

Inheritance and molecular mapping of leaf rust resistance in *Triticum turgidum* var. *durum* cv. Trinakria

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Abstract

Leaf rust of wheat, caused by *Puccinia triticina* Eriks. is the most regularly occurring rust disease that can cause severe yield losses. Due to relatively lower economic value of durum wheat compared to the common wheat, genetic analysis of leaf rust resistance in durum wheat has been carried out only to a limited extent. Durum wheat genotype Trinakria showed high level and broad spectrum seedling resistance to 18 Indian leaf rust pathotypes. Genetic analysis at seedling stage in F₂ and BC₁ populations using leaf rust pathotype 77-5 showed a single dominant gene for resistance. The leaf rust resistance gene was mapped to the short arm of 5B chromosome and Xgwm234 was the closest marker at a distance of 6.3cM. The leaf rust resistance gene present in Trinakria is tentatively named as LrTrk. In durum wheat very few Lr genes have been documented, and hence Trinakria could be a promising source of leaf rust resistance for wheat breeders. The Xgwm234 marker linked to leaf rust resistance can be used for transfer of leaf rust resistance from Trinakria to other wheat cultivars through marker assisted selection.

Key words: Leaf rust resistance, Lr52, Trinakria, SSR markers, Xgwm234

Introduction

Leaf rust caused by *Puccinia triticina* Eriks. is the most common and widespread disease of wheat. The leaf rust pathogen is adapted to a wide range of environments and the disease can be found in diverse wheat growing areas throughout the world [1]. A number of studies in different parts of the world have indicated that the leaf rust resistance functioning in durum wheat is probably unique and is different from its counterpart in bread wheat [2]. Generally, durum wheat shows resistance to the bread wheat-virulent leaf rust

pathotypes, while bread wheat to the leaf rust pathotypes which are relatively more virulent on durum wheat [3-10]. Leaf rust is a major threat to durum production worldwide especially in countries where durum specialised pathotypes are present [11-13]. Due to wide geographical distribution and regular disease occurrences, severe leaf rust incidence can cause up to 70 per cent yield losses [11, 14-16]. The deployment of resistant cultivars is an effective way to control the disease. Compared to bread wheat, leaf rust resistance in the durum wheat has been examined only to a limited extent [1]. To date, 71 Lr genes have been identified [17] but only Lr14a and Lr23 originated from tetraploid wheat [13] though Lr3, Lr14a, Lr23, Lr53, Lr61 and Lr64 occur in both tetraploid and hexaploid wheats [18]. Resistance based on single gene is short lived due to evolution of new virulent races and pyramiding of two or more genes confer long lasting resistance for different races. This necessitates the breeders to search for novel genes conferring resistance to wider spectrum of leaf rust pathotypes. The use of molecular markers offers a powerful tool to identify leaf rust resistance genes and determine their physical location on chromosome [16].

Durum wheat genotype Trinakria, an internationally known material for gluten and protein quality traits was developed from cross (B14/Capeiti 8) in Italy in 1973 [19, 20] and which showed field level resistance to leaf rust pathotypes in India [21]. The main objective, therefore, of this study was to understand the genetic basis of leaf rust resistance in Trinakria and to identify the molecular markers linked to its leaf rust resistance gene/s.

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Material and methods

Plant material

The durum wheat leaf rust resistant genotype Trinakria and a susceptible durum cultivar A-9-30-1 (Arnej) were tested against different races of leaf rust at seedling stage in natural glasshouse conditions (minimum and maximum temperature range 10-28°C). Trinakria was crossed with A-9-30-1 to develop F₁ plants which were further advanced by selfing to raise F₂ population. Besides, F₁ was also backcrossed to susceptible parent A-9-30-1 to develop BC₁ population. Inheritance of leaf rust resistance was studied in F₁, F₂ and BC₁ generations along with parents using leaf rust pathotype 77-5 (121R63-1), which is one of the most commonly prevalent and virulent leaf rust pathotypes in India. Chi-square test was employed to test the goodness of fit of observed ratios with theoretically expected ratios in F₂ and BC₁ generations.

Seedling test

Initial pure inoculum of different leaf rust races was obtained from Directorate of Wheat Research, Regional Station, Flowerdale, Shimla and was multiplied on susceptible cultivar Agra Local in isolated chambers in glass house. Fresh inoculum multiplied on Agra Local was used for artificial inoculation of parents and segregating populations at seedling stage. Fresh urediospores were suspended in water containing Tween20 (0.75 µl/ml) and sprayed on 10 days old seedlings. The inoculated seedlings were kept in moist humid chamber for 36-48 hrs. After incubation period, seedlings were kept on glasshouse benches under natural conditions. Scoring of leaf rust infection types (ITs) was done on 12th day of inoculation following the classification by Stakman *et al.* [22] on 0-4 scale.

DNA isolation and PCR conditions

Young and fresh leaf tissue collected from one month old plants was ground to fine powder using liquid nitrogen and used for DNA isolation using CTAB method [23]. The isolated DNA was quantified and diluted to the concentration of 40-50ng/ml and stored at -20°C. The PCR reaction was carried out in 10 µl reaction volume containing 4 mM Tris-HCl (pH 8.0), 20 mM KCl, 0.8 mM MgCl₂, 40 µM of each dNTP (MBI Fermentas, Germany), 1.0 unit Taq DNA polymerase (Bangalore Genei Pvt Ltd, India), 5 pmol/µl of each primer and 20 ng of genomic DNA. The Polymerase Chain Reaction (PCR) was carried out with following thermal profile: initial denaturation step

of 94°C for 4 min, followed by 45 cycles of 94°C for 1 min (denaturation), 60°C for 1 min (primer annealing) and 72°C for 1 min (primer extension) with a final extension of 72°C for 10 min. The amplified products were separated by 3.5 per cent metaphor agarose gel electrophoresis, stained with ethidium bromide and visualized on UV trans-illuminator gel documentation system (G:Box, Syngene).

Bulked segregant analysis (BSA) and molecular mapping

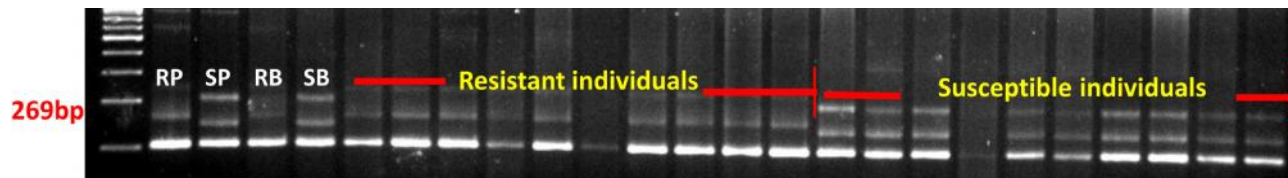
Survey of parental polymorphism was done using 537 SSR markers from A and B genomes covering all the 14 chromosomes to identify polymorphic markers between Trinakria and A-9-30-1. Bulk segregant analysis was performed in F₂ population to identify the putative markers linked to the leaf rust resistance [24]. Equal quantity of DNA from ten resistant and ten susceptible plants of F₂ population was bulked to constitute isogenically contrasting resistant and susceptible bulks, respectively. These two bulked DNA along with resistant and susceptible parents were screened using polymorphic markers to identify the putative markers linked to leaf rust resistance gene. The putative markers identified in BSA were used for genotyping of F₂ population and linkage analysis was performed using Mapmaker version 3.0 software to construct linkage map [25] with a LOD threshold of 3.0. The genetic distance between markers was calculated in cM using Kosambi function. Chi square test was performed for segregation of markers to test goodness of fit against expected ratio of 1:2:1 and also tested for linkage between markers and leaf rust resistance gene against expected ratio of 9:3:3:1.

Results and discussion

Trinakria was tested for resistance at seedling stage against 18 Indian leaf rust pathotypes along with durum cultivar A-9-30-1, which was used as susceptible parent in the present genetic analysis and mapping of leaf rust resistance along with two other check cultivars Mandsour Local, a durum cultivar and Agra Local, a bread wheat cultivar. The results (Table 1) showed that out of two check genotypes i.e. Mandsour Local and Agra Local used for characterization of leaf rust resistance, Agra Local was susceptible to all the 18 pathotypes of leaf rust, while Mandsour Local showed susceptibility to all the test pathotypes except, 12A and 104-2 where it showed slightly lower IT (3-) in comparison to 3/3+ to all other pathotypes of leaf rust. Trinakria was resistant to all the pathotypes. This

Table 1. Seedling reaction of durum wheat genotypes against different pathotypes of leaf rust

S.No.	Race	Trinakria	A-9-30-1	Mandsour Local	Agra Local
1	12-A	:1-	:1=	3-	3
2	12-1	1+2-	x	3	3
3	12-2	1	1	3	3
4	12-4	;1-	;1=	3+	3
5	12-3	;1-	;1++	3+	3
6	12-5	;1-	3	3+	3
7	12-7	;1-	1+3	3	3
8	104-2	;1=	3	3-	3
9	104-A	;	3	3	3
10	104-B	1	3	3	3
11	104-4	11-	1+	3+	3+
12	106	1=	;	3+	3
13	108	;	;1-	3	3
14	162-A	;1-	;1+	3+	3+
15	162-1	1-	;3	3+	3
16	162-2	11+	1-	3	3
17	162	;1=	1	3+	3
18	77-5	;1=	3	3	3

**Fig.1a.** Leaf rust reaction of Trinakria, F₁ and A-9-30-1 at seedling stage against race 77-5**Fig. 1b.** Leaf rust reaction in F₂ from the cross A 9-30-1/Trinakria at seedling stage against leaf rust race 77-5**RP:** Resistant parent, **SP:** Susceptible parent, **RB:** Resistant bulk, **SB:** Susceptible bulk, **M:** 100-bp DNA ladder**Fig. 2.** Putative marker *Xgwm234* identified in BSA located on chromosome 5BS in cross A-9-30-1/Trinakria

observation in the present study also confirmed the earlier report [21] which found the genotype Trinakria resistant against a set of 40 Indian leaf rust pathotypes. The genotype A-9-30-1 which was used as susceptible parent in mapping population was susceptible to the most prevalent leaf rust pathotypes in India i.e. 104-2 and 77-5. Trinakria produced an infection type (IT) ;1= whereas A-9-30-1 showed IT 3 for pathotypes, 77-5 and 104-2. The F₁ (A-9-30-1/Trinakria) showed resistant reaction against pathotypes 77-5 (IT ;1) and 104-2 (IT 1+) (Fig. 1a) indicating dominance of resistance. The F₂ population of 220 plants segregated into 156 resistant and 54 susceptible against leaf rust pathotype 77-5 at seedling stage indicating the

presence of a single dominant gene for leaf rust resistance in Trinakria (Table 2 and Fig. 1b). The observed data showed a good fit to 3 resistant : 1 susceptible ratio with χ^2 value 2.1256 ($P=0.1448$).

Table 2. Reaction of F₁, F₂ and BC₁ for leaf rust resistance at seedling stage against pathotype 77-5

Generation	Total	R	S	Expected ratio	χ^2 value	P value
F ₁	10	10	-	-	-	-
F ₂	220	156	64	3:1	2.12558	0.1448
BC ₁	86	41	45	1:1	0.18605	0.6662

R = Resistant plants; S = Susceptible plants

Table 3. Segregation of SSR markers of chromosome 5BS in F₂ generation

Marker	Fragment size(bp)	Number of plants			Total	χ^2 value	P value
		MM	Mm	mm			
<i>Xgwm234</i>	269	50	108	57	215	0.46 ^a	0.7945
<i>Xcfd20</i>	365	53	103	59	215	0.711 ^a	0.7208
<i>Xgwm191</i>	112	54		158	212	0.025 ^b	0.9875

a = tested against 1:2:1 ratio; b = tested against 1:3 ratio

The inheritance of leaf rust resistance was further confirmed in BC₁ population A-9-30-1/Trinakria//A-9-30-1 against leaf rust pathotype 77-5. Among 86 plants of BC₁ generation, 41 were resistant while 45 showed susceptible reaction with IT 3 or more ($\chi^2_{1:1} = 0.1860$, $P=0.6662$). The genetic analyses of F₂ and BC₁ population suggest the presence of a single dominant gene for leaf rust resistance in Trinakria (Table 2).

Out of 537 SSR markers used for parental polymorphism survey, 166 were found polymorphic between Trinakria and A-9-30-1. A total of 220 F₂ plants screened against leaf rust race 77-5 at seedling stage were used for molecular mapping of leaf rust resistance. BSA was performed using 166 polymorphic SSR markers between resistant and susceptible bulks, which identified only one putative SSR marker *Xgwm234* linked to leaf rust resistance gene, located on 5BS chromosome. The F₂ population was, therefore, genotyped using *Xgwm234* along with two more parental polymorphic markers *Xcfd20* and *Xgwm191* from 5B chromosome, which though were not polymorphic between resistant and susceptible bulks. In F₂ population, markers *Xgwm234* ($\chi^2_{3:1}=0.46$, $P=0.7945$), *Xcfd20* ($\chi^2_{3:1}=0.71$, $P=0.7208$), and *Xgwm191* ($\chi^2_{1:3}=0.03$, $P=0.9875$) segregated for theoretically expected ratio of 1:2:1 (Table 3). Joint segregation analysis of markers and leaf rust resistance (Table 4) showed linkage between markers *Xgwm234*. ($\chi^2_{3:6:3:1:2:1}=176.82$, $P=0$) and *Xcfd20* ($\chi^2_{3:6:3:1:2:1}=154.24$, $P=0$). However, test of joint segregation between leaf rust resistance gene *LrTrk* and *Xgwm191* when tested against expected ratio of 3:9:1:3 was non-significant. Marker *Xgwm191* behaved as a dominant marker with presence of marker allele in susceptible plants only. Linkage map was constructed using Mapmaker version 3.0 at LOD value of 3. Kosambi function was used for calculation of mapping function. The results showed that *Xgwm234* was linked to leaf rust resistance at a distance of 6.3cM whereas *Xcfd20* was linked to leaf rust resistance at the distance of 14.9cM.

Characterisation of durum wheat genotype Trinakria for leaf rust resistance at seedling stage against 18 different pathotypes showed that leaf rust resistance gene in Trinakria provides high degree of resistance against broad spectrum of leaf rust pathotypes of India. Genetic analysis of leaf rust resistance in Trinakria using leaf rust pathotype 77-5 revealed the presence of a single dominant seedling gene for leaf rust resistance. The leaf rust resistance gene in Trinakria is tentatively named as *LrTrk*. Molecular mapping and linkage analysis revealed the location of gene *LrTrk* on short arm of 5B chromosome. The linkage map of leaf rust resistance in A-9-30-1/Trinakria constructed with two SSR markers *Xgwm234* and *Xcfd20* showed the marker *Xgwm234* as nearest to the leaf rust resistance gene at a distance of 6.3 cM (Fig. 3). The markers order in linkage map of present population is in conformity with the map of Somers *et al.* [26].

Dyck and Jedel [27] identified a single dominant gene for leaf rust resistance in genotype V336 of A. E. Watkins wheat (*Triticum aestivum*) collection and transferred this leaf rust resistance gene into Thatcher to produce a near isogenic line RL6107 (Thatcher*6/V336). Hiebert *et al.* [28] mapped the leaf rust resistance in RL6107 on short arm of 5B chromosome distal to *Xgwm443* (16cM) and was named as *Lr52*. In

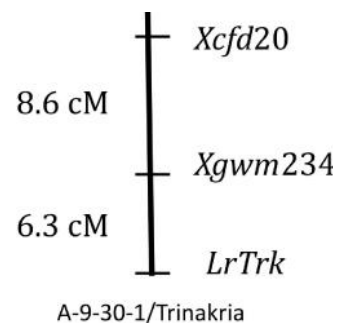
**Fig. 3.** Linkage map of leaf rust resistance gene *LrTrk* in Trinakria on 5BS chromosome of wheat

Table 4. Test of linkage between SSR markers of 5BS chromosome and leaf rust resistance in F₂ (A-9-30-1/Trinakria) population

Marker	Resistant plants			Susceptible plants			Total	χ^2 value	P value
	MM	Mm	mm	MM	Mm	mm			
<i>Xgwm234</i>	48	103	5	2	5	52	215	176.82 ^a	0
<i>Xcfd20</i>	48	99	9	5	4	50	215	154.24 ^a	0
<i>Xgwm191</i>	43	110		11	48		212	3.08 ^b	0.38

^atested against 3:6:3:1:2:1 ratio; ^btested against 3:9:1:3 ratio

'M' marker allele present in resistant parent Trinakria and 'm' marker allele present in susceptible parent A-9-30-1

another study, Obert *et al.* [29] also mapped a leaf rust resistance gene in the Iranian landrace PI 289824 on chromosome 5BS distal to *Xgwm234* (7.8cM) and the AFLP based STS marker *tmx200* was linked at 2.3cM distance, proximal to leaf rust resistance gene. In the present study, STS marker *tmx200* was found monomorphic between durum genotype Trinakria and A-9-30-1.

The pedigree analysis revealed the parents of Trinakria as B14/Capeiti 8. Capeiti 8 was selected from the cross Cappelli × Palestinian Eiti [19]. Based on available pedigree information, the parental lines of Trinakria, most likely, do not belong to ancestral lineage of *Lr52* that has been traced back to Watkins wheat collection [27-29]. As no other leaf rust resistance gene, except *Lr52* is located on 5BS chromosome and many SSR markers between *Xgwm234* and *Xgwm191* flanking *LrTrk* gene on 5B chromosome were found monomorphic, it is difficult to dissect the identity of *LrTrk* from *Lr52*. Therefore, leaf rust resistance gene in Trinakria can either be a new gene close to *Lr52* locus or is allelic to *Lr52*. Since, leaf rust resistance gene *LrTrk* is highly effective against all the Indian leaf rust pathotypes, it can be a good source for leaf rust resistance in wheat breeding. The co-dominant SSR marker *Xgwm234* linked with leaf rust resistance in Trinakria can be effectively used in introgression of leaf rust resistance in wheat improvement programme.

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