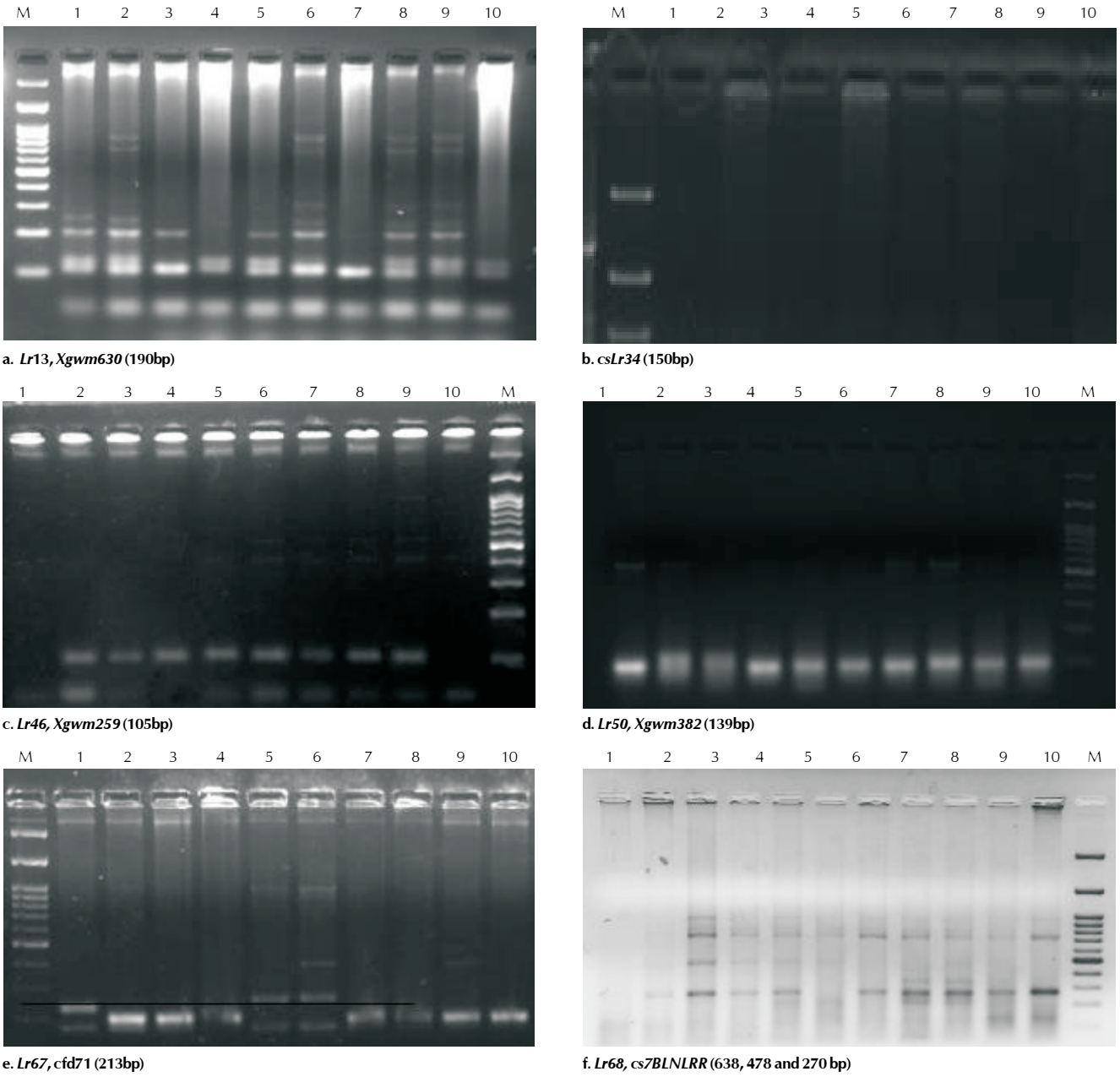
The *Lr37* gene conferring resistance against diseases such as leaf rust, yellow rust, stem rust, are located within a segment of *Triticum ventricosum* (*Ae. ventricosa*) chromosome 2NS translocated to the short arm of bread wheat chromosome 2AS (Ambrozkova *et al.,* 2002). The *Lr37* gene was identified in the *T. aestivum* on chromosome 2AS (Helguera *et al.,* 2003). We do not find out *Lr37* associated fragments in all of the 10 cultivars (Table 2 and Figure 1c). *Lr39* was transferred to wheat germplasm KS86WGRC02 from *Ae. tauschii* accession TA 1675 and was reported to be a unique gene on chromosome arm 2DS (Raupp *et al.,* 1989). *Lr39* exhibits both seedling and adult plant resistance and a SSR marker (*Xgwm210* with 190 bp fragment size) is associate with this gene (Raupp *et al.,* 2001) but *Lr39* is present in HUW234, HUW468, HUW510, Kiritati/Seri/Rayon, Ning8119, Kauz/Pasture//PBW343 and Chirya3, genotypes (Table 2 and Figure 1d).

The *Lr46* is also show rusting gene same as like *Lr34* and give better result along with the combination of *Lr*34 (Singh *et al.,* 1998). The *Lr46* locus, conferring resistance to both leaf rust and stripe rust, is located in the terminal portion of the long arm of wheat chromosome 1BL (Mateos-Hernandez *et al.,* 2006). This gene is positively associated with spot blotch resistance gene *Sb*1 (Lillemo *et al.,* 2013). The chromosome similarly, the yellow-rust resistance gene *Yr*29 is either closely linked to, or is the same as, leaf-rust resistance gene *Lr*46 (William *et al.,* 2003). A location of this gene was determined through an analysis of the substitution genotypes for the chromosomes of the resistant cultivar Pavon 76 backcrossed into the susceptible spring wheat cultivar Lalbahadur (Singh *et al.,* 1998). It was tested in the PCR conditions with the SSR markers (*Xgwm259* with the PCR product size 105bp) and it could not be amplified in the two genotype (HUW234 and Fret2/Tukru/Fret2) out of ten (Table 2 and Figure 1e).

The leaf rust resistance gene *Lr47* was transferred from chromosome 7S of *Triticum speltoides* to chromosome 7A of *Triticum aestivum* in to an interstitial translocation (Helguera *et al.,* 2000). PCR analysis using a molecular marker associated with *Lr47* showed no additional cultivars carrying *Lr47* detected in this investigation. This source of resistance has not been widely exploited in breeding; however some genotypes carried Lr47 gene have been released in California and Argentina recently (Brevis *et al.,* 2008; Bainotti *et al.,* 2009). The *Lr50* is also express at adult plant stage (Brown-Guedira, 2003). A SSR maker *Xgwm382* with the 139 bp (Table 2 and Fig. 1f)

Table2: Distribution of the markers for leaf rust (*Lr*) resistance genes among spring wheat cultivars

Genotype	Origin	*Lr13*	*Lr34*	*Lr35*	*Lr37*	*Lr39*	*Lr46*	*Lr50*	*Lr67*	*Lr68*
HUW234	India	+	+	–	–	–	–	+	–	–
HUW468	India	–	+	–	–	–	+	+	–	–
HUW510	India	+	–	–	–	–	+	+	–	+
Waxwing2*/Kiritati	CIMMYT	+	–	–	–	–	+	+	–	+
Kiritati/Seri/Rayon	CIMMYT	+	–	–	–	–	+	+	–	+
Ning8119	CIMMYT	+	–	–	–	–	+	+	–	–
Waxwing2*/Kukuna	CIMMYT	+	–	–	–	–	+	+	–	–
Kauz/Pasture//PBW343	CIMMYT	+	–	–	–	–	+	+	–	–
Chirya3	CIMMYT	+	+	–	–	–	+	+	–	–
Fret2/Tukru/Fret2	CIMMYT	+	–	–	–	–	–	+	–	–



a. *Lr*13, *Xgwm630* (190bp) **b. *csLr34* (150bp)**

c. *Lr46, Xgwm259* (105bp) **d. *Lr50, Xgwm382* (139bp)**

e. *Lr67*, cfd71 (213bp) **f. *Lr68, cs7BLNLRR* (638, 478 and 270 bp)**

Figure 1: Amplification profile of markers 1a:*Xgwm210 (Lr13);b. csLv34 (Lr34);*c. *Xgwm46 (Xgwm259); d. Xgwm382 (Lr50);* e. *cfd71 (Lr67)* and *f. cs7BLNLRR (Lr68)* associated with leaf rust resistant genes respectively. Where, M: 100-bp ladder (Fermentos). 1 = HUW234, 2 = HUW468, 3= HUW510, 4 = Waxwing2*/Kiritati, 5= Kiritati/ Seri/ Rayon, 6= Ning8119, 7= Waxwing 2*/Kukuna, 8= Kauz/Pastor// PBW343, 9 = Chirya3 and 10 = Fret2*/Tukru/Fret2*

PCR product size shows a close relationship with *Lr50* (Brown-Guedira, 2003). In our study, *Lr50* is present in all the 10 genotypes.

According to Hiebert *et al.,* 2010**,** effective APR gene for leaf rust (*Lr67*) and stripe rust located in the centromeric region of chromosome 4DL and however *Lr67* phenotypically resembled *Lr34*, the degree of resistance conferred by *Lr67* was less than that conferred by *Lr34* (Herrera-Foessel *et al.,* 2011). Furthermore, the translocation present in RL6077 did not involve chromosome 7D or *Lr34* (Herrera-Foessel *et al.,* 2011). *Lr67* and any associated genetic material present in the introgression from PI250413 had no apparent deleterious effect on agronomic performance and quality traits; thus, *Lr67* is a valuable genetic resource and also suitable for use in wheat breeding programs (Herrera-Foessel *et al.,* 2011). In this study, *Xgwm165* marker linked with *Lr67* gene have not given recommended fragment size (198bp) in any of ten genotypes, in this case amplification is also done but the fragment size of closely linked marker was not exact as appropriate recommended size (Table 2 and Figure 1g). The

molecular marker of *Lr68* gene (cs7BLNLRR with Polymorphic fragments of 738, 478 and 270 bp were obtained)is developed fromArula1 and Arula2 (CIMMYT GID 1847450 and 1847422) RILs selected from the Avocet-YrA/Parula(William *et al.,* 2007). In this study, we have found this marker in all genotypes except HUW 234 and HUW468 (Table 2 and Figure 1h).

According to Herrera-Foessel *et al.* (2012), RILs had positive alleles for markers linked to the APR QTL on 7BL but lacked positive alleles for markers linked to *Lr46/Yr29* on 1BL (*Xgwm259*) and *Lr34/Yr18* on 7DS (*Xgwm295/Xgwm130*). Thus, the use of molecular markers allowed us to detect a number of *Lr genes* in the wheat genome. We identified polymorphism genes *Lr13, Lr34, Lr46, Lr50* and *Lr68* in the different cultivars studied with the use of these markers. The marker for *Lr13* gene proved to be absent only in HUW468 out of ten genotypes. The results reported here show that the genomes of wheat HUW468 cultivars differ from one another in the spectrum of the leaf rust resistance genes.

The Indian cultivars, HUW234 and Chirya3 carries*Lr13, Lr34,* and *Lr50* genes but both are highly susceptible to leaf rust and shows 100S susceptibility under natural field condition at IARI, regional station Wellington, Tamilnadu (India) with a mixture of different races of leaf rust pathogen. This study indicates that the gene combination present in this variety is not effective. HUW468 carries *Lr34*, *Lr46* and *Lr50* genes and gives 80S susceptibility, according to this study, in the combination of all three genes in this variety have some resistance compare to HUW234. HUW510, Waxwing2*/Kiritati, Kiritati/Seri/Rayon and Kauz/Pastore//PBW343 have been carried four gene in the combinations*Lr13, Lr46, Lr50* and *Lr68* but the resistance levels was different. HUW510 and Kauz/Pastore//PBW343 shows near immunes under the field conditions while Waxwing2*/Kiritati and Kiritati/Seri/Rayon has been given 10S and 60S leaf rust susceptibility respectively. Ning8119 and Waxwing2*/Kukuna have three gene in combination *i.e., Lr13*, *Lr46* and *Lr50* with 40S rust susceptibility. Fret2/Tukru/Fret2 is also CIMMYT originated Variety having two genes*Lr13* and *Lr50*with 10S susceptibility reaction. Also the resistance of Pavon 76 is believed to be durable and it has been ascribed two genes, one of which has been identified and named *Lr46*. Similarly, Urbanovich *et al.* (2006) was validated leaf rust resistance genes like *Lr1, Lr9, Lr10, Lr19, Lr20* and *Lr26* in wheat genotypes through PCR based molecular markers. In earlier studies, The leaf rust resistance genes (*Lr13, Lr23, Lr24, Lr26* and *Lr34*) were also validated in wheat genotypes through phenotyping Gurjar *et al.* (2012). Recently, Gurjar *et al.* (2014)validated leaf rust resistance genes (*Lr1, Lr10, Lr19, Lr26* and *Lr34*) with the help of molecular markers. So we can easily validate leaf rust resistance genes with the help of PCR-based molecular marker in crop plants. In our presentstudy, molecular markers were utilized to validate Lr genes in different 10 wheat genotype. Validated markers can be easily utilized in marker assisted selection (MAS) for the early generation of selection of desirable plants which would enhance the resistance of the genotypes/lines and provide durable resistance.

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REFERENCES

Ambrozková, M., Dedryver, F., Dumalasová, V., Hanzalová, A. and Bartoš, P. 2002. Determination of the cluster of wheat rust resistance genes Yr17, Lr37 and Sr38 by a molecular marker. *Plant Protection Science*. 38, 41.

Bainotti, C. T., fraschina, J., Salines, J., Nisi, J., Dubcovsky, J., Lewis, S., Bullrich, L., Vanzetti, L. S., Cuniberti, M., Campos, P., Formica, M. B., Masiero, B., Alberione, E. and Helguera, M. 2009. Registration of 'BIOINTA 2004' wheat. *J. Plant Registration*. **3:** 165-169.

Brevis, J. C., Chicaiza, O., Khan, I. A., Jackson, L., Morris, C. F. and Dubcovsky, J. 2008. Agronomic and quality evaluation of common wheat near-isogenic genotypes carrying the leaf rust resistance gene *Lr47*. *Crop Science*. **48:** 1441-1451.

Brown-Guedira, G. L., Singh, S. and Fritz, A. K. 2003. Performance and mapping of leaf rust resistance transferred to wheat from *Triticum timopheevii* subsp. *Armeniacum*. *Phytopathology*. **93:** 784-789.

Datta, D., Prashar, M. and Bhardwaj, S. C. 2006. Pyramiding of leaf rust resistance genes Lr9 and Lr24 through molecular marker assisted selection in wheat (*Triticum aestivum*). *Indian J. Genetics*. **66:** 332-334.

Datta, D., Prashar, M. and Bhardwaj, S. C. 2007. Validationand incorporation of leaf rust resistance genes Lr9, Lr19,Lr24 and Lr26 through molecular markers in wheat (*Triticum aestivum*). *Indian J. Genetics*. **67:** 7-11.

Datta, D., Prashar, M., Bhardwaj, S. C. and Singh, S. 2011. Alternate schemes for combining leaf rust resistance genesthrough molecular marker. *Indian J. Agriculture Sciences*. **81:** 602-605.

Dyck, P. L. 1977. Genetics of leaf rust reaction in three introductions of common wheat. *Canadian J. Genetics and Cytology*. **19:** 711-716.

Dyck, P. L. 1987. The association of a gene for leaf rust resistance with the chromosome 7D suppressor of stem rust resistance in common wheat. Genome. **29:** 467-469.

Dyck, P. L. 1994. The transfer of leaf rust resistance from *Triticum turgidum* ssp. Dicoccoides to hexaploid wheat. *Canadian J.Plant Science*. **74:** 671-673.

Ezzahiri, B. and Roelfs, A. P. 1989. Inheritance and expression of adult plant resistance to leaf rust in era wheat. *Plant Disease*. **73:** 549-551.

FAO 2012. FAOSTAT agriculture data. Agricultural production 2009. Available at http://faostat.fao.org (accessed 22 April 2012). *FAO, Rome, Italy*.

Gurjar, M. S., Aggarwal, Rashmi and Prashar, M. 2012. Phenotyping of Indian wheat (Triticum aestivum) genotypes for identification of rust resistance genes. Indian *Phytopathology*. **65:** 345-348.

Gurjar, M. S., Aggarwal, R.,Banerjee, S., Bag, T. K. and Prashar, M. 2014. Identification and validation of leaf rust resistance genes in Indian wheat genotypes using molecular markers. *Indian Phytopathology*. **67:** 358-364.

Helguera, M., Khan, I. A. and Dubcovsky, J. 2000. Development of PCR markers for wheat leaf rust resistance gene *Lr47*. *Theoretical and Applied Genetics*. **100:** 1137-1143.

Helguera, M., Khan, I. A., Kolmer, J., Lijavetzki, D., Zhong-Qi, L. and 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.

Herrera-Foessel, S. A., Lagudah, E. S., Huerta-Espino, J., Hayden, M. J., Bariana, H. S., Singh, D. and Singh, R. P. 2011. New slow-rusting leaf rust and stripe rust resistance genes *Lr67* and *Yr46* in wheat are pleiotropic or closely linked. *Theoretical and Applied Genetics*.**122:** 239-249.

Herrera-Foessel, S. A., Singh, R. P., Huerta-Espino, J., Rosewarne, G. M., Periyannan, S. K., Viccar, L., Calvo-Salazar, V., Lan, C. and Lagudah, E. S. 2012. Lr68: a new gene conferring slow rusting resistance to leaf rust in wheat.*Theoretical and Applied Genetics*. **124:** 1475-1486. DOI 10.1007/s00122-012-1802-1.

Hiebert, C, W., Thomas, J. B., McCallum, B. D., Humphreys, G. D., DePauw, R. M., Hayden, M. J., Mago, R., Schnipenkoetter, W. and Hayden, M. 2010. An introgression on wheat chromosome 4DL in RL6077 (Thatcher*6/PI 250413) confers adult plant resistance to stripe rust and leaf rust (Lr67). *Theoretical and Applied Genetics*.**121:** 1083-1091.

Hiebert, C. W., Thomas, J. B., Somers, D. J., Mccallum, B. D. and Fox, S. L. 2007. Microsatellite mapping of adult-plant leaf rust resistance gene *Lr22a* in wheat. *Theoretical and Applied Genetics*. **115:** 877-884.

Kandan, A., Akhtar, J., Singh, B., Dixit, D., Chand, D., Agarwal, P. C., Roy, A. and Rajkumar, S. 2013. Population genetic diversity analysis of *Bipolarisoryzae* fungi infecting *Oryzasativa* in India using URP markers. *The Ecoscan*. **7(3&4):** 123-128.

Kerber, E. R. and Dyck, P. L. 1990. Transfer to hexaploid wheat of linked genes for adult-plant leaf rust and seedling stem rust resistance from an amphiploid of *Aegilopsspeltoides* x *Triticum monococcum*. *Genome*. **33:** 530-537.

Kolmer, J. A., Jin, Y. and Long, D. L. 2007. Wheat leaf and stem rust in the United States. *Crop and Pasture Science*. **58(6):** 631-638.

Kolmer, J. A. 1996. Genetics of resistance to wheat leaf rust. *Annual Review of Phytopathology*. **34(1):** 435-455.

Krattinger, S. G., Lagudah, E. S., Spielmeyer, W., Singh, R. P., Huerta-Espino, J., McFadden, H., Bossolini, E., Selter, L. L. and Keller, B. 2009. A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. *Science*. **323:** 1360-1363.

Lagudah, E. S., Krattinger, S. G., Herrera-Foessel, S., Singh, R. P., Huerta-Espino, J., Spielmeyer, W., Brown-Guedira, G., Selter, L. L. and Keller, B. 2009. Gene-specific markers for the wheat gene Lr34/Yr18/Pm38 which confers resistance to multiple fungal pathogens. *Theoretical and Applied Genetics*. **119:** 889-898.

Lagudah, E. S., Mcfadden, H., Singh, R. P., Huerta-Espino, J., Bariana, H. S. and Spielmeyer, W. 2006. Molecular genetic characterisation of the *Lr34/Yr18* slow rusting resistance gene region in wheat. *Theoretical and Applied Genetics*.**114:** 21-30.

Lillemo, M., Joshi, A. K., Prasad, R., Chand, R. and Singh, R. P. 2013. QTL for spot blotch resistance in bread wheat line Saar co-locate to the biotrophic disease resistance loci Lr34 and Lr46. *Theoretical and Applied Genetics*. **126:** 711-719.

MacKey, J. 1963. Autogamous plant breeding based on already high bred material. In: Akerberg, E. and Hagberg, E. (Eds.). *Recent Plant Breeding Research Svalöf* 1946-1961. Uppsala. pp. 73-88.

Mateos-Hernandez, M., Singh, R., Hulbert, S. H., Bowden, R. L., Huerta-Espino, J., Gill, B. S. and Brown-Guedira, G. 2006. Targeted mapping of ESTs linked to the adult plant resistance gene Lr46 in wheat using synteny with rice. *Functional Integrative Genomics*. **6:** 122-131.

McIntosh, R. A., Dubcovsky, J., Rogers, W. J., Morris, C. F., Appels, R., Xia, X. C. 2013. V. Catalogue of gene symbols for wheat: 2012 Supplement Available online: www.shigen.nig.ac.jp/wheat/komugi/genes (accessed 7 June 2013).

Mcintosh, R. A., Wellings, C. R. and Park, R. F. 1995.*Wheat rusts: An atlas of resistance genes*, Melbourne, CSIRO, 1995, 208 p. ISBN 0-643-05428-6.

Rajaram, S., Singh, R. P. and Torres, E. 1988. Current CIMMYT approaches in breeding wheat for rust resistance. In: Simmonds NW, Rajaram S (eds) Breeding strategies forresistance to the rust of wheat. *CIMMYT, Mexico, D.F*. pp.101-118.

Raupp, W. J., Gill, B. S., Browder, L. E. and Wilson, D. 1989. Chromosomal location of two leaf rust resistance genes transferred from *Aegilopssquarrosa* to hexaploid wheat. AgronAbstr, p 96.**Raupp, Singh, G. L. W. J., Brown-Guedira, S. and Gill, B. S. 2001.** Cytogenetic and molecular mapping of the leaf rust resistance gene Lr39 in wheat. *Theoretical and Applied Genetics*. doi: 10.1007/s001220051652. **102:** 347-352.

Saghai-Maroof, M. A., Solima, K. M., Jorgenson, R. A. and Allard, R. W. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. *ProcNatlAcadSci USA*. **81:** 8014-8018.

Sayre, K. D., Singh, R. P., Huerta-Espino, J. and Rajaram, S. 1998. Genetic progress in reducing losses to leaf rust in CIMMYT-derived Mexican spring wheat cultivars. *Crop Science*. **38:** 654-659.

Slikova, R., Gregova, E., Bartos, P., hanzalova, A., Hudcovicova, M. and Kraic, J. 2004. Development of wheat genotypes possessing a combination of leaf rust resistance genes Lr19 and Lr24. *Plant Soil Environment*. **50:** 434-438.

Singh, D. P., Sharma, A. K. and Grewal, A. S. 2001. Loose smut resistant lines in wheat with combined resistance to Karnal bunt, rusts, powdery mildew and leaf blight. *Wheat Information Service*. **92:** 27-29.

Singh, D. P., Sharma, A. K., Kumar, J., Goel, L. B., Karwasra, S. S., Beniwal, M. S. and Grewal, A. S. 2002. Resistant lines to loose smut (*Ustilagosegetum* var. *tritici*) in wheats (*Triticum aestivum*, *T. durum*, *T. dicoccum*) and triticale. *Indian J. Agriculture Sciences*. **72:** 308-310.

Singh, R. P., Mujeeb-Kazi, A. and Huerta-Espino, J. 1998. Lr46: a gene conferring slow-rusting resistance to leaf rust in wheat. *Phytopathology*. **88:** 890-894.

Singh, R. V., Gupta, P. C. and Singh, Y. 1991. Production potential of under limited water supply condition. *Indian J. Agriculture Sciences*. **61(11):** 835-837.

Urbanovich, O. Y., Malyshev, S. V., Dolmatovich, T. V. and Kartel, N. A. 2006. Identification of leaf rust resistance genes in wheat (*Triticum aestivum* L.) cultivars using molecular markers. *Russian J. Genetics*. **42:** 546-554.

William, H. M., Trethowan, R. and Crosby-Gal Van, E. M. 2007. Wheat breeding assisted by markers: CIMMYT's. Experience. *Euphytica*.**157:** 307-319.

Williams, K. J., Lewis, J. G., Bogacki, P., Pallotta, M., Willsmore, K. L., Kuchel, H. and Wallwork, H. 2003. Mapping of a QTL contributing to cereal cyst nematode tolerance and resistance in wheat. *Australian J. Agriculture Research*. **54:** 731-737.

Zala, H., Bosamia, T., Kulkarni, K. and Shukla, Y. 2014. Assessment of molecular diversity in wheat (*Triticum aestivum* L. and *Triticum durum* L.) genotypes cultivated in semi-arid region of Gujarat. *The Bioscan*. **9(2):** 831-837.