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# Validation and identification of molecular markers linked to the leaf rust resistance gene *Lr*28 in wheat

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**Abstract** Leaf rust resistance gene *Lr*28 controls one of the important resistances in the Indian subcontinent against the most prevalent *Puccinia triticina* pathotype 77-5. Pyramiding *Lr*28 with other resistance genes would therefore, provide durable resistance against rust, a process that can be facilitated by DNA markers. A microsatellite marker wmc313 linked to *Lr*28 at a distance of 5.0 cM was identified in the population HD2329 × HW2037. The marker was validated in another population developed from WL711× CS + *Lr*28: 2D/2M 3/8 (acc. 2956) as well as in a few near- isogenic lines (NILs) carrying gene *Lr*28. Compared to the previously reported marker TPSCAR $SCS421_{570}$, wmc313 is more closely linked to *Lr*28. Both these markers flanking the gene should be useful in the deployment of *Lr*28 into the breeding program using marker-assisted selection allowing pyramiding with other effective genes to confer durable resistance.

**Keywords** *Lr*28 · Marker assisted selection · Microsatellite markers · *Puccinia triticina* · *Triticum aestivum*

Leaf rust of wheat (*Triticum aestivum* L.) caused by *Puccinia triticina* Eriks., is found worldwide in all wheat growing areas, often resulting in the yield losses up to 50%. In India too, it causes substantial economic losses during epidemic years (Nayar et al. 2002). Although more than 60 leaf rust resistance genes have been identified at the seedling and adult plant stages (McIntosh et al. 2008), these resistances are very often overcome by the pathogen; therefore, incorporation of resistant genes is a continuous process to counter evolving pathotypes. Combining several genes, into one cultivar, ('pyramiding') is an effective strategy to enhance the durability of the resistance against rusts. Identification of molecular markers for the resistance genes and their use in breeding can facilitate gene pyramiding into valuable backgrounds in short time, making it more cost effective.

Leaf rust resistance (*Lr*) genes such as *Lr*9, *Lr*19, *Lr*24, *Lr*28 and *Lr*32 provide complete protection against leaf rust pathotypes in wheat-growing regions of Asia (Tomar and Menon 2001). The resistance gene *Lr*28 located on chromosome 4AL was transferred into wheat from *Aegilops speltoides* (McIntosh et al. 2008). It has been transferred into different genetic backgrounds of Indian collection (Tomar and Menon 1998), and no undesirable linkage drag or negative effect on yield has been observed (Kumar and Raghavaiah 2004). Therefore *Lr*28, in combination with other resistance genes, has a potential to reduce yield losses caused by *P. triticina*. Three markers tagging *Lr*28 have been hitherto reported by Naik et al. (1998), Vikal et al. (2004) and Cherukuri et al. (2005). For widespread utilization of the molecular markers in marker assisted breeding, validation in multiple backgrounds is essential. The STS marker derived by Naik et al. (1998) was found to be loosely linked to *Lr*28 in previous studies (Sharp et al. 2001; Chełkowski et al. 2003) and hence not considered in our study. The objectives of this study were to (1) test available microsatellite markers for *Lr*28, (2) identify

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**Table 1** Seedling reaction in $F_2$ and $F_3$ generations of HD2329 × HW2037 and WL711 × acc. 2956 to the pathotype 77-5 under controlled conditions

| Cross | Generation (ratio) | Reaction of seedlings | | | $\chi^2$ | P value |
|---|---|---|---|---|---|---|
| | | HR | Seg | HS | | |
| HD2329 × HW2037 | $F_2$ (3:1) | 86 | 0 | 24 | 0.440 | 0.506 |
| | $F_3$ (1:2:1) | 26 | 60 | 24 | 0.612 | 0.736 |
| WL711 × acc. 2956 | $F_2$ (3:1) | 118 | 0 | 27 | 0.076 | 0.782 |
| | $F_3$ (1:2:1) | 42 | 76 | 27 | 0.178 | 0.914 |

HR: Homozygous resistant; Seg: Segregating; HS: Homozygous susceptible

markers closely linked to *Lr*28 and (3) validate their potential in marker-assisted selection.

In the study, two $F_2$ derived $F_3$ populations were used. The first population consisted of 110 $F_{2:3}$ families from a cross between a near- isogenic line (NIL) HW2037 and its recurrent parent HD2329. Secondly, a $F_1$ harvest of 145 seeds of a cross between cultivar WL711 and CS + *Lr*28: 2D/2M 3/8 (acc. 2956) (kindly provided by Prof. R. G. Saini, Punjab Agricultural University, Ludhiana, India) was advanced to generate a $F_{2:3}$ population. Six NILs for *Lr*28 developed at the Regional Research Station, IARI, Wellington, India, namely HW2031 (C306 + *Lr*28), HW2032 (LOK1 + *Lr*28), HW2033 (WH147 + *Lr*28), HW2035 (NI5439 + *Lr*28), HW2037 (HD2329 + *Lr*28) and HW2061 (Sonalika + *Lr*28), along with their recurrent parents were used in validation studies. Additionally a set of 48 wheat cultivars (postulated to carry leaf rust resistance genes) from advanced varietal trials under the All India Co-ordinated Wheat and Barley Program were used in the final marker validation studies.

The leaf rust pathotype 77-5 (121R63-1), avirulent on *Lr*9, *Lr*18, *Lr*19, *Lr*24, *Lr*25, *Lr*28, *Lr*29, *Lr*32, *Lr*39, *Lr*41, *Lr*42, *Lr*45 genes was used for phenotyping. Seedlings were grown at 18 ± 2°C and >85% RH in the controlled condition facility. Seven-day-old seedlings were inoculated using the standard procedure as described by Browder (1971)). Reaction to the disease, i.e. infection type (IT) was noted 14 days after inoculation, using the 0 - 4 Stakman's scale (Stakman et al. 1962). For testing $F_{2:3}$ families, about 16 - 30 plants of each family were inoculated. Based on IT of $F_{2:3}$ families, the $F_2$ genotypes were classified as homozygous resistant (HR), segregating (Seg) and homozygous susceptible (HS).

Total DNA was extracted from healthy leaves, using the modified CTAB method (Rogers and Bendich 1985). Based on available wheat genetic maps of chromosome 4AL (Somers et al. 2004; Song et al. 2005), a total of 26 microsatellite markers viz. barc70, barc78, cfa2256, cfd2,

**Table 2** Test of linkage between *Lr28*, wmc313 and $SCS421_{570}$ marker in the $F_2$ generation of crosses HD2329 × HW2037 and WL711 × acc. 2956

| Marker | Segregants* | | | | Linkage (cM) |
|---|---|---|---|---|---|
| | R/+ | R/- | S/+ | S/- | |
| Population HD2329 × HW2037 | | | | | |
| wmc313 | 24 | 2 | 2 | 21 | 5.0 |
| $SCS421_{570}$ | 21 | 5 | 5 | 18 | 15.3 |
| Population WL711× acc. 2956 | | | | | |
| wmc313 | 34 | 4 | 1 | 26 | 4.5 |
| $SCS421_{570}$ | 36 | 2 | 5 | 22 | 6.7 |

*R: resistant; S: susceptible; '+': presence of the marker; '−': absence of the marker.

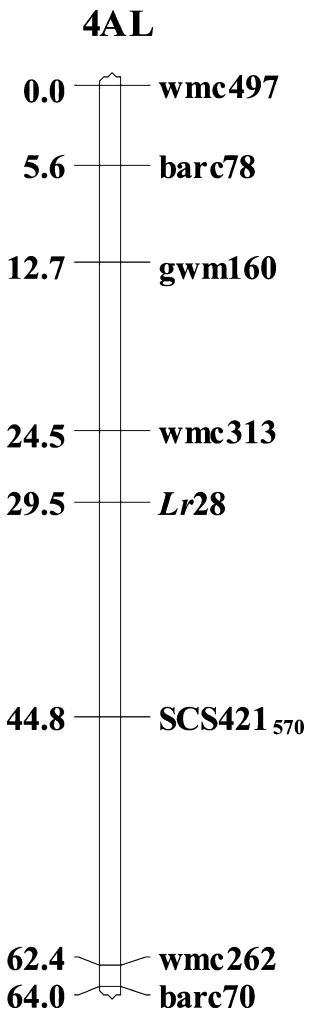


**Fig. 1** Linkage map of the microsatellite and SCAR markers linked to the *Lr*28 locus in the population HD2329 × HW2037 of wheat. The locus names are indicated on right and Kosambi map distance (cM) on left side of map

cfd30, cfd88, cfd257, gwm162, gwm397, gwm494, gwm610, gwm637, psp3030, wmc96, wmc161, wmc219, wmc232, wmc262, wmc313, wmc497, wmc500, wmc513, wmc617, wmc722, wmc776 along with the reported linked marker gwm160 (Vikal et al. 2004) were screened. Amplifications were performed in 10 µl reaction volume, using protocol reported in GrainGenes database (http://wheat.pw.usda.gov). The amplification products were electrophoresed on 5% native polyacrylamide gels (PAGE) stained in ethidium bromide and documented using digital gel documentation system. For amplification of marker TPSCAR $SCS421_{570}$, the reaction conditions reported by Cherukuri et al. (2005) were used. Only HR and HS plants were considered for linkage analysis. Chi squared distribution analyses were carried out to test if the observed segregation ratios for leaf rust resistance and marker loci fit in the expected ratio. Genetic linkage analysis was performed using Mapmaker program version 3.0 (Lander et al. 1987).

The parental lines HW2037 and CS + *Lr*28: 2D/2M 3/8 (acc. 2956) were highly resistant to race 77-5 (IT fleck ;), while HD2329 and WL711 were susceptible (IT 3+ and 4). Both the populations segregated in a 3:1 ratio in the $F_2$ generation indicating a monogenic inheritance of the resistance (Table 1). In the marker analysis, seven out of the 26 tested microsatellite markers, showed polymorphism between HD2329 and HW2037. In selective genotyping using homozygous plants, six promising markers were identified and further tested in the population.

With marker wmc313, the resistant parent and resistant $F_2$ plants showed two bands (320 bp and 309 bp), while the susceptible plants amplified only a single band (309 bp). The polymorphic fragment of 320 bp was amplified only in the resistant $F_2$-segregants and co-segregated with the resistance. Based on the segregation data (Table 2), wmc313 was linked at a distance of 5.0 cM in the population HD2329 ×HW2037 (Fig. 1). The results were further validated in the population WL711 × CS + *Lr*28:

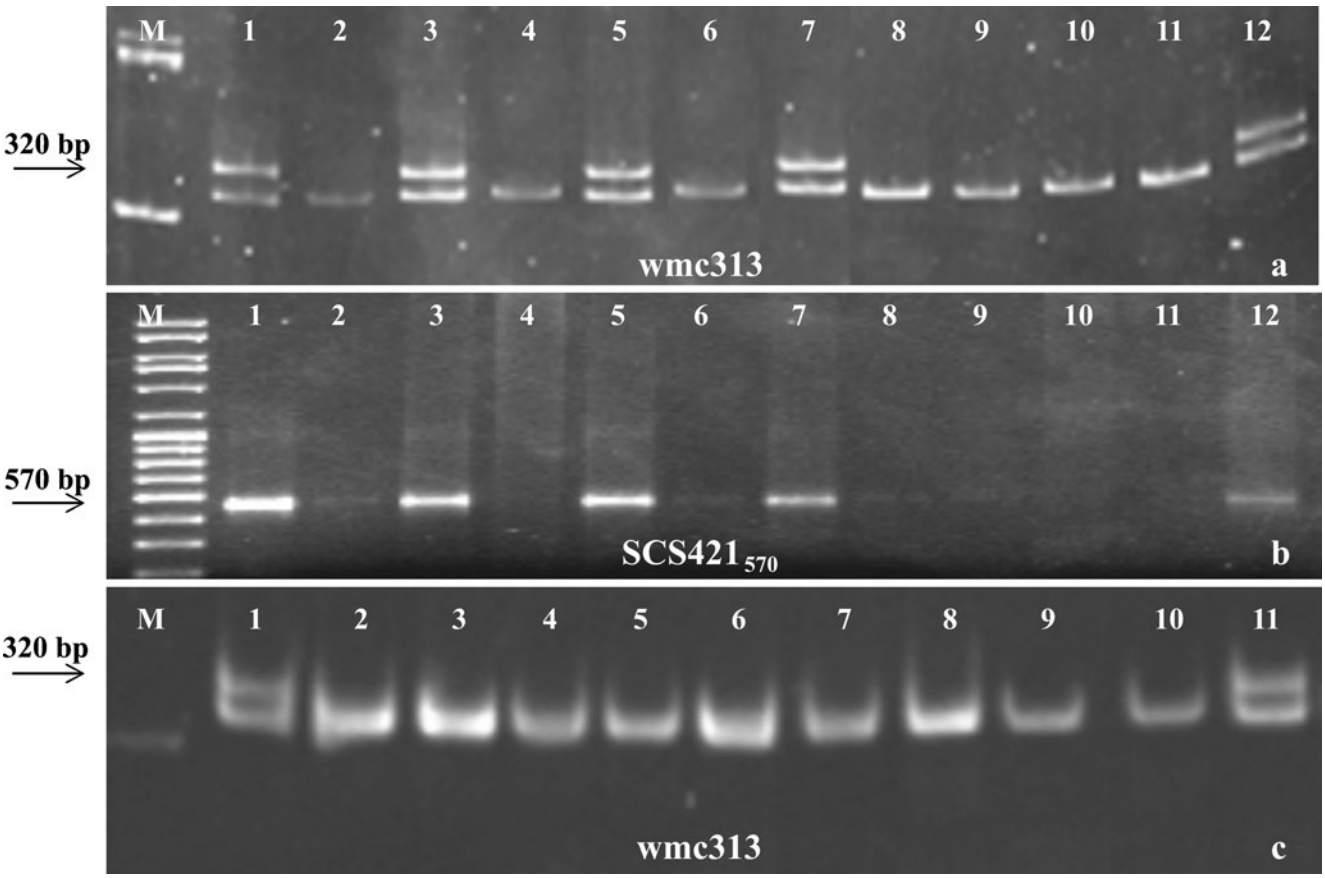


**Fig. 2** Validation of markers linked to gene *Lr*28 on a set of NILs carrying *Lr*28 (**a**, **b**) and wheat cultivars (**c**). Marker wmc313 is resolved on 5% native PAGE and TPSCAR $SCS421_{570}$ on 1.5% agarose gel. The polymorphic band is indicated by an arrow. The postulated genes are given in the parentheses. The sample order for Figs. 2a, b: M. 100 bp ladder / Low range DNA ruler plus (Bangalore Genei); (1) HW2031; (2) C306; (3) HW2032; (4) LOK1; (5) HW2037; (6) HD2329; (7) HW2061; (8) Sonalika; (9) HW2033; (10) WH147; (11) HW2035; (12) *Lr*28 source (CS2D/2M 3/8). The order for Fig. 2c: M. 100 bp ladder; (1) *Lr*28 source (CS2D/2M 3/8); (2) HD2189 (*Lr*13 + *Lr*34); (3) HI977 (*Lr*23 + *Lr*24); (4) NI5439 (*Lr*34); (5) MACS2496 (*Lr*23 + *Lr*26); (6) WH147 (*Lr*13); (7) HW2044 (*Lr*24); (8) UAS305; (9) Kalyansona (*Lr*34); (10) HD4672 (*Lr*23); (11) MACS6145 (*Lr*28)

2D/2M 3/8 (acc. 2956) where a linkage distance of 4.5 cM was observed (Table 2). Marker gwm160 (Vikal et al. 2004) was loosely linked at a distance of 16.8 cM in the HD2329 × HW2037 population and remained unlinked in the second population (data not shown). Marker $SCS421_{570}$ showed a polymorphic band of 570 bp in the resistant lines and mapped at 15.3 cM and 6.7 cM in the populations HD2329 × HW 2037 and WL711 × CS + *Lr*28: 2D/2M 3/8 (acc. 2956), respectively (Table 2). The distance of about 11 cM has also been reported in the previous study using HW4002 × Agra local population (Cherukuri et al. 2005).

The markers wmc313 and $SCS421_{570}$ were further validated in a few NILs carrying the gene *Lr*28 in different genetic backgrounds (Figs. 2a, b). Both the markers amplified the expected polymorphic bands in the NILs except HW2033 and HW2035. Additionally, a set of 48 wheat cultivars, postulated to carry different *Lr* genes, was genotyped with the markers wmc313 and $SCS421_{570}$. With wmc313, expected double band pattern was observed only in the cultivar MACS6145 carrying the gene *Lr*28, whereas other cultivars not carrying the gene showed a single band (Fig. 2c). No cross amplification with other resistance genes was observed. With marker $SCS421_{570}$, the expected band at 570 bp was also observed only in MACS6145.

The markers $SCS421_{570}$ and wmc313 (identified in this study) were able to identify the gene *Lr*28 in diverse genetic backgrounds. Based on the linkage distances of 4.5 cM and 5.0 cM in both populations, wmc313 is the most closely linked marker for *Lr*28 reported so far. The accuracy of wmc313 was established on a set of 48 cultivars as well as NILs for *Lr*28. Among the NILs, HW2033 and HW2035 failed to amplify the expected amplicon with either marker. The failure of amplification in HW2035 has also been reported by Prabhu et al. (2003). However, the absence of the polymorphic amplicon in HW2033 was unexpected. The results for HW2033 were consistent for two different seed stocks in our collection, indicating the need to verify every seed stock. Such misidentity of NILs has been reported earlier by Prabhu et al. (2003). Although the marker $SCS421_{570}$ gave accurate amplification in the NILs and the elite germplasm, when tested in the two populations it showed more distance from the gene as compared to wmc313. Since $SCS421_{570}$ acts as a dominant marker, chances of misclassification are high. In contrast, although wmc313 also acts as a dominant marker, it still gives a common amplicon (309 bp) in all genotypes and thus has an advantage of reducing false negatives. Interestingly, in both the populations $SCS421_{570}$ and wmc313 were found to flank the *Lr*28 gene. Hence, the use of such flanking markers makes the selection for the target gene in a breeding programme more accurate in comparison to a single marker.

The *Lr*28 gene provides a high level of resistance against 77-5, the most predominant pathotype in India. Under a heavy leaf rust infection, it contributes to a higher 1000-grain weight and number of effective tillers per plant with no negative effects on yield and bread-making quality (Kumar and Raghavaiah 2004). However, this gene has not been widely used in breeding programmes in India. So far only one cultivar, MACS6145, carrying *Lr*28 has been released in 2005 by the Agharkar Research Institute, India and Indian Agricultural Research Institute, Regional Station, Wellington, India. This could be due to the fact that conventional pathological testing is a tedious and time-consuming task. Results of the present study suggest that this can be effectively overcome by the use of markers wmc313 and $SCS421_{570}$ in screening breeding materials for *Lr*28. Since tight linkage of a marker with the desired gene is important for success of MAS in breeding, the marker wmc313 should be more effective in MAS for *Lr*28 and facilitate pyramiding to confer a durable resistance to leaf rust.

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