RESEARCH ARTICLE

Genetics and mapping of a new leaf rust resistance gene in *Triticum aestivum* L. × *Triticum timopheevii* Zhuk. derivative 'Selection G12'

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Abstract

A *Triticum timopheevii*-derived bread wheat line, Selection G12, was screened with 40 pathotypes of leaf rust pathogen, *Puccinia triticina* at seedling stage and with two most commonly prevalent pathotypes 77-5 and 104-2 at adult plant stage. Selection G12 showed resistance at both seedling and adult plant stages. Genetic analysis in F_1, F_2 and $F_{2:3}$ families at the seedling stage revealed that leaf rust resistance in Selection G12 is conditioned by a single incompletely dominant gene. The leaf rust resistance gene was mapped to chromosome 3BL with SSR markers *Xgwm114* and *Xgwm547* flanking the gene at a distance of 28.3 cM and 6 cM, respectively. Based on the nature of resistance and chromosomal location, it is inferred that Selection G12 carries a new gene for leaf rust resistance, tentatively named as *LrSelG12*.

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Introduction

Leaf rust caused by the fungus *Puccinia triticina*, is one of the most wide spread diseases of bread wheat (*Triticum aestivum* L.). Although, rust diseases have chemical control, genetic resistance in the host is the most economical and environment-friendly method. Wild relatives of wheat are reservoir of useful genes, including genes for rust resistance. To date, 74 leaf rust resistance genes have been designated and about half of them have originated from various closely or distantly related species of wheat (McIntosh *et al*. 2013; Naik *et al*. 2015). Among closely related species, tetraploid wheats are important sources of resistance to diseases. Tetraploid wheat includes not only the emmer wheats (*T. turgidum* L., AABB), but also the timopheevi wheats (*T. timopheevii* Zhuk., A^tA^tGG) besides other distantly related species. *T. timopheevii* is a cultivated form, and its wild form is *T. araraticum.* Resistance to a number of diseases and insect pests have been reported in *T. timopheevii* and *T. araraticum* (Brown-Guedira *et al.* 1996, 1997; Friebe *et al.* 1996; McIntosh and Gyarfas 1971; Sharma and Gill 1983; Tomar *et al.* 1988).

Four leaf rust resistance genes (*Lr18*, *Lr50*, *LrTt1* and *LrTt2*) from *T. timopheevii* have been described until now (McIntosh *et al*. 2012, 2013; http://www.shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp). Leaf rust resistance gene *Lr18* (Dyck and Samborski 1968) on chromosome 5BL is associated with a *T. timopheevii*-derived telomeric band (Dyck and Samborski 1968; McIntosh 1983). *Lr50* was introgressed from *T. timopheevii* ssp. *armeniacum* and mapped on the chromosome 2BL with flanking SSR markers *Xgwm382* and *Xgdm87* (Brown-Guedira *et al.* 2003). Two other leaf rust resistance loci *LrTt1* (Leonova *et al.* 2004) and *LrTt2* from *T. timopheevii* have also been documented (Leonova *et al.* 2010). Recently, Laikova *et al.* (2013) have developed synthetic hexaploids by crossing *T. timopheevii* and *T. tauschii*, which showed resistance to leaf rust, stem rust, powdery mildew and loose smut.

At IARI, New Delhi, *T. timopheevii* accessions have been used to develop rust resistant bread wheat lines. One such line, PTD 12 (Pusa timopheevii-derivative 12), was derived from a cross between bread wheat line CM 108-31 (a hybrid-none-necrotic semi-dwarf mutant of C306) and *Triticum timopheevii* (Guha *et al.* 1996). PTD 12 was later renamed as Selection G12, where G represents the G genome of *Triticum timopheevii*. Selection G12 was screened for leaf rust resistance under epiphytotic conditions at adult plant stage for 5 years and showed a high degree of resistance with maximum rust score of 5MR. The present study was undertaken to determine the effectiveness of resistance in Selection G12

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against an array of *Puccinia triticina* pathotypes at seedling stage and to understand the mode of inheritance of leaf rust resistance. An effort was also made to determine the chromosomal location of the leaf rust resistance gene by molecular mapping with microsatellite markers.

Materials and methods

Host material and pathogen

Plant material included a susceptible cultivar Agra Local (AL) and leaf rust resistant line, Selection G12. A cross was made between Selection G12 and AL. Spikes of F_1 plants were selfed to produce F_2 generation for genetic studies and molecular mapping. Seeds were harvested from each F_2 plants to make $F_{2:3}$ families. Urediniospores of *Puccinia triticina* pathotypes were obtained from Indian Institute of Wheat and Barley Research, Regional Station, Flowerdale, Shimla. The susceptible check AL was used to get continuous harvest of inoculums under the controlled condition as described by Joshi *et al.* (1988).

Testing for leaf rust resistance

The parents (Selection G12 and AL), F_1, F_2 and $F_{2:3}$ populations were tested for leaf rust resistance at the seedling stage. Ten-day-old seedlings were inoculated by spraying an aqueous suspension of urediniospores with a drop of Tween-20 as the surfactant. The inoculated seedlings were kept in humidity chambers for 48 h and then moved to greenhouse for rust development. Infection types (ITs) were scored 12 days after inoculation on a 0–4 scale as described by Stakman *et al.* (1962). Selection G12 and AL were tested with 40 pathotypes (table 1). Pathotype 77-5 (121R63-1), currently the most predominant one in India, was used for genetic analysis. Parental lines were also tested with pathotypes 77-5 (121R63-1) and 104-2 (21R55) at the adult plant stage in 2012–13 and 2013–14. Disease levels on adult plants were recorded at growth stage Z80 (Zadoks *et al.* 1974) as percentage leaf area covered with uredinia according to the modified Cobb scale (Peterson *et al.* 1948), by which rust severity is recorded on a 0–100 scale, combined with infection response (Joshi *et al.* 1988).

DNA extraction and molecular analysis

Fresh leaf tissues collected from 40–45 days-old plants were pulverized in liquid nitrogen with mortar and pestle. DNA isolation was done following CTAB method (Murray and Thompson 1980). DNA was quantified and diluted to the working concentration of 20–30 ng/μL. PCR amplification was carried out in 10 μL reaction volumes containing 4 mM Tris-HCl (pH 8.0), 20 mM KCl, 0.8 mM $MgCl_2$, 40 μM of each dNTP (MBI Fermentas, Germany), 1.0 unit *Taq* DNA polymerase (Bangalore Genei, Bengaluru, India), 5 pmol/μL of each primer and 20 ng of genomic DNA. PCR amplification was performed with following thermal profile: 94°C for 4 min, followed by 45 cycles of 1 min at 94°C (denaturation), 1 min at 60°C for primer annealing (vary with different primers), 1 min and 72°C and final extension at 72°C for 10 min. Amplified products were resolved on 3.5% MetaPhor (Lonza, Rockland, USA) gels stained with ethidium bromide. Gels were visualized with a UV transilluminator gel documentation system (Syngene G-Box, Cambridge, UK).

Table 1. Infection types on Selection G12 and Agra Local against 40 pathotypes of *Puccinia triticina* when tested at seedling stage at mean temperature range of 20–28°C.

S. no.	Pathotype	Host lines		S. no.	Pathotype	Host lines	
		Selection G12	AL			Selection G12	AL
1	10	;1$^{=}$	33^{+}	21	77-6	;1^{N}	3^{+}
2	11	;1$^{=}$	33^{+}	22	77-8	12	3^{+}
3	12A	;$^{-}$	33^{+}	23	77-9	1$^{=}$	33^{+}
4	12-1	;1$^{=N}$	3^{+}	24	77-11	;12	33^{+}
5	12-2	12	3^{+}	25	104	;1^{-N}	33^{+}
6	12-3	;1$^{=}$	3^{+}	26	104A	;1$^{=N}$	3^{+}
7	12-4	0;	3^{+}	27	104B	12	3^{+}
8	12-5	;1^{-N}	33^{+}	28	104-1	;12^{+}	33^{+}
9	12-6	;$^{-}$	33^{+}	29	104-2	;1^{+}	3^{+}
10	12-7	;1^{-}	3^{+}	30	104-3	;12^{-}	3^{+}
11	12-8	;1$^{=}$	33^{+}	31	104-4	;1$^{=}$	33^{+}
12	12-9	;1	33^{+}	32	106	;1	33^{+}
13	77	;^{N}	33^{+}	33	107	;1–	33^{+}
14	77A	;^{N}	33^{+}	34	107-1	;12^{-N}	33^{+}
15	77A-1	;1	3^{+}	35	108	;1$^{=}$	33^{+}
16	77-1	;$^{=}$	33^{+}	36	108-1	;1	3^{+}
17	77-2	0;	3^{+}	37	162	;N	3^{+}
18	77-3	;1	3^{+}	38	162A	1^{-}	33^{+}
19	77-4	;	3^{+}	39	162-1	;1^{-}	3^{+}
20	77-5	;1$^{=}$	3^{+}	40	162-2	;1	3^{+}

Polymorphic survey between the parents was performed with 793 simple sequence repeat markers, including 203 GWM (Röder *et al.* 1998a, b; Gupta *et al.* 2002), 290 WMC (Gupta *et al.* 2002; Somers *et al.* 2004), 61 BARC (Song *et al*. 2005), 55 CFA and 132 CFD (Sourdille *et al.* 2001; Guyomarc'h *et al.* 2002) and 52 GDM markers (Pestsova *et al*. 2000), sufficiently covering the whole genome (a minimum number of 22 SSR markers was included for the chromosome 4B). DNA from 10 homozygous resistant and 10 homozygous susceptible F_2 plants was pooled to get the resistant and susceptible bulks, respectively. Polymorphic markers were used for bulked segregant analysis (Michelmore *et al.* 1991) to identify the markers that are putatively linked with rust resistance gene in Selection G12. All the information about markers was obtained from the GrainGenes website (http://www.graingenes.org).

Statistical analysis and linkage map construction

The entire F_2 population was genotyped with putatively linked markers identified in bulked segregant analysis (BSA). Linkage analysis was done using MAPMAKER ver. 3.0 (Lander *et al.* 1987) with a minimum LOD score of 3.0 and a maximum genetic distance of 30 cM. The genetic distances (cM) were calculated using the mapping function of Kosambi (1943). χ^2-tests were done to find the goodness of fit for segregation of the resistance gene and also to test independence between molecular markers and the leaf rust resistance gene (Mather 1951).

Results

Multipathotype tests for leaf rust response

Selection G12 expressed a high degree of seedling resistance with ITs ranging from 0 to 12^+ against different pathotypes, whereas the susceptible parent AL showed ITs 33^+–3^+ against all the pathotypes tested (table 1). At the adult plant stage, Selection G12 showed a high degree of adult plant resistance (5R to 5MR/TMR) respectively, during two years of testing against the most prevalent pathogypes 77-5 and 104-2. The other parent AL produced a susceptible response of 70S–80S with both the pathotypes.

Inheritance of leaf rust resistance

At seedling stage, Selection G12 exhibited a high degree of resistance (IT;$1^=$), while AL was susceptible (IT 3^+).

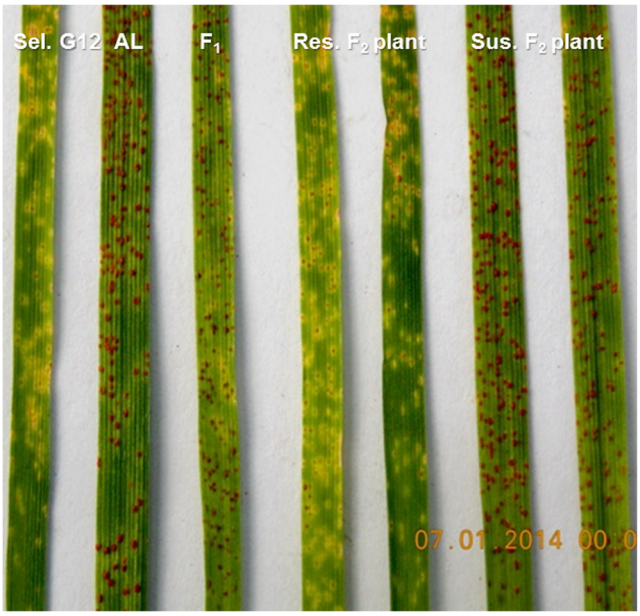

Figure 1. Infection types (ITs) of pathotype 77-5 on Selection G12, Agra Local, F_1 and for F_2 plants of the cross, Selection G12/AL.

F_1 of the cross, Selection G12/AL produced an intermediate phenotype (IT 2^+3, mostly susceptible pustules with a few smaller pustules having some indications of necrosis/chlorosis), indicating incomplete dominance (figure 1). The F_2 population showed the monogenic segregation ratio of 1:3 ($\chi^2_{1:3} = 1.48$, $P_{1df} = 0.22$) with 37 resistant and 139 susceptible plants (including intermediate types). In F_2, the intermediate phenotype was difficult to be differentiated clearly from the susceptible ones on individual plant basis. Therefore, the intermediate category was merged with the susceptible category. These results suggest that the leaf rust resistance in Selection G12 is conditioned by a single incompletely dominant gene. The $F_{2:3}$ families also segregated into 1 homozygous resistant: 2 segregating : 1 homozygous susceptible ratio (table 2), confirming the F_2 segregation results.

Molecular mapping

One hundred and ninety-three microsatellite markers were found polymorphic between the parents. BSA with parental polymorphic markers showed that the markers *Xgwm547* and *Xgwm114* located on chromosome 3BL were also polymorphic between the bulks and were putatively linked to

Table 2. Segregation of leaf rust resistance in F_2 and F_3 seedling populations against pathotype 77-5 at temperatures range of 20–28°C.

Generation	Number of seedlings/families			Expected ratio	χ^2	*P* value
	Resistant	Segregating	Susceptible			
F_2	37	–	139*	1 : 3	1.48	0.22
F_3	37	92	47	1 : 2 : 1	1.50	0.47

*Including plants with intermediate reactions.

the leaf rust resistance gene (figure 2). The marker *Xgwm547* was inherited as a dominant allele, whereas *Xgwm114* as codominant.

Linkage was detected between the resistance gene and markers *Xgwm547* and *Xgwm114* as well as between the markers (table 3). Joint segregation analysis of marker *Xgwm547* and rust resistance gene showed deviation from independent assortment ratio of 3 : 6 : 3 : 1 : 2 : 1 with χ^2 value of 136.42 ($P < 0.0001$). Similarly, the second marker *Xgwm114* also showed deviation from the theoretically expected independent assortment ratio of 1 : 2 : 1 : 2 : 4 : 2 : 1 : 2 : 1 (χ^2 value 66.96, $P < 0.0001$). The results of joint segregation showed that both markers *Xgwm547* and *Xgwm114* were linked with rust resistance gene in Selection G12. The three χ^2 values for linkage (table 3) were compared. The lowest χ^2 value ($\chi^2_{3:6:3:1:2:1} = 39.83$) for independent

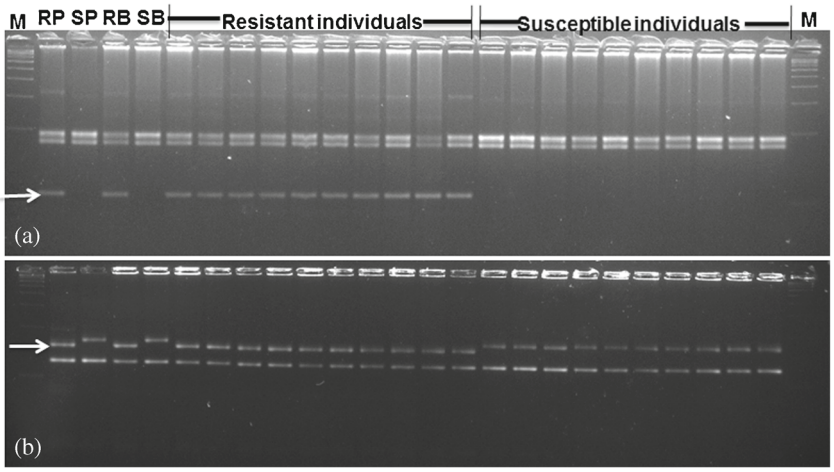

Figure 2. Bulked segregant analysis and co-segregation of SSR marker (a) *Xgwm547* and (b) *Xgwm114* with the leaf rust resistant gene; M, 100 bp ladder; RP, resistant parent (Selection G12); SP, susceptible parent (Agra Local); RB, resistant bulk; SB, susceptible bulk; bulked sample is based on homozygous F_2 plants.

Table 3. Joint segregation of the resistance locus in Selection G12 with SSR markers *Xgwm547* and *Xgwm114*.

Marker		F_2 genotypes based on F_3 HR	H	HS	Total	χ^2 values
Xgwm547	P	37	84	5	126	χ^2 1 : 2 : 1 (LR) = 1.50, P_{2df} 0.4724
						χ^2 3 : 1 (marker) = 1.09, P_{1df} 0.2963
	A	0	8	42	50	χ^2 3 : 6 : 3 : 1 : 2 : 1 = 136.42, $P_{5df} < 0.0001$
						χ^2 linkage = 133.83, $P_{2df} < 0.0001$
	Total	37	92	47	176	
Xgwm114	L	21	16	5	42	χ^2 1 : 2 : 1 (LR) = 1.50, P_{2df} 0.4724
						χ^2 1 : 2 : 1 (marker) = 7.12, P_{2df} 0.0284
						χ^2 (1 : 2 : 1 : 2 : 4 : 2 : 1 : 2 : 1) = 66.96, $P_{8df} < 0.0001$
	H	10	54	11	75	
	U	6	22	31	59	χ^2 linkage = 58.34, $P_{4df} < 0.0001$
	Total	37	92	47	176	
		Xgwm114 L	H	U	Total	
Xgwm547	P	37	60	29	126	χ^2 1 : 2 : 1 = 7.12, P_{2df} 0.0284
						χ^2 3 : 1 = 1.09, P_{1df} 0.2963
						χ^2 3 : 6 : 3 : 1 : 2 : 1 = 39.83, $P_{5df} < 0.0001$
	A	5	15	30	50	χ^2 linkage = 31.62, $P_{2df} < 0.0001$
	Total	42	75	59	176	

L, lower fragment; U, upper fragment; P, presence of fragment; A, absence of fragment; HR, homozygous resistant; H, heterozygous; HS, homozygous susceptible; LR, leaf rust.

assortment between markers *Xgwm547* and *Xgwm114* indicated that the two markers were far and the gene for leaf rust resistance in Selection G12 was located between the two SSR markers, but relatively close to *Xgwm547*. The linkage map (figure 3) constructed by software MAPMAKER ver. 3.0 (Lander *et al.* 1987) showed that *Xgwm547* is at a distance of 6 cM distal to the resistance gene. The other marker *Xgwm114* was 28.3 cM proximal to the resistance gene. Thus, resistance gene was flanked by markers *Xgwm547* and *Xgwm114* at 6 cM and 28.3 cM, respectively.

Discussion

The tetraploid species, *T. timopheevii* and its wild relative *T. araraticum* have been used as sources of resistance to diseases such as powdery mildew (*Pm6*, *Pm27* and *Pm37)*; leaf rust (*Lr18*, *Lr50*, *LrTt1* and *LrTt2*) and stem rust (*Sr36*, *Sr37*, *Sr40* and *SrTt3*) (McIntosh and Gyarfas 1971; Jorgensen and Jensen 1973; McIntosh *et al.* 1995; Järve *et al.* 2000; Brown-Guedira *et al.* 2003; Leonova *et al.* 2004, 2010; Perugini *et al.* 2008). Bread wheat introgression line, Selection G12, was reported to possess a high degree of leaf rust resistance (Guha *et al.* 1996). The present study confirmed that Selection G12 possesses broad spectrum seedling resistance against an array of *P. triticina* pathotypes. Genetic analysis confirmed that a single gene confers resistance in this line. However, the resistance gene in Selection G12 showed incomplete dominance with susceptible as well as resistant pustules in F_1.

Out of 191 polymorphic markers between Selection G12 and AL, two markers *Xgwm547* and *Xgwm114* from the long arm of chromosome 3B were found to be polymorphic between the resistant and susceptible bulks and thus, putatively linked to rust resistance gene in Selection G12. According to Somers *et al.* (2004), there is no other marker between *Xgwm547* and *Xgwm114*. Therefore, linkage map was constructed using these two markers only. The segregating resistant gene in Selection G12 was mapped on the chromosome 3BL. The close linked marker *Xgwm547* (a dominant marker) was positioned distal to the resistance gene with a map distance of 6.0 cM, whereas codominant marker *Xgwm114* is proximal to it at 28.3 cM (figure 3).

The three χ^2 linkage values (table 3) indicated that the resistance gene was flanked by the two markers. This supports the order of markers and resistance gene observed in linkage map. The marker order in the present study is in conformity with the Somers' map (Somers *et al.* 2004), although there was an anomaly in the map distances between the two marker loci. This deviation was expected due to the difference in the nature and size of the mapping population.

Till date, four leaf rust resistance genes (*Lr18*, *Lr50*, *LrTt1*, *LrTt2*) have been introgressed from *T. timopheevii*. One of them, *Lr18*, located on chromosome 5BL (Dyck and Samborski 1968; McIntosh 1983) is a temperature-sensitive gene (Dyck and Johnson 1983). The second (*Lr50*) transferred from *T. timopheevii* is dominant in nature and

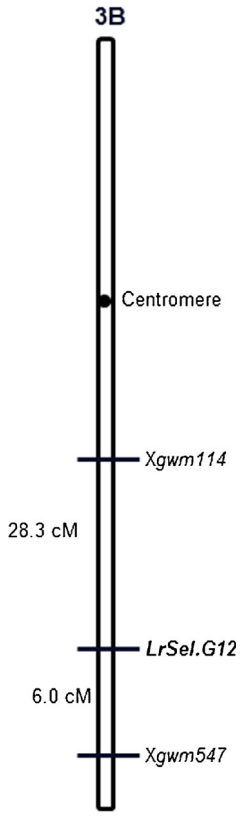

Figure 3. Position of *LrG12* on the genetic map of wheat chromosome 3BL.

located on chromosome 2BL (Brown-Guedira *et al.* 2003). Two other leaf rust resistance loci *LrTt1* and *LrTt2* from *T. timopheevii*, were mapped on chromosome 2A and 5B, respectively (Leonova *et al.* 2004). In our study, the resistance gene has been located on chromosome 3BL. Based on genetic nature of resistance and genomic position of the resistance gene in Selection G12, it can be concluded that Selection G12 harbours a new leaf rust resistance gene. Therefore, this gene may be tentatively named as *LrG12*.

A large number of leaf rust genes have become ineffective due to the evolution of new virulent races in India (Tomar *et al.* 2014). The resistance gene *LrG12* is expected to be of significant importance in diversifying the genetic base of rust resistance. The microsatellite marker *Xgwm547* is linked to *LrG12* at a distance of 6.0 cM can also be used for marker-assisted selection. However, there is the need to develop tightly linked markers for effective utilization in marker-assisted breeding.

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