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GENETIC CHARACTERIZATION OF NOVEL *LR* GENE STACK IN SPRING WHEAT VARIETY CHAKWAL86 AND ITS EFFECTIVENESS AGAINST LEAF RUST IN RAIN FED AREAS OF PAKISTAN

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Abstract

Disease resistance remains a prime focus in crop improvement. Based on the field observations and marker assisted screening, we report the gene stack *Lr10*, *Lr17a* and *Lr27+31* as a resistant combination for spring wheat cultivated in rain fed areas. To demonstrate this, a rain fed leaf rust resistant variety *'Chakwal86'*, has been genetically characterized following its cross with a susceptible variety '*Inqilab91'*. The parents, NILs and F_2 population studied in the greenhouse through inoculation and in the field under natural conditions revealed a 3:1 resistance to susceptible ratio, while the $F_{2:3}$ populations revealed a 1:2:1 ratio suggesting the dominant mode of resistance. The PCR-based markers developed to characterize individuals by selecting SSR based markers specific to *Lr10*; *Lr17a* and *Lr27*, revealed all three genes stacked in *Chakwal86*, while *Lr10* and *Lr27+31* in *Inqilab91*. The individual lines carrying gene combinations as found in *Chakwal86* remained resistant in the field while all those not carrying *Lr17a* in the stack were susceptible. Hence the gene stack *Lr10*, *Lr17a* and *Lr27+31* has been found an effective resistant combination for spring wheat in rain fed areas.

Introduction

Unlike stripe and stem rusts, wheat leaf rust has a much extensive distribution (Wiese, 1987; Gupta *et al*., 2006) and global occurrence (Laudson, 1973) while its severity depends on the geographic location and prevailing environmental conditions. An effective and sustainable method to combat such a disease is through breeding resistant varieties. However, the dynamic nature of pathogen (*Puccinia* spp.) and the ever increasing global demand for wheat has made this a continuous effort. Deploying resistant genes in cultivars has been an effective means to control leaf rust (Kolmer, 2003). Taken this further, the concept of gene pyramiding has gained much importance lately. Hitherto, more than sixty leaf rust resistance genes have been catalogued (McIntosh *et al.*, 2008) catering both race-specific and race non-specific resistance. However the race specific genes lose effectiveness quickly, often within few years of introduction (Kilpatrick, 1975). Thus, pyramiding or combining effective race-specific genes in a single cultivar prolongs effectiveness. As in doing so, the pathogen is forced to undergo a sequence of mutations corresponding to the genes stacked thus reducing the probability of rapid breakdown in resistance (McDonald & Linde, 2002).

Once effective, several genes deployed individually have lost their effectiveness, for example *Lr10*, *Lr27*, *Lr31* etc. The latter two, known as complementary genes in combination *Lr27+31*, commonly found in wheat varieties in Pakistan and elsewhere, have lost their effectiveness. However, certain genes such as *Lr17a*, a race specific seedling resistance gene (Dyck & Samborski, 1970), have been an important component of resistance to a number of wheat cultivars in Australia, South Asia (India, Pakistan), Europe (United Kingdom) (McIntosh *et al*., 1995) and United States (Kolmer *et al.*, 2005). The gene has been found potentially effective if deployed in combination with other resistance genes (Rattu, 2006), this requires stabilizing the stacked genes and assessing their composition through breeding procedures.

Development of elite cultivars with potential resistance against prevailing pathogens (especially rusts), is possible through: a) probing effective resistant genes in the primary gene pool. The primary gene pool would mean the crosses made between local varieties or the genetic stock for combining resistance without losing promising agronomic traits. Theoretically, this provides an opportunity for gene stacking in novel combinations. One such effective gene stack probed in the present study is *Lr10*, *Lr17a* and *Lr27+31* found in the variety *Chakwal86*. The effectiveness of this novel stack has been assessed in a mapping population developed by crossing '*Chakwal86'* with '*Inqilab91'*, the latter a high yielding susceptible cultivar, most likely carrying *Lr10* and *Lr27+31* (Mirza *et al*., 2000; Rattu, 2006). The purpose of the present study was: to screen the presence of *Lr10*, *Lr27* or *Lr31* and *Lr17a*; to identify the effective gene combination present in *Chakwal86*.

Materials and Methods

Plant material: Based on our previous gene postulations, the variety '*Chakwal86'* (parent 1), was characterized as resistant at five different rain-fed locations across the country. Based on this information it was crossed with '*Inqilab91'* a high yielding susceptible cultivar. Ninety two individual plants from the resulting F_2 progeny were evaluated for leaf rust resistance at seedling and adult plant stages. The wheat seedlings at 2-4 leaf stages were inoculated with uridiniospores of two virulent isolates of *Puccinia triticina* collected from the susceptible parent previously. The observations were carried out 10-12 days after inoculation. The leaf rust infection type was recorded according to the standard 0-4 scale developed by Stakman *et al*., (1962) and modified by Roelfs *et al*., (1992). The adult plant tests were carried out in the field during February and March in randomized complete block design along with the susceptible check variety Morocco. The goodness of fit was assessed through chi-square analysis in the F_2 progeny, both at seedling and adult plant stages.

DNA extraction and screening of *Lr10*, *Lr17a*, *Lr27* and *Lr31*: Genomic DNA was extracted from leaves of the parents and each of the F_2 individual plants following an earlier described protocol (Liu *et al*., 2006). For screening *Lr10*, a previously described STS marker *Lrk10* (Shashermayr *et al*., 1997) linked to *Lr10* was used to screen parents, the resistant and susceptible bulks comprised of 5 resistant and 5 susceptible F_2 plants respectively. The PCR products were resolved identifying 310bp amplified product linked to *Lr10*. For screening *Lr17a*, we probed fifteen SSR markers specific to the wheat chromosome 2AS (Roder *et al*., 1998, Somers *et al*., 2004, Song *et al*., 2005) in the parents for polymorphism. Only polymorphic markers were assayed in the F_2 progeny. Polymerase chain reaction was performed in 20µl reaction volumes, each containing 30-45ng genomic DNA, 10x reaction buffer, 2.5mM $MgCl_2$, 2.5mM dNTPs and 1U TAQ polymerase. The reaction was carried out in a T-personal thermal cycler (Biometra USA) with an initial denaturing at 94°C for 3 min, followed by 35 cycles of 94°C for 1min, annealing as described earlier (Roder *et al*., 1998), 72°C for 1min, and a final extension at 72°C for 10mins.

Electrophoresis and data analysis: PCR products were resolved on 8% polyacrylamide gel containing 3.2% (v/v) formamide (Litt *et al*., 1993). The gels were stained with ethidium bromide and visualized in a gel documentation system (Bio Rad). The Linkage analysis was performed using Mapmaker v. 3.0 (Lander *et al*., 1987) at LOD 3.0.

Results

The rust resistance in parent 1 and parent 2 were assessed against two virulent isolates *QAUPr-05* and *QAUPr-16* obtained locally from the susceptible parent in the previous years. At seedling stage the parent 1 showed resistance with IT ;0 and parent 2 showed a susceptible reaction against these isolates with IT 4. At adult plant stage, a similar response was revealed with parent 1 showing rust severity '5R' while the parent 2 showed a susceptible reaction '80S'. These parents were crossed producing F_1 hybrids; all were resistant at seedling and adult plant stages. The assessment of ninety two F_2 individuals obtained by selfing the F_1 revealed 65 (70%) as resistant while the remaining 25 (27%) as susceptible (Fig. 1). These plants under field conditions showed a similar segregating ratio (Fig. 2) that fitted well with 3:1 resistant to susceptible ratio ($\chi^2_{(3:1)} = 0.232$, df $\geq$ 0.05) as described in Table 1. These observations suggested the presence of a dominant gene conferring resistance against these isolates and under natural field conditions. Based on the pedigree information, gene postulations from previous years and rust reaction data, three genes *Lr10*, *Lr17a* and *Lr27+31* were anticipated in parent 1 and two genes *Lr10* and *Lr27+31* in parent 2.

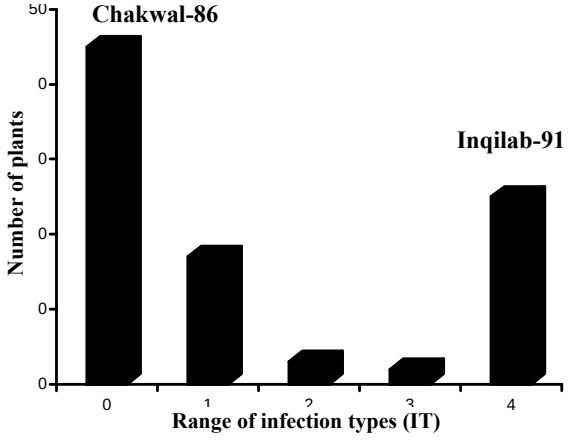

Fig. 1. Frequency distribution of F_2 individual plants against leaf rust at seedling stage.

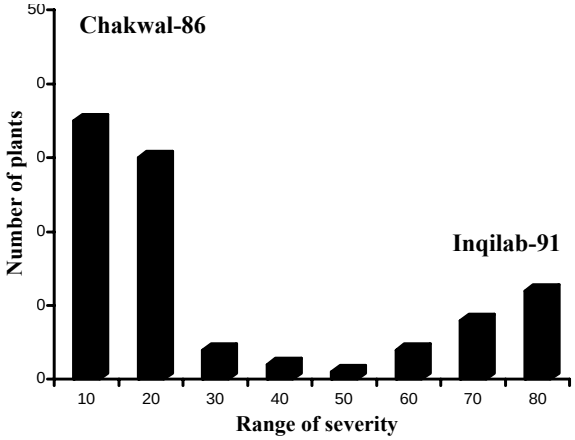

Fig. 2. Frequency distribution of F_2 individual plants against leaf rust at adult plant stage.

Table 1. The goodness of fit assessed by the chi-square analysis for the F_2 progeny at seedling and adult plant stages.

Progeny stages	Resistant (IT 0 or 5R)	Susceptible (IT 4 or 70S)	Total	Ratio	$\chi^2_{(3:1)}$ P = 5.99
Seedling stage	68	24	92	3:1	0.232
Adult plant stage	63	23	87	3:1	0.215

We screened the presence of *Lr10* in parents, the resistant and susceptible bulks and the individual plants from the F_2 segregating population. The marker assisted screening for *Lr10* revealed a monomorphic product at 282bp indicating a uniform genetic background with no recombination at this locus either in parents or in the F_2 progeny. A very similar behavior was anticipated for the complementary gene combination *Lr27+31*. It could be proposed that the parents and the progeny would only differ at the locus *Lr17a*, which has been probed further.

Among fifteen microsatellite markers specific to chromosome 2AS tested on parents, only six (40%) revealed polymorphism with respective allelic variation (Table 2). These polymorphic markers were used to screen the F_2 population for close marker-trait (*Lr17a*) association. Among these, *Xgwm512* explained only

4.62% phenotypic variation and revealed the maximum distance (11.34 cM) from the gene *Lr17a*. On the contrary, two markers *Xgwm636* and *Xwmc667* were found flanking to the gene *Lr17a*. However *Xgwm636* explained the maximum phenotypic variability i.e. 95.8% with an observed proximity of 2.1cM, close enough to be co-segregated. Further details on these markers have been summarized in Table 3. Hence based on the data, the marker *Xgwm*636 was found the most appropriate flanking marker to screen all F_2 progeny. Furthermore, a linkage map was constructed for the chromosome 2AS along with the marker positions (Fig. 3).

Table 2. Allelic variations recorded in parents and the F_2 progeny for six polymorphic microsatellite loci. Type 1 refers to Parent 1 (*Chakwal86*) while type 2 refers to Parent 2 (*Inqilab91*).

Marker	*Chakwal86*	*Inqilab91*	Type 1	Type 2	Type 1 & 2
Xwmc382	215	210	55	19	18
Xwmc407	145	165	50	23	19
Xgwm614	125	110	57	21	14
Xgwm636	95	110	58	24	10
Xwmc667	131	125	57	25	10
Xgwm512	180	170	52	22	18

Table 3. A summary of associated marker mapping data revealed from the F_2 population.

Marker	Position (cM)	Interval (cM)	Distance from *Lr17a*	LR	LOD	Phenotypic Variation (%)
Xwmc382	0	0	11.34	14	3.04	4.62
Xwmc407	2.1	2.1	9.24	96.2	20.9	33.86
Xgwm614	6.4	4.3	4.94	148.5	23.2	44.56
Xgwm636	9.3	2.9	2.1	267.6	58.1	95.7
Xwmc667	12.9	3.6	1.56	223.8	34.9	61.33
Xgwm512	20.7	7.8	9.36	21.6	4.7	9.56

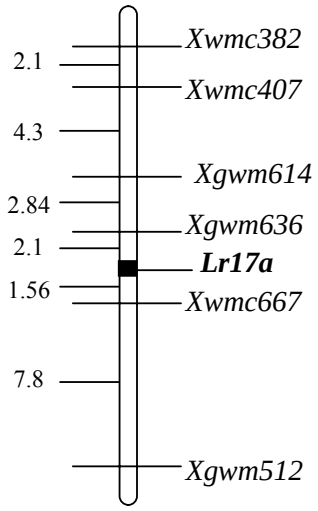

Fig. 3. Linkage map of chromosome 2AS, with relative marker positions and intervals between the markers indicated as numbers (in cM) on the left.

Discussion

Molecular methods find much wider application, for example in wild species (Yousaf *et al*., 2009) as well as in other economically important crops as in rice (Masood *et al*., 2005), cotton (Mumtaz *et al*., 2010) and sugarcane (Mumtaz *et al*., 2011). For the present purpose our focus remains the pyramiding of resistant genes in wheat. Gene pyramiding is a breeding strategy where two or more genes may be combined in one genotype. The complex wheat genome organization probably limits the success in gene pyramiding. Nevertheless a number of studies reported the success in combining genes, for example *Lr13* and *Lr16* (Samborski & Dyck, 1982); *Lr9* and *Lr24* (Long *et al*., 1994); *Lr13*, *Lr34* and *Lr37* (Kloppers & Pretorious, 1997) etc. with a demonstrated control for leaf rust. Here we report the success in pyramiding the genes *Lr10*, *Lr17a* and *Lr27+31*, a resistance conferring combination, as demonstrated in the Pakistan cultivar '*Chakwal86*'. The triple gene stack is a result of the efforts of breeders in our national program through conventional breeding. To take this further, we crossed the resulting variety '*Chakwal86'* with '*Inqilab91'*, the latter having *Lr10* and the gene combination *Lr27+31*, providing a similar genetic background (except *Lr17a*) yet with superior agronomic (yield) potential. The resulting F_2 population was evaluated under natural conditions as well as through marker assisted screening in the laboratory. The seedling and field-based adult plant response proved the effectiveness of the triple gene combination while the F_2 progeny was further screened to map *Lr17a* in the population.

Lr17a is a hypersensitive leaf rust resistance gene mapped on chromosome 2AS of wheat. Other leaf rust resistance genes mapped on this chromosome included *Lr37* linked to *Sr38* and *Yr17* (Bariana & McIntosh, 1993), *Lr45* (McIntosh *et al*., 1995) and *Lr49* (Siani *et al*., 2002). In our study *Lr17a* was mapped to chromosome 2AS, a region corresponding to a cluster of closely linked SSRs spanning out 20.7 cM region in wheat microsatellite consensus map of Somers *et al.*, (2004). The microsatellite markers *Xgwm636* and *Xwmc-667* were found flanking to the leaf rust resistance gene *Lr17a* as reported previously (Roder *et al*., 1998; Bremenkamp-Barrett *et al*., 2008). However, the allele *Xgwm636* revealing a 95bp band was found more closely linked to *Lr17a* in our population. This conclusion was based on its

agreement with the infection type (IT) data assessed in the F_2 population. Hence the F_2 population was screened on the basis *Xgwm636*$_{95bp}$. Our experience from this and other related studies endorsed the significance of molecular markers linked to the desired *Lr* gene(s). Such linked markers are helpful in selecting individuals with pyramided or introgressed genes in an early F_2 and $F_{2:3}$ segregating populations.

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