Pak. J. Bot., 39(3): 953-959, 2007.

EVIDENCE OF ISOLATE-SPECIFICITY IN NON-HYPERSENSITIVE RESISTANCE IN SPRING WHEAT (*TRITICUM AESTIVUM*) TO WHEAT LEAF RUST

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Abstract

Isolate-specific aspect of non-hypersensitive resistance in wheat to wheat leaf rust was studied at seedling stage in the green house. Isolate-specific response of non-hypersensitive resistance was assessed from latency period (LP) and infection frequency (IF) of two single-pustule isolates of *Puccinia triticina* in 26 spring wheat cultivars/lines. Small but significant cultivar x isolate interactions were observed for LP and IF in seedlings of host genotypes. Isolate specific effect for LP at seedling stage was consistent and reproduced in a repeated experiment; however, the interaction for IF was inconsistent and was not reproducible. The inconsistency in cultivar x isolate interaction may be due to some non-genetic origin. The result suggested that a gene-for-gene relationship could exist between non-hypersensitive resistance genes in the host and genes in pathogen.

Introduction

Rusts are the most destructive and also the most widely recognised diseases of wheat crop. Wheat leaf rust caused by *Puccinia triticina,* is the commonest, most widely distributed of the cereal rusts. It occurs worldwide, where wheat is grown (Chester, 1946).

Systematic breeding for disease resistance started after the discovery of genetics of resistance (Biffen, 1905). Since the time, resistance, based on hypersensitive host response, dominated the wheat breeding for resistance against leaf rust. The hypersensitive is characterised by discrete phenotypes and is conferred by a single or a few major genes (*Lr*). Race specificity is a prominent characteristic of such resistance type where every host resistance is elicited by recognition of a certain avirulence factor produced by pathogen as postulated in the gene-for-gene model (Flor, 1956, 1971) and coined as vertical resistance by Van der Plank (1963). Resistance based on single, major, race–specific genes often become ineffective within 5 years after its introduction in commercial cultivars (Kilpatrick, 1975).

Because of lack of durability of hypersensitive resistance genes, research was initiated to investigate other ways to protect the crops against such pathogens. Non-hypersensitive resistance also called partial resistance (Parlevliet, 1975), is considered to be more durable (Parlevliet, 1985), the most valuable alternative to hypersensitive resistance. Partial resistance is characterised by reduced epidemic build-up though its infection type indicates the absence of hypersensitive resistance (Parlevliet & Van Ommeren, 1975). Partial resistance is assumed to be due to joint effect of longer latency period, lower infection frequency and smaller spore production, latency period being the most important component (Shaner & Finney, 1980, Teng *et al*., 1977).

Table 1. The list of 26 wheat cultivar/ lines used in the present study and level of non-hypersensitive resistance.

	Cultivar/line	Level of PR	Author and year
1	Baldus	High	List of recommended Cultivars in the Netherlands.
2	Anemos	Medium	List of recommended Cultivars in the Netherlands
3	Minaret	Rather poor	List of recommended Cultivars in the Netherlands
4	Akabozu	Fairly high	Broers, 1989a
5	Thatcher	Low	Rubiales & Niks, 1995
6	Thatcher *Lr34*	Moderate	Rubiales & Niks, 1995
7	BH1146	High	Broers, 1989a
8	Lalbahadur	Susceptible	Singh *et al.*, 1998
9	Lalbahadur *Lr34*	Fairly High	Singh & Rajaram, 1992
10	Lalbahadur *Lr46*	Fairly High	Singh *et al.*, 1998
11	Morocco	Low	Jacobs, 1990
12	Pavon 76	High, depending on isolate	Singh *et al.*, 1998
13	Skalavatis Bearded	Low	Broers, 1989a
14	Skalavatis Unbearded	Low	Broers, 1989a
15-26	*CBRG 19-CBRG30	Very high	Singh *et al*., 2000

*GBRG19-CBRG30 were obtained from Dr. R.P. Singh (CIMMYT), and bred to be near immune to wheat leaf rust by compiling genes for non-hypersensitive resistance.

It is often assumed that partial resistance is quantitatively expressed and non-race specific, fitting the concept of the "horizontal" resistance (Van der Plank, 1963). However, race specific effects have been reported in some plant-pathogen systems (Parlevliet, 1977; Parlevliet, 1979; Todorova, 2000; Van Silfhout, 1993). The underlying cause or such interaction was proposed as a "minor gene-for-minor gene" model (Parlevliet & Zadoks, 1977). More detailed investigation is needed to understand better its complexity and specific characters. This will help to improve and develop better selection strategies for developing non-hypersensitive resistant cultivars. This study was aimed to investigate the race-specific aspect of non-hypersensitive resistance in wheat to leaf rust.

Materials and Methods

Materials

The research was carried out in the Laboratory of Plant Breeding, Wageningen University, The Netherlands during 2001-2002.

Wheat genotypes: The experiments were performed on 26 wheat cultivars/lines. Which included 5 susceptible cultivars, 3 partially resistance cultivars, 12 CIMMYT lines, 3 cultivars from the list of recommended cultivars in the Netherlands and 4 near isogenic lines (Table 1).

Pathogen: Two isolates of *P. triticina* viz., INRA and Ventas were used in the experiments. Dr. Gouyot provided single uredinials of French isolate "INRA". The Spanish isolate "Ventas" and French isolate "INRA" were multiplied in Laboratory of Plant Breeding, Wageningen University.

Methodology: The research comprised three experiments. First experiment contained 12 lines obtained from CIMMYT while second experiment contained 14 cultivars/lines. On the basis of result of first two experiments, 12 cultivars/lines showing cultivar x isolate interaction and/or with high level of partial resistance were selected for 3rd experiment. The experimental design was completely randomise design with two isolates and four seedlings of each cultivar/line. The plants were sown in wooden boxes of about 30 x 30 cm and each cultivar or line was represented by four seedlings.

Ten days after sowing, the primary leaves of the seedlings were fixed in horizontal position with adaxial side upward. Per box 4 mg of urediospore was mixed with lycopodium spores (1:10), vol./vol.), and applied using a settling tower. In each box a greased glass slide was placed for later determination of inoculation density. The spore density per cm ^{2} was measured and statistically analysed by using F-test. The average inoculation density was about 231/cm^2. After inoculation, the plants were kept overnight (19 hours) in a high humidity chamber at 100% R.H., and in complete darkness and then transferred to a green house.

Latency period (LP), infection frequency (IF) and infection type (IT) were measured on primary leaf. For latency period, observations started 5 days after inoculation. Infection frequency and infection type was recorded after completion of sporulation.

The LP was measured on the basis of daily pustule counts (Parlevliet, 1975). When light green flecks, which precede pustules, became visible, on the central part of leaves, a segment containing about 30-60 flecks was marked. The number of pustules (urediosori) in these segments was counted every day, using a10x pocket lenses, until the number did not increase anymore.

IF was measured using a metal strip with 2 x 0.5 cm^2 window (Parlevliet & Kuiper, 1977). The metal strip was placed on the central part of the primary leaf. The number of pustules within the window was a direct estimate for IF in number of pustules per cm^2. IT was determined according to a scale from 0-9 (McNeal *et al*., 1971).

Data were analysed using statistical procedure, analysis of variance (Steel & Torrie, 1981). Duncan's multiple range test (Duncan, 1955) was used to compare the isolates and cultivars/lines.

Results

Latency period: The most important component of non-hypersensitive resistance LP can only be determined on the genotypes that exhibit a compatible infection type (IT, 7 or high). In the present study all CIMMYT lines and all other spring wheat cultivars studied exhibited a high IT at primary leaf to *P. triticina*. The percentage of early aborted infection units with plant cell necrosis was lower than 8%. So, it can be concluded that there was not hypersensitive reaction in this material.

The analysis of variance for LP on CIMMYT lines (experiment 1) at primary leaf showed that effect of Cultivar x isolate interaction was highly significant. For example, a strong cultivar x isolate interaction was found between CBRG 19 and CBRG 20 for isolate "INRA" and isolate "Ventas" (Fig. 1). A similar cultivar x isolate interaction was observed between CBRG 22 and CBRG 24 with both isolates (Fig. 1). However, in both cases there was not a clear differential interaction.

Analysis of variance for LP in experiment 2 did not show a significant cultivar x isolate interaction but in the repeated experiment (experiment 3) cultivar x isolate interaction was significant. For example, significant interaction was observed between CBRG 19 and CBRG 20 and between CBRG 22 and CBRG 24 with both isolates (Fig. 2).

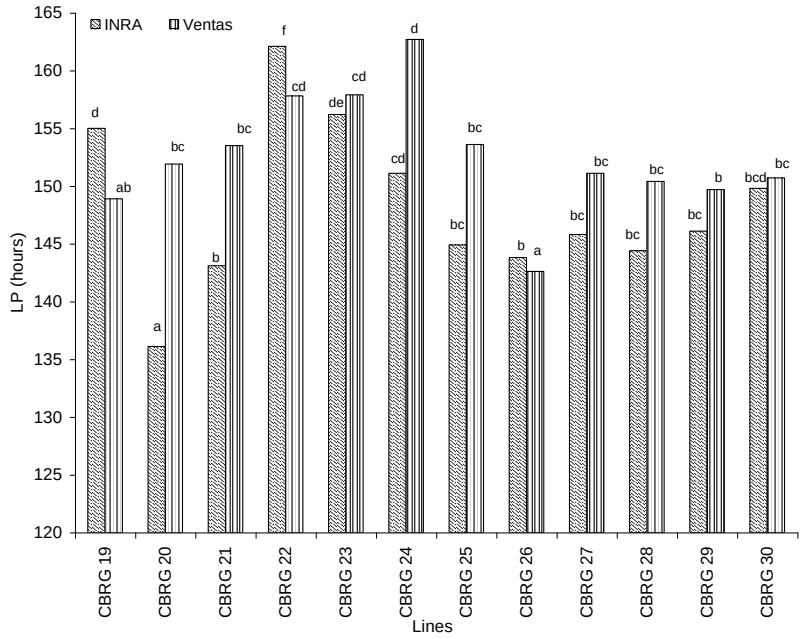

Fig. 1. Mean LP of two isolates of *P. triticina* on twelve CIMMYT wheat lines, the bars indicating the same isolate with same letters are not significantly different (Duncan's multiple range test, p = 0.05).

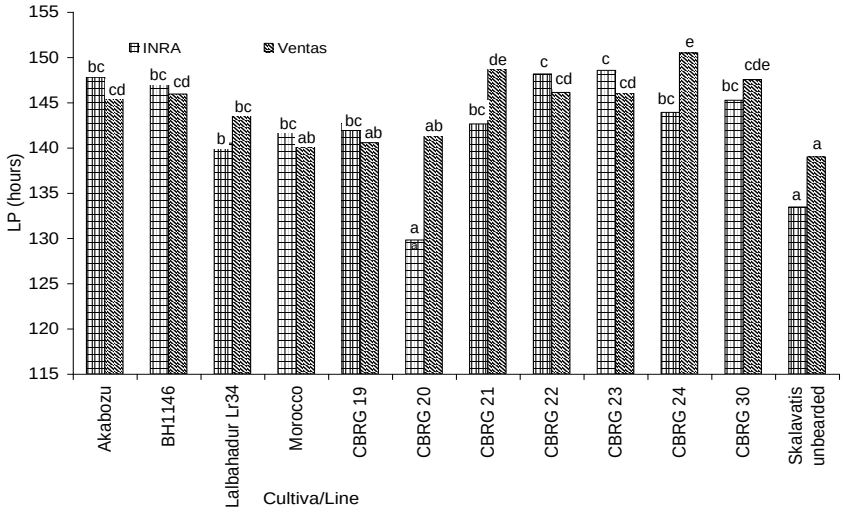

Fig. 2. Mean of LP of two *P. triticina* isolates on the wheat cultivars/lines included in experiment 3 and the bars indicating same isolate with a letter in common are not significantly different (Duncan's multiple range test, p≤ 0.05).

Table 2. Mean IF (number of pustules per square centimeter of leaf area) of two isolates of *P. triticina* on some spring wheat cultivars/CIMMYT lines in three seedling stage experiments.

Cultivar/line	Experiment 1 and 2		Experiment 3	
	INRA	Ventas	INRA	Ventas
CBRG 20	22.5a[x]	27.8c[x]	77.3abcd[x]	87.0abc[x]
CBRG 24	17.3a	6.5a	84.0bcd	70.0a
CBRG 23	19.3ab	5.7a	59.0a	100.8cd
BH1146[*]	52.5ab[x]	28.5abcd[x]	82.0bcd	92.0abcd
Lalbahadur *Lr34*[*]	89.0def	28.8 abcd	89.5cd	92.0abcd

[x] Numbers within a column and with in an experiment followed by a letter in common are not significantly different (Duncan multiple range test, $p \leq 0.05$).
[*]Data have been taken from experiment 2.

Infection frequency: Infection frequency of the isolates (INRA and Ventas) on primary leaf of wheat in these three experiments was also analysed. In all three experiments the cultivar x isolate interaction was highly significant. The most extreme example for 12 CIMMYT lines (experiment 1) was the interaction between CBRG 20 and CBRG 24 with both isolates (Table 2).

In the seedling stage experiment 2, BH1146 and Lalbahadur *Lr34* showed a clear interaction for IF (Table 2). Accordingly, no clear, reproducible interaction could be detected in experiment 3. The interaction between CBRG 20 and CBRG 24 as well as the interaction between BH 1146 and Lalbahadur *Lr34* did not reappear in the experiment 3 (Table 2). A very clear cultivar x isolate interaction was observed between CBRG 23 and CBRG 24 with both isolates in experiment 3 (Table 2).

Discussion

The small but significant interactions between host genotype and pathogen isolate for single components of non-hypersensitive resistance detected suggest that the resistance is not completely race-non-specific. Isolate specific effect for LP at seedling stage was consistent and reproducible (Fig. 1). For example, LP of isolate "INRA" on CBRG 19 was longer than LP of isolate "Ventas" but LP of isolate "INRA" was significantly shorter on CBRG 20 than LP of isolate "Ventas". Accordingly, the resistance in the line CBRG 24 was more effective to isolate "Ventas" than to isolate "INRA" but for line CBRG 22 the resistance is more effective to isolate "INRA" than to isolate "Ventas". These interactions reappeared in the third experiment. The research findings here are in agreement with those observed in barley/barely leaf rust by Parlevliet (1977) and in wheat by Kuhn *et al.* (1978). Former observed a significant and reproducible but small deferential interaction for non-hypersensitive resistance. In the data of Kuhn *et al.* one can identify a small interaction for LP of wheat leaf rust in seedling stage. These results corroborate the “integrated concept” of Parlevliet & Zadoks (1977) in which both vertical and horizontal resistance are supposed to act on gene-for-gene base. The interactions found here are rather small; indicating that gene effects may be quite small and non-hypersensitive resistance may of a polygenic nature.

Although in individual experiments isolate specific effects for IF was observed (Table 2), however, the results were inconsistent and did not reappear in the third experiment. Broers (1989b) found similar results for non-hypersensitive resistance in wheat to leaf rust

and came to conclusion that the race specific effect of partial resistance is fairly and regularly occurs but inconsistent and hard to repeat. These results lead to question why cultivar x isolate interaction is not consistent. There are at least three reasons, like wrong statistical decisions, differences in the amount of initial inoculum and differences in environmental conditions that may influence the cultivar x isolate interaction. The non-hypersensitive resistance is a complex system with an expression that is highly dependent on cultivar, race and environmental conditions (Broers, 1989b). When different cultivars have different optimal environmental conditions (especially temperature) for expression of their resistance, a cultivar–environment effect may appear. Additionally, isolates may have different optimum temperature for expression of their aggressiveness. These differences might affect reappearance of small significant cultivar x isolate interactions for non-hypersensitive resistance.

To study consistency of the race-specific effects of non-hypersensitive resistance, the experiments should be performed under similar environmental conditions with same incubation period. Further more, full proof of minor gene -for-minor gene hypothesis (Flor, 1956, 1971) requires a genetic analysis of avirulence genes in pathogen.

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(Received for publication 29 January 2004)