

## Characterization of *Lr46*, a gene conferring partial resistance to wheat leaf rust

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Components of resistance conferred by the *Lr46* gene, reported as causing “slow rusting” resistance to leaf rust in wheat, were studied and compared with the effects of *Lr34* and genes for quantitative resistance in cv. Akabozu. *Lr34* is a gene that confers non-hypersensitive type of resistance. The effect of *Lr46* resembles that of *Lr34* and other wheats reported with partial resistance. At macroscopic level, *Lr46* produced a longer latency period than observed on the susceptible recurrent parent Lalbahadur, and a reduction of the infection frequency not associated with hypersensitivity. Microscopically, *Lr46* increased the percentage of early aborted infection units not associated with host cell necrosis and decreased the colony size. The effect of *Lr46* is comparable to that of *Lr34* in adult plant stage, but in seedling stage its effect is weaker than that of *Lr34*.

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Leaf rust, caused by *Puccinia triticina*, is an important disease in most wheat growing areas. The use of genetic resistance is the most economical and environmentally friendly way to combat this disease. Breeders commonly have relied on race specific *Lr* genes for hypersensitive resistance (HR) which are very effective in reducing the epidemic build-up and easy to manage in breeding programmes because of their monogenic nature. But new races of the rust fungus frequently defeat HR. There is a great interest in improving the durability of the intrinsically “non-durable” types of resistance by gene pyramiding or by use of multilines. Another possibility would be to identify and introduce resistance types that are intrinsically durable like partial resistance (PR). PR is characterized by a slow epidemic build-up despite a high infection type (non-hypersensitive type of resistance) indicating a compatible host-pathogen interaction (PARLEVLIET 1975). In most cases, PR is inherited polygenically (PARLEVLIET 1979; QI et al. 1998) and hence selection for higher levels of resistance is less straightforward than for monogenically inherited traits. There are indications that PR of some wheat cultivars against wheat leaf rust may rely on a combination of few genes. One of them is *Lr34*, located on chromosome 7DS of wheat. This gene, formerly known as *LrT2*, is a gene that confers partial resistance to wheat leaf rust sensu Parlevliet (RUBIALES and NIKS 1995).

Pavón 76 is a Mexican bread wheat cultivar and carries slow rusting to leaf rust since its release in

1976. This slow rusting is based on two genes with additive effects. One of these genes has been identified and named as *Lr46*. It is located on chromosome 1B of Pavón 76 (SINGH et al. 1998).

Our aim in this paper is to characterise the phenotypic expression of *Lr46* and to compare it with that of *Lr34* and with the effect of the quantitative resistance genes present in partially resistant cv. Akabozu.

### MATERIALS AND METHODS

#### *Plant material*

This study was performed on the susceptible cv. Lalbahadur (*Lr1*) and the lines Lalbahadur – *Lr34* (Lalbahadur “Parula 7D”, chromosome substitution line) and Lalbahadur – *Lr46* (Lalbahadur “Pavón 76 1B” chromosome substitution line) (SINGH et al. 1998). The cultivars Little Club and Akabozu were included as susceptible and partially resistant checks.

#### *Inoculations*

The isolate of *Puccinia triticina* used in the experiments was “B9414-1CA3”, kindly provided by Dr. H. Goyeau (INRA, Lab. Pathologie Végétale, Thiverval-Grignon, France). It is virulent in seedling on *Lr1*, *2c*, *3*, *3bg*, *11*, *12*, *13*, *14a*, *14b*, *16*, *18*, *21*, *22*, *26*, *33*, *34*, *37*, *44(I)* and *B(I)*.

For the seedling tests, plants were grown in soil in plant boxes (37 × 39 × 5 cm). Seven replications of 4–6 plants of each line each were performed. Eleven days after sowing, first leaves were fixed adaxial side

upwards in the box with metallic clips. Leaves were inoculated in a settling tower with 4 mg of rust urediospores mixed with *Lycopodium* spores (1:20 v/v) resulting in a deposition of about 120 spores/cm<sup>2</sup>. The plants were incubated overnight, about 9 hours, in a mist chamber at 20°C with 100% relative humidity. They were then transferred to a climate room (21°C and 70% RH) during the latency of rust. When the first uredia appeared, these plants were transferred to a greenhouse compartment (18°–23°C day-night range).

For the adult plant test, plants were grown in 12 × 12 cm pots in a greenhouse. Adult plants were inoculated when they had just expanded the flag leaf. Three replications were carried out of four plants each. Inoculation was performed on flag leaves fixed to a board with their adaxial side up. The inoculum consisted of a 1:20 (vol./vol.) mixture of spores and *Lycopodium* spores. For each pot the quantity of urediospores applied was 1 mg. The mixture was evenly dusted over the plants. Incubation was as described before.

We used four to six leaves per line per replication for macroscopic observations and three leaves per line per replication for microscopic observations. The experimental units for the statistical analysis were the average over all leaves of a genotype within a replication.

#### Macroscopic observations

Latency period, infection frequency and infection type were recorded. Latency period was taken as the time period from the beginning of incubation to the time at which the 50% of the uredia had appeared (PARLEVLIE 1975). Latency period was determined by daily counting the number of uredia visible in a marked area until the number no longer increased. Infection frequency was determined on the marked area of the leaves. The final number of uredia was used to calculate the number of uredia per cm<sup>2</sup>. Infection type was recorded 12 days after inoculation, according to a 0–9 scale (MCNEAL et al. 1971).

The means of the observed values of latency period and infection frequency were converted into relative values per replication, setting the observed values obtained on Lalbahadur at 100%.

#### Microscopic observations

Central segments of about 3 cm<sup>2</sup> of inoculated leaves were collected five days after inoculation. Three leaves per accession were harvested from each replication. The segments were processed for fluorescence microscopy (ROHRINGER et al. 1977) but instead of Calcofluor we used UVITEX 2B (Ciba-Geigy). The preparations were examined at 200× with a Leica

epifluorescence microscope (DM LB, 330 to 380 nm wavelength transmission). Sporelings that had not formed any haustorial mother cell were excluded. At least 100 sporelings per leaf segment were scored and classified according to their stage of development (NIXS 1982). Infection units that formed a primary infection hypha and no more than six haustorial mother cells were considered as early aborted. Sporelings with more than six haustorial mother cells were classified as established. Necrosis of host cells was visible by using a filter with a range between 420 and 490 nm transmission, which displays a golden yellow autofluorescence. The length (L) and width (W) of ten arbitrarily chosen established colonies per leaf were measured with an eyepiece micrometer. The shape of the colony was considered as an ellipse where L and W were major and minor axes. Colony size (CS) was calculated as the geometric mean of L and W. Therefore:  $CS = \text{SQRT}(1/4 \times L \times W)$ .

## RESULTS

### Macroscopic observations

Macroscopic components of the resistance are shown in Table 1. All lines displayed a high infection type both in seedlings and adult plants. In seedlings, latency period was significantly longer in Lalbahadur-Lr34 than in Lalbahadur. The prolongation of the latency period was not significant in seedlings of Lalbahadur-Lr46. In adult plants, latency period on both Lalbahadur-Lr34 and Lalbahadur-Lr46 was significantly longer than on Lalbahadur. For none of the lines the latency period was as long as that for Akabozu. The lines did not differ significantly in infection frequency in seedling nor in adult plant, except for Akabozu, on which the infection frequency tended to be reduced.

Disease severity was highly reduced in Lalbahadur-Lr34 and Lalbahadur-Lr46 in field studies under multicyclic disease progress situation (data not shown).

### Microscopic observations

Data from the microscopic observations are shown in Table 2. Only Akabozu displayed a higher percentage of early aborted colonies without plant cell necrosis in seedlings. In adult plants also, Lalbahadur-Lr34 and Lalbahadur-Lr46 showed an increase in the percentage of early aborted colonies that were not associated to cell necrosis. The colonies of *Puccinia triticina* in seedlings of Lalbahadur-Lr34 were smaller than those of Lalbahadur but those of Lalbahadur-Lr46 did not differ significantly from Lalbahadur. In adult plants, the size of colonies supported by

Table 1. Macroscopic components of resistance of wheat to *Puccinia triticina* "B9414-1CA3" induced by *Lr34* and *Lr46* in seedling (primary leaf) and adult plant (flag leaf) stage

| Lines              | IT <sup>1</sup> |           | RLP <sup>2</sup>            |                             | RIF <sup>2</sup>           |                            |
|--------------------|-----------------|-----------|-----------------------------|-----------------------------|----------------------------|----------------------------|
|                    | Primary leaf    | Flag leaf | Primary leaf                | Flag leaf                   | Primary leaf               | Flag leaf                  |
| Lalbahadur         | 9               | 9         | 100 c <sup>3</sup><br>(137) | 100 d <sup>2</sup><br>(175) | 100 a <sup>2</sup><br>(84) | 100 a <sup>2</sup><br>(28) |
| Lalb.- <i>Lr34</i> | 9               | 9         | 107 b                       | 116 c                       | 86 a                       | 84 a                       |
| Lalb.- <i>Lr46</i> | 9               | 9         | 103 bc                      | 109 b                       | 94 a                       | 104 a                      |
| Little Club        | 9               | 9         | 103 bc                      | 100 d                       | 90 a                       | 100 a                      |
| Akaboza            | 9               | 9         | 120 a                       | 129 a                       | 66 b                       | 71 a                       |

<sup>1</sup> Infection type (IT) according to MCNEAL et al. (1971).

<sup>2</sup> Relative latency period (RLP) and relative infection frequency (RIF) expressed as values relatives to Lalbahadur (= 100 %). The actual average values for Lalbahadur of latency period (hours) and infection frequency (uredia per cm<sup>2</sup>) are presented in brackets.

<sup>3</sup> Letters in common per column indicate that differences are not statistically significant (Duncan, P < 0.05).

Table 2. Microscopic components of resistance of wheat to *Puccinia triticina* "B9414-1CA3" induced by *Lr34* and *Lr46* in seedling and adult plant stage

| Lines              | % EA <sup>-1</sup> |           | CS (mm <sup>2</sup> ) <sup>2</sup> |           | % Necrosis <sup>3</sup> |           |
|--------------------|--------------------|-----------|------------------------------------|-----------|-------------------------|-----------|
|                    | Seedling           | Flag leaf | Seedling                           | Flag leaf | Seedling                | Flag leaf |
| Lalbahadur         | 2 c <sup>4</sup>   | 1 d       | 0.30 a                             | 0.070 a   | 2 a                     | 17 a      |
| Lalb.- <i>Lr34</i> | 3 bc               | 16 bc     | 0.20 b                             | 0.025 b   | 2 a                     | 12 a      |
| Lalb.- <i>Lr46</i> | 6 abc              | 16 bc     | 0.25 ab                            | 0.032 b   | 1 a                     | 19 a      |
| Little Club        | 4 c                | 7 cd      | 0.32 a                             | 0.043 ab  | 0 b                     | 7 ab      |
| Akaboza            | 11 a               | 29 b      | 0.17 b                             | 0.011 c   | 1 a                     | 3 b       |

<sup>1</sup> % EA-: Percentage of early aborted infection units not associated with plant cell necrosis.

<sup>2</sup> CS: Mean colony size (mm<sup>2</sup>).

<sup>3</sup> % Necrosis: percentage of infection units associated with plant cell necrosis.

<sup>4</sup> Letters in common per column indicate that differences are not statistically significant (Duncan, P < 0.05).

Lalbahadur-*Lr34* and Lalbahadur-*Lr46* was significantly smaller than of the colonies in Lalbahadur. The percentage of infection units associated with host cell necrosis was in all lines negligible (less than 2 % of the infection units).

## DISCUSSION

*Lr34* is a gene has been reported to cause an increase in latency period, in percentage of early aborted colonies not associated with cell necrosis and a decrease of colony size (RUBIALES and NIKS 1995). Its effects are more pronounced in adult plants than in seedlings. These parameters are typical to partial resistance (JACOBS 1990; JACOBS and BUURLAGE 1990). The effect of *Lr46* resembled that of the *Lr34* in adult plants. Both prolong the latency period and cause a higher percentage of abortion, a reduced colony size and a lower disease severity relative to the check Lalbahadur. However, in seedling stage the effect of *Lr46* appeared as a tendency, and was

not sufficiently substantial to be statistically significant.

Our results indicate that *Lr46* confers a similar non-hypersensitive type of defence to wheat leaf rust as *Lr34*, but its effect is smaller than that of *Lr34*. It has been reported that *Lr34* in combination with other genes enhances resistance and durability (RUBIALES and NIKS 2000). Other genes are reported to have the similar effects in other pathosystems. The gene *Sr2* is a gene conferring "slow rusting" too and it also enhances resistance and durability to stem rust in combination with other genes (ROELFS 1988). *Lr34* is widely distributed in the commercial bread wheats (SINGH 1993). Recently, KOLMER and LIU (2001) reported that wheat lines BH1146 and Westphal 12A contained *Lr34* among other genes, indicating that this gene is widespread, and responsible for a substantial part of the partial resistance found in wheat against wheat leaf rust. It would be very interesting to determine whether the *Lr46* would be useful to enhance the effect of the *Lr34* gene substantially.

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