

Expression of Two Wheat Leaf Rust Resistance Gene Combinations Involving *Lr34*

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ABSTRACT

Drijepondt, S. C., Pretorius, Z. A., and Rijkenberg, F. H. J. 1991. Expression of two wheat leaf rust resistance gene combinations involving *Lr34*. *Plant Dis.* 75:526-528.

The effects of temperature and different isolates of *Puccinia recondita* f. sp. *tritici* on the expression of leaf rust resistance genes *Lr33*, *LrT3*, and *Lr34* and gene combinations *Lr33* + *Lr34* and *LrT3* + *Lr34*, all in Thatcher wheat background, were studied in seedling and adult plants. In reaction to isolates 3SA122, 3SA132, and 3SA135, the expression of low infection type by the individual genes and the two combinations in the seedling stage was best expressed at 7 C. These low infection types were largely negated at 15, 20, and 25 C. All three individual host genes, as well as the two combinations, expressed race-specific characteristics. Enhanced seedling resistance attributable to the combination of *Lr* genes was observed only with certain isolates. Infection types displayed on flag leaves suggested that levels of enhanced resistance were more easily detected in adult plants. Leaf rust infection types in adult plants were not as influenced by elevated temperatures as seedlings. In adult plants, the low infection types were best observed at 15 C. The effectiveness of gene combinations involving *Lr34* depends on the second gene, the rust culture used, environment, and growth stage of the host plant.

Additional keywords: resistance enhancement

The accumulation or pyramiding of resistance genes into a single host plant cultivar has been proposed as a method of attaining durable resistance (13). In the wheat (*Triticum aestivum* L.) leaf rust (caused by *Puccinia recondita* Roberge ex Desmaz. f. sp. *tritici* (Eriks. & E. Henn.) D. M. Henderson) system, Singh and McIntosh (19) provided genetic evidence for complementary resistance (*Lr*) gene action in the wheat cultivar Gatcher, showing that both *Lr27* and *Lr31* needed to be present to confer resistance. On the basis of phenotypic expression of infection types, Samborski and Dyck (17) showed that the gene combinations *Lr13* + *Lr16*, *Lr30* + *Lr3ka*, *Lr30* + *Lr11*, and *Lr33* + *Lr34* exhibited higher levels of resistance than either of the respective *Lr* genes alone, especially those expressing adult plant resistance. Similarly, Dyck and Samborski (10) found that the genes *Lr34* and *LrT3*, both present in the cultivars Terenzio and Lageadinho, interacted to produce an enhanced adult plant resistance. Other reports of resistance enhancement attributable to gene combinations also exist (6,11,18).

Little information regarding the stability of *Lr* gene combinations to variation in environment or pathogen race is available. Dyck and Johnson (8) provided evidence that *Lr33* + *Lr34* in line RL6059 were more resistant to certain races of *P. r. f. sp. tritici* than to others. It is important to establish how genes function in combination and what cultures and environments are useful in detecting resistance in newly developed lines or cultivars. This is true especially for *Lr34*, which, in combination with other *Lr* genes, most probably forms the basis of currently known sources of durable resistance to leaf rust (15).

The expression of infection types in cereal rust interactions is generally dependent on the environment in which the resistance mechanisms function (3). Therefore, manipulation of the ambient testing temperature could facilitate phenotypic identification of important gene combinations. In this paper, we report the reactions of wheat lines with *Lr33*, *LrT3*, and *Lr34* and the combinations *Lr33* + *Lr34* and *LrT3* + *Lr34*, at two growth stages and different temperatures, to three races of *P. r. f. sp. tritici*.

MATERIALS AND METHODS

Seedling tests. Seedlings of Thatcher wheat lines near-isogenic for *Lr34* (RL6058) (Tc*6/PI58548), *Lr33* (RL6057) (Tc*6/PI58548), *Lr33* + *Lr34* (RL6059) (Tc*6/PI58548), *LrT3* (896) (Tc*6/Terenzio), and *LrT3* + *Lr34* (RL6050) (Tc*6/Terenzio) (6,7) and of Thatcher (susceptible control cultivar)

were grown in soil in 10-cm-diameter plastic pots. Approximately 15–20 seedlings of each genotype were grown per pot at 15–25 C in a room with illumination of about 300 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Seven-day-old plants were inoculated with isolates 3SA122, 3SA132, and 3SA135 of *P. r. f. sp. tritici*. These isolates were chosen because they represented variation in virulence observed in preliminary studies (S. C. Drijepondt, unpublished data). Their avirulence/virulence formulae are *Lr1*, 2a, 2b, 3ka, 11, 15, 17, 20, 24, 26, 30/*Lr2c*, 3a, 3bg, 10, 14a, 16 for 3SA122; *Lr3a*, 3bg, 3ka, 11, 16, 20, 26, 30/*Lr1*, 2a, 2b, 2c, 10, 14a, 15, 17, 24 for 3SA132; and *Lr1*, 2a, 2b, 2c, 3ka, 11, 14a, 15, 20, 24, 26, 30/*Lr3a*, 3bg, 10, 16, 17 for 3SA135. Primary leaves were inoculated with a suspension of 0.4 mg of fresh urediniospores per milliliter of Soltrol 130 (Philips Chemical Company, Borger, TX) light mineral oil.

Approximately 1 hr after the inoculation of seedlings, the plants were placed in a dew chamber at 18–20 C in darkness for 18 hr. Leaves were then allowed to dry gradually before the plants were transferred to air-conditioned greenhouse compartments where the ambient temperature was maintained at 14–16, 19–21, and 24–26 C, respectively. Daylight was supplemented with 900 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ emitted by cool-white fluorescent tubes for 12 hr each day. A set of plants was also placed in a cold room at 5–9 C where 200 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ light from cool-white fluorescent tubes was emitted for 8 hr daily. Preliminary work had indicated that healthy seedlings, displaying distinctive infection types, could be maintained at 5–9 C (S. C. Drijepondt, unpublished data). The different temperature regimes will be referred to as 7, 15, 20, and 25 C. Pots were randomly arranged in all four environments.

For seedlings evaluated in the greenhouse, infection types were scored 8–14 days after inoculation, whereas those produced on plants at 7 C were recorded after 37–45 days. According to the 0–4 infection type scale (16), a “0” denotes the absence of uredinia or any macroscopic sign of infection and “;” indicates no uredinia but visible flecks. A “4” indicates large uredinia without necrosis or chlorosis. Infection types “1”, “2”, and “3” are allocated on a basis of increasing pustule size and decreasing chlorosis and

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Accepted for publication 24 October 1990 (submitted for electronic processing).

Table 1. Infection types produced by the interaction of three isolates of *Puccinia recondita* f. sp. *tritici* at four temperatures on seedlings of five Thatcher near-isogenic *Lr* lines

Line	Infection type ^a											
	3SA122				3SA135				3SA132			
	7 C	15 C	20 C	25 C	7 C	15 C	20 C	25 C	7 C	15 C	20 C	25 C
RL6057 (<i>Lr33</i>)	3c	3++	3++	3++	3+	3++	4	3++	2	2	2++	2+
RL6059 (<i>Lr33</i> + <i>Lr34</i>)	;1c	3+	3+	3++	;12	2+	4	3++	;	;1	;1++	2-
RL6058 (<i>Lr34</i>)	3-	3++	3++	3++	;12	4	4	3++	;12	3	3++	3++
896 (<i>LrT3</i>)	;12	3++	3++	3++	2=	4	4	3++	3	3++	4	3++
RL6050 (<i>LrT3</i> + <i>Lr34</i>)	;	3+	3+	3++	;	4	4	3++	;12	3+	3++	3++
Thatcher (susceptible control)	3++	3++	3++	3++	3+	4	4	4	3	3++	3++	3++

^aInfection type (16) is the mean reaction observed on 10–15 plants in each of three replicate experiments.

necrosis surrounding uredinia. Plus or minus signs show that uredinia are at the upper or lower size limit of an established infection type class (16). All seedling experiments were conducted three times.

Adult plant tests. The near-isogenic Thatcher lines, gene combinations, and Thatcher were grown in 18-cm-diameter plastic pots (two plants per pot) containing 4 kg of soil in a greenhouse set at 19–23 C. Daylight was supplemented with light from cool-white fluorescent tubes emitting 900 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for 12 hr daily. At the time of planting, a soil drench of 0.5 g of urea (46% N) and 0.8 g of superphosphate (10.5% P) per pot was applied. Sixty-six days after planting, when the ears of all lines had completely emerged (Zadoks growth stage 59 [20]), the adaxial surface of flag leaves was inoculated according to the procedure of Browder (1). Fungal isolates, inoculum concentration, and incubation of inoculated plants were similar to that of the seedling tests. The flag leaves of 10 plants (five replicate pots) per genotype were inoculated in each isolate-environment combination. Plants were evaluated in greenhouse compartments with temperature and illumination as described for seedlings. The 7 C regime was inadequate for adult plant evaluations and therefore omitted. Infection types were scored according to the description of Roelfs (16) 11–15 days after inoculation. All adult plant tests were repeated in a duplicate experiment.

RESULTS

Only slight variation in infection types, not indicative of changes between discrete infection type classes, was observed between repetitions of seedling and adult plant experiments. The infection types presented in Tables 1 and 2 are those most commonly observed in each host-isolate-environment combination.

Seedling tests. Infection types of host-isolate-temperature combinations are presented in Table 1. At 7 C, low infection types to isolate 3SA122 were expressed by near-isogenic lines having *LrT3*, *LrT3* + *34*, or *Lr33* + *34*. Lines with *Lr33* + *34* or *LrT3* + *34* had lower infection types to 3SA122 than *LrT3*, *Lr33*, and *Lr34* individually. All lines

Table 2. Infection types produced by the interaction of three isolates of *Puccinia recondita* f. sp. *tritici* at three temperatures on flag leaves of five Thatcher near-isogenic *Lr* lines

Line	Infection type ^a								
	3SA122			3SA135			3SA132		
	15 C	20 C	25 C	15 C	20 C	25 C	15 C	20 C	25 C
RL6057 (<i>Lr33</i>)	2+	3+	3+	2++	3++	3++	;1+	2	2+
RL6059 (<i>Lr33</i> + <i>Lr34</i>)	;1=	;1	2++	2	2++	2++	;1	1+2	2+3
RL6058 (<i>Lr34</i>)	1++	3+	3++	1++	3++	3++	1++	3+	3+
896 (<i>LrT3</i>)	2++	3	3++	2++	3	3++	3	3	3
RL6050 (<i>LrT3</i> + <i>Lr34</i>)	;	2+	3	;12	2+	3	2+	2+	3
Thatcher (susceptible control)	3++	3++	3++	3++	3++	3++	3++	3++	3++

^aInfection type (16) is the mean reaction observed on 10 plants in each of two replicate experiments.

were susceptible to isolate 3SA122 at 15, 20, and 25 C.

Inoculations with isolate 3SA135 showed that at 7 C, the infection type of the line with *Lr33* + *34* was similar to that conditioned by *Lr34*. The combination of *LrT3* with *Lr34* resulted in a lower infection type (;) at 7 C to 3SA135 than either *LrT3* or *Lr34* independently (Table 1). At 15 C, inoculation of *Lr33* + *34* with isolate 3SA135 produced infection type 2+, which was lower than that of lines with either *Lr33* or *Lr34*. No resistance was observed at 15, 20, or 25 C for RL6057 and RL6058 inoculated with isolate 3SA135.

The *Lr33* + *34* combination had a lower infection type (;) at 7 C to isolate 3SA132 than did the lines with only *Lr33* or *Lr34*. At this temperature, the infection type of the line with *LrT3* + *34* corresponded to the infection type for *Lr34* alone (Table 1). With isolate 3SA132, resistance enhancement in the line with *Lr33* + *34* was visible at all four temperatures.

Adult plant tests. Infection types produced on the flag leaves are shown in Table 2. At 15 C, low infection types to isolate 3SA122 were expressed by all genes individually, as well as in combination. Both *Lr33* + *34* (infection type ;1=) and *LrT3* + *34* (infection type ;) had lower infection types compared with the individual genes. *Lr33* + *34* had improved resistance at all three temperatures to 3SA122, and although *LrT3* + *34* reacted in a similar fashion, the enhancement of resistance was very slight at 25 C.

Similar to the seedling reactions at 7 C

to isolate 3SA135, flag leaves of the line with *Lr33* + *34* did not show a lower infection type at 15 C than that of *Lr34*. However, at 20 and 25 C, uredinia on *Lr33* + *34* were smaller than on the lines with *Lr33* or *Lr34*. The infection type of *LrT3* + *34* was lower to 3SA135 than those of *LrT3* and *Lr34* alone at all three temperatures, with the lowest infection type occurring at 15 C.

The infection type expressed by *Lr33* + *34* to 3SA132 was slightly lower at 15 and 20 C than those of the individual *Lr* genes, but this improved resistance diminished at 25 C. *LrT3* + *34* produced a 2+ infection type, which was intermediate between *LrT3* (infection type 3) and *Lr34* (infection type 1++) at 15 C. In comparison with the individual genes, a slight improvement of resistance was observed in *LrT3* + *34* at 20 C. At 25 C, a loss of effectiveness attributable to the combination of *LrT3* and *Lr34* was evident. The background cultivar Thatcher was susceptible (infection type 3–4) with all isolates and at all temperatures tested (Tables 1 and 2).

DISCUSSION

The detection of rust resistance in seedlings of small grains by means of a single qualitative observation is a valuable asset in resistance breeding programs. Not only does it save time and space, but it is possible to strictly regulate the environment and evaluate a large number of genotypes. Not all resistance is readily recognizable in seedlings. Slow rusting for example, is usually most clearly expressed by disease development rates in the field or by quantitative

measurement of components of resistance (21). Temperatures lower than 12 C have been successfully applied to detect seedling resistance in the slow leaf rusting wheat cultivar Suwon 85 (5). Conversely, a temperature of 25.5 C is used to detect high-temperature seedling resistance conferred by *Lr13* (14). Our results emphasized the value of using test temperatures considerably lower than the commonly used 20 C (4).

With certain isolates of *P. r. f. sp. tritici*, Dyck and Johnson (8) could identify *Lr34* alone in seedlings and in combination with *Lr33* at 10, 15, 20, or 25 C. No South African isolate could, however, detect *Lr34* in seedlings at 20–25 C (S. C. Drijepondt, unpublished data). We have demonstrated that *Lr34* can be selected for at 7 C, providing the intensity and duration of illumination is conducive to the expression of *Lr34* in primary leaves. Because *Lr34* genotypes are known to produce lower infection types under conditions of low light intensity and cool temperatures (10), our results cannot be attributed to temperature alone. Seedling plants in the 7 C environment received considerably less illumination than those in the greenhouse, suggesting that both temperature and light are important in eliciting *Lr34* expression. When selecting for *Lr34* in complex backgrounds, knowledge of the expression of other *Lr* genes, under similar conditions to a range of cultures, should facilitate detection of specific leaf rust resistance genes. A disadvantage of the low-temperature procedure is, however, the time required for optimum development of infection types.

The resistance conditioned by the two *Lr* gene combinations investigated was affected by the environment and the leaf rust isolate used. Expression of resistance in *Lr33 + 34* and *LrT3 + 34* depended, furthermore, on the growth stage of the plant. Enhanced resistance attributable to gene combinations was evident at 25 C in adult plants compared with the genes individually, while temperatures of 15 C and above generally negated the expression of resistance in seedlings. Knott and Weller (12) also recognized that additive effects among *Sr* genes in wheat may occur at one growth stage but not at another. In their study, interactions among *Sr* genes were much more common in adult plants than in seedlings.

From the adult plant tests, it appeared that resistance expressed by *Lr33 + 34* and *LrT3 + 34* was most improved if both genes of the gene pairs were individually effective against the pathogen isolate. This confirms the results of Schafer et al (18) that combinations of resistance genes in a genotype would be most effective if all individual resistance genes conferred high levels of resistance.

Although infection type 3 was observed in certain *LrT3 + 34* adult plant evaluations, susceptible responses comparable to Thatcher were not found at any temperature with any culture. The influence of the adult plant resistance gene *Lr22b* in the recurrent parent Thatcher (2) on *LrT3*, *Lr33*, and *Lr34*, as well as on *LrT3 + 34* and *Lr33 + 34*, is not known. Slight variation in the seedling infection type of Thatcher was observed, but adult plants responded similarly across isolates and temperatures. The possibility that *Lr22b* influenced infection types should, however, not be disregarded.

The two resistance gene combinations tested in our study reacted differentially to the same isolate, thus suggesting that not all combinations involving *Lr34* will be equally effective. Differential reactions toward isolates, more commonly referred to as race-specificity (9), was also exhibited at 7 C by seedlings of lines near isogenic for *Lr33*, *Lr34*, and *LrT3*. Previously, Samborski and Dyck (17) reported race-specificity for *Lr34* in seedling tests. In the adult plant stage at 15 C, we did not observe race-specificity for *Lr34*. Further studies on the influence of different races on quantitative aspects of adult plant resistance of *Lr34* should prove useful.

ACKNOWLEDGMENTS

We thank P. L. Dyck, Agriculture Canada, for providing seed of lines RL6050, RL6057, RL6058, RL6059, and 896, and A. P. Roelfs, Cereal Rust Laboratory, USDA-ARS, St. Paul, MN, for comments during manuscript preparation.

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