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Selection of a breeder friendly marker for durable wheat leaf rust resistance gene *Lr34*

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ABSTRACT

Molecular as well as morphological markers reported to be linked with the durable wheat leaf rust resistance gene *Lr34* were subjected to verification in a set of selected wheat genotypes. The area under disease progress curve (AUDPC) scores observed in the selected lines, Leaf tip necrosis (LTN) and profile generated using the recently reported linked molecular marker *csLV34*, were analyzed. Study indicated that LTN, once believed to be dependable marker for the gene was found not as reliable as *csLV34* marker for utilization as a selection criterion for the *Lr34* gene by the breeders.

Key words: *Lr34* gene, Marker, LTN, AUDPC

The *Lr34* gene known as "slow rusting gene" has been important to wheat breeders because of durable nature to leaf rust and its association with stripe rust resistance gene *Yr18* as well as tolerance to Barley yellow dwarf virus. The *Lr34* gene is not effective at seedling stage, however adult plant resistance, slow rusting impact and interaction with other genes has made this gene very useful for making a durable rust resistance platform for wheat. Because of widespread effectiveness of *Lr34* gene as a source of resistance under field conditions and its interactive effects (German and Kolmer 1992), this has attracted the attention of breeders for incorporating durable leaf rust resistance in wheat. Selection for this gene in segregating population is tedious due to lack of proper selection criteria. Earlier the presence of *Lr34* could be indicated by the presence of leaf tip necrosis (LTN) in adult plants, which is closely linked with it (Singh 1992). Leaf tip necrosis is reported to be governed by several QTLs and its expression is variable in different backgrounds and environmental conditions (Messmer *et al.* 2000). Lagudah *et al.* (2006) developed one STS marker, *csLV34* closely linked with *Lr34* gene. Present study was undertaken to demonstrate whether morphological (LTN) or genetic marker (*csLV34*) proves a better reliable marker to rely upon for introgression of *Lr34* gene in breeding programmes.

MATERIAL AND METHODS

Under the present study, a set of 40 wheat genotypes (39 Indian genotypes) were selected (Table1). Recently

Table 1 Genotypes taken for the study with their LTN and AUDPC values

Sr. No.	Genotype	LTN	AUDPC	Sr. No.	Genotype	LTN	AUDPC
1	A. LOCAL	-	3000	22	HUW 468	+	1000
2	A-9-30-1	-	1800	23	IWP-72	+	1300
3	BW11	+	562	24	K 8962	+	425
4	DL-153-2	+	950	25	K 9006	-	440
5	DWR 39	-	950	26	Kalyansona	-	1450
6	GW-173	+	1450	27	K9107	+	175
7	HD 2329	-	980	28	NIAW-34	+	1700
8	HD 2601	-	1650	29	Pavon76	+	750
9	HD2189	+	1450	30	PBW 299	-	850
10	HD2687	-	600	31	PBW 65	-	750
11	HI 977	+	1500	32	UP 262	-	700
12	HI-1077	+	825	33	UP115	+	1500
13	HPW 42	-	475	34	VL 401	+	500
14	HPW-56	+	850	35	VL 616	-	1000
15	HS 207	-	750	36	VL 738	-	1500
16	HS 295	-	725	37	WH 147	+	1750
17	HS1097	-	725	38	WH 2265	+	1200
18	HS-240	-	950	39	WH 542	-	1300
19	HS-277	+	475	40	WL 711	+	1190
20	HS386	-	610				
21	HUW 234	+	2400				

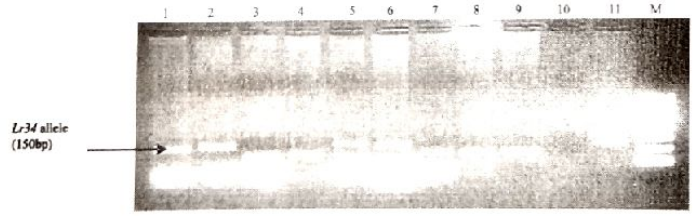
+ Indicates presence of LTN - Indicates absence of LTN

developed STS marker, *csLV34* was used to confirm the presence of *Lr34* gene in these genotypes.

Genomic DNA of all 40 wheat genotypes was extracted using CTAB method (Doyle and Doyle 1987). Polymerase chain reaction (PCR) was performed in 25µl of 10X PCR buffer, 2.0 µl of dNTPs (2.5mM each), 1 µl each of forward and reverse primer (100 pmol/ µl) and 1µl of 100 ng of DNA in a PTC-200 thermal cycler (MJ Research). Primer annealing temperature was 50°C. PCR products were resolved on 2.5% high-resolution agarose gel (Bangalore Genei) and visualized by ethidium bromide staining under Geldoc system (SYNGENE). The LTN trait associated with *Lr34/Yr18* locus was recorded by visual observation on the leaves at Zadok's stage 65-69 (Zadoks *et al.* 1974). Leaf rust recordings were done at equal intervals and area under disease progress curve (AUDPC) was calculated using a computer package (CIMMYT 1988).

RESULT AND DISCUSSION

Morphological marker *i.e.* LTN was found present in 20 genotypes (BW 11, DL 153-2, GW 173, HD 2189, HI 977, HI 1077, HPW 56, HS 277, HUW 234, HUW 468, IWP 72, K 8962, K 9107, NIAW 34, Pavon 76, UP 115, VL 401, WH 147, WH 2265, WL 711) (Table1). Genetic marker *csLV34* showed the presence of marker allele (150bp fragment) in 12 genotypes (BW 11, NIAW 34, HD 2189, K 8962, K 9107, HPW 42, HUW 468, HS 277, HD 2329, UP 115, HS 207 and VL 616) (Fig.1). Out of 12 genotypes found positive by molecular marker (*csLV34*), 8 genotypes namely BW 11, HD 2189, HS 277, HUW 468, K 8962, K 9107, NIAW 34 and UP 115 were found to possess LTN, whereas, 4 genotypes HD 2329, HPW 42, HS 207 and VL 616 did not possess LTN. Among 28 genotypes which did not show the presence of *Lr34* gene by marker, 12 genotypes namely DL 153-2, GW 173, HI 977, HI 1077, HPW 56, HUW 234, IWP 72, Pavon 76, VL 401, WHI 47, WH 2265, WL 711



1- BW11 2 -NIAW34 3 -HI977 4-HI-1077 5-K8962 6-K9107 7-VL401 8-WH147 9-WL711 10-IWP-72 11-UP115 M-100 bp Ladder

Fig.1 Gel profile of csLV34 marker in wheat genotypes

showed LTN. Other 16 genotypes lacked LTN as well as *Lr34* gene by marker. Based on the presence or absence of *Lr34* gene (by *csLV34* marker) and LTN, genotypes were grouped in two groups. The first group consisting of 12 genotypes (positive for *Lr34* gene) had mean AUDPC score of 874.3±138.1 (SE), whereas the other group comprising of genotypes negative by *csLV34* marker as well as for LTN, showed mean AUDPC value of 1166.6±110.7 (SE). Another group of genotypes having LTN only showed mean value of AUDPC as 1222.1. These results indicate better association of *csLV34* marker with slow disease development *i.e.* presence of *Lr34* gene. However, presence of LTN alone could not show its significant effect in reducing the disease severity, thereby indicating its poor association with *Lr34* gene.

Although marker (*csLV34*) showed good linkage with *Lr34* gene but it is not tightly linked to the LTN trait, as 4 genotypes that showed *Lr34* gene by molecular marker lacked LTN, whereas 12 genotypes that possess LTN lacked *Lr34* gene. Earlier studies showed that LTN is not always associated with *Lr34* gene as it was found to be associated with *Lr46/Yr29* locus (Rosewarne *et al.* 2006) and in genotype 'C306' the LTN was not due to *Lr34* gene presence (Mishra *et al.* 2005). Hence, leaf tip necrosis cannot be considered as an exclusive marker for selecting *Lr34* in wheat improvement. Linked molecular markers such as *csLV34* are advocated for utilization in breeding programme for bringing durable rust resistance.

REFERENCES

CIMMYT. 1988. A programme for calculation of AUDPC. CIMMYT, Mexico DF: a software package.

Doyle J J and Doyle J L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin* **19:** 11-15.

German S E and Kolmer J A.1992. Effect of gene *Lr34* on the enhancement of resistance to leaf rust of wheat. *Theoretical Applied Genetics* **84**: 97–105.

Lagudah, E S, McFadden H, Singh R P, Huerta-Espino J, Bariana, H S and Spielmeyer W. 2006. Molecular genetic characterization of the *Lr34 /Yr18* slow rusting resistance gene region in wheat. *Theoretical Applied Genetics* **11**: 421 – 30.

Messmer M M, Seyfarth R, Keller M, Schachermayr G, Winzeler M, Zanetti S, Feuillet C and Keller B. 2000. Genetic analysis of durable leaf rust resistance in winter wheat. *Theoretical Applied Genetics* **100**: 419-431.

Mishra A N, Kaushal K, Yadav S R, Shirsekar G S and Pandey H N. 2005. A leaf rust resistance gene, different from *Lr34*, associated with leaf tip necrosis in wheat. *Plant Breeding* **124**: 517–519.

Rosewarne G M., Singh R P, Huerta-Espino J, William H M, Bouchet S, Cloutier S, McFadden, H and Lagudah E S. 2006. Leaf tip necrosis, molecular markers and 1-proteasome subunits associated with the slow rusting resistance genes *Lr46/ Yr29. Theoretical Applied Genetics.* **112**: 500–508.

Singh R P. 1992. Association between gene *Lr34* for leaf rust resistance and leaf tip necrosis in wheat. *Crop Sciences* **32**: 874–878.

Zadoks J C, Chang T T and Konzak C F. 1974. A decimal code for the growth stages of cereals. *Weed Research* **14**: 415-421.