RESEARCH NOTE

Genetics of leaf and stripe rust resistance in a bread wheat cultivar Tonichi

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Introduction

Leaf rust caused by *Puccinia triticina* (=*P. recondita* Roberage ex Desmaz f. sp. *tritici* Eriks and E. Henn.) and stripe rust caused by *P. striiformis* Westend f. sp. *tritici* are the major foliar diseases of wheat, resulting in yield loss all over the world (Eversmeyer and Browder 1974; Kolmer 1996). The wheat cultivars become susceptible to rusts due to their narrow genetic base for resistance and the rapid rate of evolution of the pathogen, making it necessary to search for new source(s) for resistance. Thus, the wheat production has been largely dependent on the development and the use of resistant cultivars having diverse and well characterized genes. So far, nearly 58 leaf rust and 40 stripe rust resistance genes have been identified and designated as *Lr1* through *Lr58* and *Yr1* through *Yr40*, respectively (McIntosh *et al*. 2005; Kuraparthy *et al*. 2007). Resistance based on single major gene is often considered short lived due to the genetic shifts or the emergence of new virulence in the pathogen population in response to selection imposed by the host. It is believed that, in wheat, certain gene combinations give better and long lasting resistance to rust diseases than given by any of the genes individually (Dyck and Samborski 1982). There are evidence that gene *Lr34* in combination with other *Lr* genes, provide durable resistance to leaf rust (German and Kolmer 1992). The combination of *Lr34* with *Lr12* and/or *Lr13* provided durable leaf rust resistant cultivars worldwide (Roelfs 1988). The gene *Lr34* and *LrT3* interacted to produce enhanced adult plant resistance (Dyck and Samborski 1982) and the *Lr33*+*Lr34* in combination resulted in more resistance than individual genes (Dyck and Johnson 1983). The *Lr34* gene has been widely used in wheat breeding programmes because of its durable resistance to leaf rust and its association with *Yr18*, a stripe rust resistance gene.

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Cultivar, Tonichi (CAR422/Anahuac75), developed at the international maize and wheat improvement centre (CIMMYT), Mexico, has shown resistance to leaf rust and stripe rust in the Indian subcontinent during the past 15 years of testing (Saini *et al*. 2002). This cultivar has also been reported to carry *Lr34* (Singh and Gupta 1991). The present report describes nature and inheritance of leaf rust resistance gene(s) of cultivar Tonichi, their interaction and contribution towards durable rust resistance. Inheritance of stripe rust resistance was also studied at adult plant stage.

Materials and methods

The resistant cultivar Tonichi, the susceptible cultivars WL711 and Agra Local and the resource lines for *APR* genes *Lr12, Lr13, Lr22a, Lr22b, Lr34, Lr48*, and *Lr49* were used for this investigation. The leaf rust and stripe rust reaction, origin, source and parentage of all the cultivars and resource lines used for this study is given in table 1.

Screening against leaf rust

Multipathotype tests were conducted on seedlings and adult plants of all cultivars and NILs against single spore cultures of the variant 77-5 of Indian leaf rust race 77, the most virulent and frequently occurring race from the Indian subcontinent. The seedling avirulence/virulence formula of this race is given below:

77-5: P *Lr9, Lr18, Lr19, Lr24, Lr25, Lr28, Lr29, Lr32, Lr41, Lr45/pLr1, Lr2, Lr3, Lr10, Lr11, Lr12, Lr13, Lr14, Lr15, Lr16, Lr17, Lr18, Lr20, Lr21, Lr22, Lr23, Lr26, Lr27+Lr31, Lr33, Lr34, Lr36, Lr37, Lr42, Lr43, Lr44, Lr46, Lr48, Lr49.*

The leaf of seven-day-old seedling(s) and three flag leaves of adult plant of each cultivar were inoculated separately with uridiniospores, talc mixture of each race keeping inoculum density of 6–8 uridiniospores over a microscopic field area of 2.92 mm^2. The inoculated leaves were

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Table 1. List of cultivar or near-isogenic lines used, their rust reaction, origin, source and parentage.

Cultivar or near -isogenic line	Known leaf rust resistance genes present	Average coefficient of infection against		Origin	Source	Parentage
		Leaf rust	Stripe rust			
Tonichi	–	TR	10.0	Mexico	RAMC	CAR422/Anahuac75
CSP44	*Lr48*	4.0	5.0	Australia	RAMC	WW80/2*WW15//Kalyansona
VL404	*Lr49*	0.5	TR	India	AICWIP	Kentana/Bage/Frontana//General Urquiza/3/ ST464 / PI741061
WL711	–	80.0	70.0	India	PAU	S308/Chris//Kalyansona
Agra Local	–	70.0	70.0	India	PAU	A land race from north Indian state Uttar Pradesh (UP)
Near-isogenic lines in Thatcher background						
RL6011	*Lr12*	60.0	60.0	Canada	PLD	Exchange/6*Thatcher
RL6001	*Lr13*	70.0	20.0	Canada	PLD	7*Thatcher/Frontana//6*Thatcher/ KenyaFarmer//6*Thatcher/PI170925
RL6044	*Lr22a*	4.0	20.0	Canada	PLD	6*Thatcher/Tetra Canthatch/Aegilops squarrosa var. strangulate
Thatcher	*Lr22b*	60.0	20.0	Canada	PLD	Thatcher 78 P^6 T^6 Inc113
RL6058	*Lr34*	40.0	40.0	Canada	PLD	6*Thatcher/PI158548

TR, traces of resistance; RAMC, Dr R. A. McIntosh Plant Breeding Institute, 107 Cobbitty Road, Cobbitty NSW 2570, Australia; AICWIP, All India Coordinated Wheat Improvement Project, Karnal, Haryana, India; PLD, Dr P. L. Dyck, Research Station, Agriculture Canada, Winnipeg, Manitoba, Canada; PAU, Punjab Agricultural University, Ludhiana, Punjab; AICWIP, All India Coordinated Wheat Improvement Project, Karnal, Haryana, India; '–', not known.

incubated in a dark chamber maintained at 20 ± 1°C at 100 per cent relative humidity for 16 h and were shifted to 20°C ± 2°C. After 14 days of inoculation the types of infection were recorded using the scale proposed by Stakman *et al*. (1962). Disease severity for all cultivars and NILs was assessed by growing adult plants of these in open experimental field.

For inheritance studies, cultivar Tonichi was crossed with the susceptible cultivar WL711 and for allelic tests this cultivar was crossed with VL404(*Lr49*), CSP44(*Lr48*) and RL6058(*Lr34*). The F_1, F_2 and F_3 generations from the cross of cultivar Tonichi/WL711, and only the F_1 and F_2 generations from the crosses of Tonichi/VL404, CSP44 and RL6058(*Lr34*), were sown in open experimental field in paired rows. Two rows of each of the two susceptible cultivars Agra Local and WL711 were planted after every twenty experimental rows as well as, all around the experimental plots to ensure quick spread of the disease. Three flag leaves of each plant were inoculated using urediniospore-talc mixture of race 77-5. The types of infection on these plants were recorded for 21 days after the inoculation using the scale of Stakman *et al*. (1962), and they were later assessed for terminal disease severity.

Screening against stripe rust

The adult plants for stripe rust studies were sown in an isolated plot approximately 3 km away from the leaf-rust plot, to avoid contamination with leaf-rust spores. Stripe rust race 46S119 having the following avirulence/virulence formula was used to determine the nature and number of stripe rust resistance genes in field studies: 46S119: P *Yr1, Yr5, Yr10, Yr15, Yr17, Yr24, Yr26, Yr27*/p *Yr1, Yr6, Yr7, Yr8, Yr9, Yr11, Yr12, Yr18*. This material for leaf rust and stripe rust was repeatedly spray inoculated every evening with urediniospores of race 77-5 and 46S119, respectively suspended in water (1 g of inoculum per 10 l water, using one drop of Tween-20 as dispersant). The observations on the leaf rust and stripe rust severity were recorded as percentage of leaf area covered with rust according to the modification of the Cobb scale as described by Peterson *et al*. (1948). The plants with disease severity less than susceptible parent were classified as resistant and those with severity equal to or higher than the susceptible parent were classified as susceptible. Simple chi-square (χ^2) test was applied to fit appropriate genetic ratio in F_2 and F_3 generations.

Results and discussion

Studies on leaf rust resistance

The seedlings of cultivars Tonichi, CSP44(*Lr48*), VL404(*Lr49*), WL711, Agra Local and NILs Tc+*Lr12*, Tc+*Lr13*, Tc+*Lr22a*, Tc+*Lr22b* and Tc+*Lr34* developed high infection types (3 to 33+). The adult plants of cultivars Tonichi, CSP44(*Lr48*), VL404(*Lr49*) and NIL Tc+*Lr22a* developed resistant infection types varying from ';' to ';1^+2'. It is inferred that cultivar Tonichi has hypersensitive adult plant resistance gene(s) effective against leaf rust race 77-5 which may be either of the genes *Lr22a, Lr48, Lr49* or some unknown gene(s). The gene *Lr22a* has been derived from *Triticum tauschii* (Dyck and Kerber 1970), and therefore presence of *Lr22a* in cultivar Tonichi is unlikely. The presence of genes *Lr48* and *Lr49* in cultivar Tonichi

was determined by allelic tests with cultivar CSP44(*Lr48*) and VL404(*Lr49*). The terminal disease severity for cultivars/NILs Tc+*Lr12*, Tc+*Lr13*, Tc+*Lr22b*, WL711 and Agra Local under field conditions varied between 60S and 80S. While the remaining cultivars and NILs were resistant with severity from TR to 40S.

In the inheritance studies, the F_1 adult plants from the cross of Tonichi/WL711 showed resistant infection types (;1^+) while the F_2 generation contained 160 resistant (R) and 54 susceptible (S) plants ($\chi^2_{3R:1S}$ = 0.006). The F_3 generation had 27 homozygous resistant (HR), 86 segregating (segr.) and 46 homozygous susceptible (HS) families ($\chi^2_{1HR:2segr:1HS}$ = 5.60). These observations suggest that low adult plant reaction of cultivar Tonichi to race 77-5 is conferred by a dominant hypersensitive resistance gene. These results ruled out the presence of recessive gene *Lr48* (Saini *et al*. 2002). Therefore the F_2 generation from the cross of Tonichi/CSP44 (*Lr48*) was not studied. Allelic tests with VL404(*Lr49*) showed all the 185 F_2 plants were resistant which confirmed that hypersensitive adult plant resistance gene of Tonichi is *Lr49*.

After the infection type data, these plants were scored for terminal disease severity. The F_1 plants showed disease severity of TR-5MR while the F_2 and F_3 generations segregated into 154R : 6S plants ($\chi^2_{15R:1S}$ = 1.71) and 116 HR : 152 segr. : 11 HS progenies ($\chi^2_{7HR:8segr:1HS}$ = 3.79), respectively. These results imply that two genes determine the low terminal disease severity of cultivar Tonichi. The second resistance gene of cutivar Tonichi is proposed to be *Lr34* and was confirmed by allelic tests with RL6058 (*Lr34*). Cultivar Tonichi also showed leaf tip necrosis, a morphological marker linked to the nonhypersensitive leaf rust resistance gene *Lr34* (Singh 1992). One important observation in scoring of rust severity is that the 154 resistant F_2 plants showed a wide variation in leaf rust severity (from TR to 40S), based on which resistant plants were categorized into three different groups i.e., plants with leaf rust severity of TR-5MR, 10MR–20MR and 30S–40S (table 2). Along with six susceptible plants F_2 generation segragated into 9 (TR-5MR):3 (10MR–20MR):3 (30S–40S):1 (70S–80S) ratio, as expected for two gene segregation. These observations indicated that when present singly fixed before and after ratio gene *Lr49* and *Lr34* gave leaf rust score 10MR–20MR and 30S–40S, respectively. But when present together, leaf rust score reduces to TR-5MR. Similar observations were further confirmed in F_3 generation. There was difference of leaf rust severity in different homozygous resistant and segregating families. There were five different types of HR families segregating in 1:2:1:2:1 ratio and three different type of segregating families in 1:2:1 ratio. Thus, the combination of *Lr49* and *Lr34* enhance the level of resistance. The additive nature of genes is useful in lowering the disease severity to its acceptable level for wheat breeding.

This kind of interaction where the combination of genes give higher level of resistance than when present separately have been reported earlier by several authors

Table 2. Observed and expected number of resistant F_2 plants, F_3 families, their genotype and leaf rust severity against race 77-5, in the cross of Tonichi with WL711.

Genotypic class	Disease severity	Number of plants/families		χ^2
		Observed	Expected	
F_2 generation				
A_B_(9)	TR-5MR	99	90	0.90
A_bb(3)	30S–40S	24	30	1.20
aaB_(3)	10MR–20MR	31	30	0.03
aabb(1)	70S–80S	6	10	1.60
Total		160	160	$\chi^2(9:3:3:1) = 3.73; P = 0.2921$
F_3 (HR families)				
AABB(1)	TR-5MR	13	16.57	0.76
AABb(2) (1AABB:2AABb:1AAbb)	TR–40S	30	33.14	0.29
AAbb(1)	30S–40S	12	16.57	1.26
AaBB(2) (1AABB:2AaBB:1aaBB)	TR-20MR	43	33.14	2.9
aaBB(1)	10MR–20MR	18	16.57	0.09
Total		116	116	$\chi^2(1:2:1:2:1) = 5.27; P = 0.2607$
F_3 (segregating families)				
aaBb(2) (1aaBB:2aaBb:1aabb)	10MR–80S	43	38	0.66
AaBb(4) (9A_B_:3A_bb:3aaB_:1aabb)	TR–80S	65	76	1.59
Aabb(2) (1AAbb:2Aabb:1aabb)	40S–80S	44	38	0.95
Total		152	152	$\chi^2(1:2:1) = 3.20; P = 0.2019$
F_3 (susceptible families)				
Aabb	70S–80S	11	11	0

A, *Lr34*; B, *Lr49*.

(Kolmer *et al*. 1991; Kloppers and Pretorius 1997; Samborski and Dyck 1982) and often forms the basis of durable resistance. The adult plant resistance gene *Lr34* has already received attention of the wheat breeders because of its association with durable resistance and its presence in large number of culivars throughout the world (Shiwani and Saini 1993). Unlike the seedling resistance genes, the adult plant resistance express only when the wheat plants enter into reproductive phase, thus sustaining the pathogen races even when the resistance shown by the carrier wheat is very high at adult plant stage. This decreases the selection pressure on pathogen, reducing the chances of evolution of new virulence.

Studies on stripe rust resistance

The F_1 adult plants from cross of Tonichi/WL711 showed stripe rust severity of 30S–40S in field. The F_2 generation had 150R and 25S plants ($\chi^2_{13R:3S}$ = 2.2). The F_3 generation contained 123HR, 131segr. and 25HS families ($\chi^2_{7HR:8segr:1HS}$ = 3.7). This distribution of F_2 plants and F_3 generations indicate the presence of one dominant and one recessive gene for stripe rust resistance in cultivar Tonichi. The dominant gene is *Yr18*, because Tonichi has the linked leaf rust resistance gene *Lr34* which is confirmed by allelic tests with RL6058(*Lr34–Yr18*). The identity of second stripe rust resistance gene was not established. This recessive gene is given a temporary symbol *YrT*. Further testing at seedling and adult plant stage is required to designate this gene.

Based on the present results, it is concluded that the long lasting resistance of cultivar Tonichi to leaf and stripe rust in the Indian subcontinent and elsewhere in the world may be due to interactive action of the leaf rust and stripe rust resistance genes carried by this cultivar. These type of gene combinations may be a good alternative for durable resistance as it behaves like horizontal resistance thus avoiding the evolution of new races of pathogen. Singh *et al*. (2004, 2005) also reported two to five more genes for leaf rust and stripe rust resistance from many cultivars in addition to *Lr34* and *Yr18* that are contributing towards their durable resistance. The recessive stripe rust resistance gene identified from cultivar Tonichi in present investigations may be similar to one of the genes reported by Singh *et al*. 2004 from some cultivars having proven record of durable resistance. Therefore, cultivar Tonichi may be useful in developing cultivars with long lasting resistance to leaf rust and stripe rust diseases in Indian subcontinent.

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