# Genetic analysis of partial resistance to bacterial leaf streak (*Xanthomonas campestris* pv. *cerealis*) in wheat

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Genetic variability of partial resistance to bacterial leaf streak was investigated in hexaploid winter wheat (*Triticum aestivum*.), using 16 parental genotypes and 48 pure lines (F10) derived from a composite cross programme. Two experiments were undertaken in a controlled growth chamber. Seeds of all genotypes were grown under controlled conditions using a randomized block design with three replications. Each replication consisted of a row of 20 seedlings of each parent and pure line. An Iranian strain of bacterial leaf streak was used for the inoculation of 12-day-old seedlings. In a third experiment, eight genotypes from parents and F10 pure lines representing a large variability for partial resistance were inoculated with four other Iranian strains of bacterial leaf streak. A large genetic variability was observed amongst the 64 genotypes for partial resistance to the disease. Partial resistance heritability estimates were rather high (70%), indicating that the resistance factors may be transmitted by crossing. Amongst all genotypes investigated, 'DC$^2$-30-N2' and 'IBPT-66' displayed the highest partial resistance to the disease. Significant correlations between strains in the third experiment show that a genotype resistant or susceptible to one strain will have similar reactions with other strains. No significant genetic gain was observed for partial resistance in the best pure line of the 48 lines studied, when compared with the best parental line. Increasing the number of pure lines is likely to result in the identification of genotypes that might prove to be more resistant.

## INTRODUCTION

Bacterial leaf streak caused by various pathovars of *Xanthomonas campestris* is a serious worldwide bacterial disease of wheat (Boosalis, 1952; Duveiller, 1989). Losses attributed to bacterial leaf streak can be quite high under conditions favourable to the pathogen; up to 40% yield reductions were reported in susceptible wheat cultivars (Schaad & Forster, 1985). The disease also affects grain quality (Mehta, 1990). Bacterial leaf streak has been increasing on a world scale, and has now become one of the most important diseases of cereals (Mehta, 1990).

Foliar expression of the disease is characterized by longitudinal stripes that extend between the leaf veins and by the production of milky exudates under humid conditions (Duveiller *et al*., 1993). Contaminated seed is the main source of inoculum (Sands *et al*., 1986; Forster & Schaad, 1988; Duveiller, 1989). Eradication of the bacterial inoculum from the wheat seed would be helpful, but known chemical and physical methods of seed treatment are not sufficiently effective (Forster & Schaad, 1988; Sun *et al*., 1988; Fourest *et al*., 1990). The susceptibility of wheat to bacterial leaf streak is similar under field and greenhouse conditions (Akhtar & Aslam, 1986). The disease reactions on the primary leaves are the same as those observed on the flag leaf (Milus & Mirlohi, 1994). Resistance amongst 19 wheat cultivars to 81 bacterial strains isolated from different cereals was highly variable, but the cultivar $\times$ strain interaction was not significant and there was thus no evidence for races of the pathogen (Milus & Chalkly, 1994)

There is an increasing interest in wheat improvement programmes and in the development of cultivars with improved resistance to this disease.

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The development of easier, cheaper and widely applicable methods of integrated control requires the identification and incorporation of resistance genes into wheat breeding material. However, the genetic component of resistance to the disease is still not clearly understood. According to Woo & Smith (1962), resistance to bacterial leaf streak in wheat is controlled by a single dominant gene. The inheritance of resistance was also investigated by Duveiller *et al.* (1993) who showed that resistance to bacterial leaf streak or black chaff in five wheat lines was conditioned by five genes which differed in magnitude of expression of resistance. The aim of the investigations presented below was to assess the genetic variability of partial resistance to bacterial leaf streak in 64 hexaploid winter wheat (*Triticum aestivum*) genotypes: 16 parental genotypes and 48 pure lines (F10) derived from a composite cross programme.

## MATERIALS AND METHODS

The experiments were carried out with 64 genotypes of hexaploid winter wheat (*T. aestivum*), including 48 randomized F10 pure lines derived from a composite cross population (IBPT) and their 16 parental genotypes. The composite cross population (IBPT) is a genetic pool developed by INRA (Institut National de la Recherche Agronomique, France). This pool was obtained from a pyramidal cross of 16 pure lines (Thomas *et al.*, 1991). A bulk was made with the hybrid plants obtained after the last cross and was multiplied for 3 years) giving B0 population. Random samples of B0 were used in 1984 to found the B population in a multilocal network in France in the framework of a programme of dynamic management of wheat genetic resources. This population has been growing since 1984 in the same location at normal cultural density. At least 4000 individuals are grown each year. In the F10 generation of this composite population grown at our Department of Biotechnology and Plant Breeding (IBPT), 100 plants were randomly selected in order to study the genetic control of different agronomic traits. From the 100 above-mentioned F10 pure lines, 48 were selected at random for use in this study. The resistance or susceptibility of the F10 pure lines and their parents had not been studied previously.

Two successive experiments, with three replicates in each case, were performed in a growth chamber. Seeds were sterilized with sodium hypochlorite solution (1·56% of available chlorine) for 5 min, washed in sterile distilled water and planted in plastic containers ($60\times\ 40 \times 8$ cm) containing moistened vermiculite.

The bacterial strain (IBLS 20), which had been isolated from a wheat variety grown in Iran, and identified as *Xanthomonas campestris* pv. *cerealis* (Alizadeh & Rahimian, 1989) was used in our programme. The bacterial strain was grown at $26 \pm 1^{\circ}$C and maintained on slants of nutrient agar medium (Difco) at $3 \pm 1^{\circ}$C or was lyophilized for long-term storage. Each replication included one row of 20 seedlings per parent or pure lines and each tray contained eight rows. Seedlings were watered with Knop's nutrient solution (Arabi *et al*., 1992) every day throughout the experiment. The containers were placed in a growth chamber at temperatures of $25 \pm 1^{\circ}$C (day), $23 \pm 1^{\circ}$C (night), and a relative humidity of 75–85%. Light intensity was 90 mol $m^{-2}$ $s^{-1}$, with a day length of 12 h.

One day before inoculation, the seedlings were sprayed with sterile distilled water and each container covered with a Perspex translucent lid to maintain near-saturated humidity, which is favourable to bacterial inoculation. Twelve-day-old seedlings were sprayed with a bacterial suspension standardized at about $10^9$ colony-forming units per mL in sterile distilled water (CFU $mL^{-1}$ SDW), prepared from a 24-h-old culture.

Twelve days after inoculation, the first leaves of the seedlings were scrutinized to determine the proportion of diseased leaf area (water soaking and translucent spots or stripes). Host reaction was rated from 1 (resistant) to 9 (susceptible) in relation to the proportion of leaf area showing disease symptoms as proposed by Alizadeh *et al.* (1994): 1: 0–5%; 2: 5–10%; 3: 10–20%; 4: 20–30%; 5: 30–40%; 6: 40–50%; 7: 50–60%; 8: 60–75% and 9: 75–100% of leaf area infected.

Statistical analyses were carried out in order to determine the genotype effect on the studied trait. The Newman–Keuls test was used for comparing means for partial resistance. Heritability was determined according to the equations:

$$\mathrm{MSG} = \mathrm{r}\delta^2\mathrm{G} + \delta^2\mathrm{E},$$

$$\delta^2\mathrm{p} = \delta^2\mathrm{G} + \delta^2\mathrm{E},$$

$$\mathrm{h}^{2=}(\delta^2\mathrm{G}/\delta^2\mathrm{p})'100,$$

where MSG is the mean square of genotypes, $\delta^2$G is the genetic variance, $\delta^2$E is the environmental

variance, $\delta^2$p is the phenotypic variance, r is the number of blocks and $h^2$ is the heritability.

The mean ($\bar{x}$) of the 48 F10 pure lines and that of the 16 parents were compared. The best pure line was also compared with the best parent for partial resistance.

In order to determine the resistance and susceptibility of this material to other strains of bacterial leaf streak, a third experiment was made under the same conditions as before. In this experiment, eight genotypes (parents and F10 pure lines), representing a large variability for resistance and susceptibility identified in the two previous experiments, were inoculated with four Iranian strains of bacterial leaf streak (IBLS 22, IBLS25, IBLS 29 et IBLS 41). Means ($\bar{x}$) of partial resistance were compared and correlations between the strains used in all experiments for partial resistance were estimated.

## RESULTS AND DISCUSSION

The genetic variability of parental genotypes

**Table 1** Genetic variability for resistance of parental genotypes and F10 pure lines of a composite cross in hexaploid wheat

| Varieties or F10 lines | Experiment I | Experiment II | Varieties or F10 lines | Experiment I | Experiment II |
|---|---|---|---|---|---|
| *Parents* | | | | | |
| C45 | 5·79a | 5·58a | Kaukaz | 4·02b | 3·03a |
| Clement 2 | 6·05a | 5·24ab | Tjb36 | 3·93b | 4·33abcd |
| Rivoli | 4·64ab | 3·47cd | Vc75Rv53 | 3·97b | 3·60cd |
| Weihemphe | 4·55ab | 3·90bcd | Oxley | 3·95b | 3·91bcd |
| Loros cr | 4·60ab | 3·21cd | Top | 3·29bc | 3·22cd |
| Mironovskaïa | 4·38ab | 4·78abc | Clément 1 | 3·18bc | 2·84d |
| C3275 | 4·55ab | 4·19abcd | C6156 | 2·00c | 1·60e |
| D48 | 4·60ab | 4·54cd | $Dc^2$30N2 | 1·93c | 1·58e |
| *F10 lines* | | | | | |
| IBPT 1 | 5·83cde | 4·50fgh | IBPT 23 | 6·95ab | 5·73def |
| IBPT 2 | 5·19efg | 4·62fgh | IBPT 25 | 3·72ijk | 4·23ghi |
| IBPT 4 | 4·48ghi | 6·86abc | IBPT 28 | 3·91ghi | 3·49jk |
| IBPT 6 | 4·02ghi | 3·47jk | IBPT 29 | 5·73cde | 4·24ghi |
| IBPT 7 | 6·35bcd | 3·95ijk | IBPT 33 | 5·72cdef | 6·07cde |
| IBPT 10 | 4·50fgh | 4·47fgh | IBPT 34 | 3·55klm | 3·40jk |
| IBPT 12 | 4·86fgh | 5·66def | IBPT 37 | 5·14efg | 5·70def |
| IBPT 16 | 5·52def | 5·69def | IBPT 38 | 5·05efg | 6·32bcd |
| IBPT 17 | 6·24bcd | 4·09ijk | IBPT 40 | 3·69klm | 4·75gh |
| IBPT 19 | 4·60fgh | 3·94ijk | IBPT 43 | 5·24def | 5·67def |
| IBPT 22 | 5·62cde | 5·73def | IBPT 44 | 4·12ghi | 4·30ghi |
| IBPT 45 | 4·81fgh | 5·65def | IBPT 72 | 4·36ghi | 6·18bcd |
| IBPT 48 | 3·76ijk | 4·67fgh | IBPT 74 | 5·19efg | 5·05fgh |
| IBPT 50 | 5·17efg | 5·04fgh | IBPT 75 | 5·57def | 5·38efg |
| IBPT 51 | 4·67fgh | 5·77def | IBPT 76 | 5·44def | 4·40ghi |
| IBPT 54 | 5·26def | 6·38bcd | IBPT 81 | 4·88fgh | 4·54fgh |
| IBPT 55 | 5·67cde | 5·2fgh | IBPT 84 | 3·28lm | 3·51jk |
| IBPT 57 | 6·29bcd | 4·92fgh | IBPT 85 | 5·17efg | 4·50fgh |
| IBPT 59 | 4·83fgh | 4·52fgh | IBPT 86 | 6·43bcd | 6·83abc |
| IBPT 60 | 3·29lm | 5·51efg | IBPT 87 | 6·83abc | 5·98cde |
| IBPT 63 | 4·07ghi | 5·58def | IBPT 88 | 4·71fgh | 4·58fgh |
| IBPT 64 | 3·79hij | 5·25efg | IBPT 89 | 7·31a | 7·67a |
| IBPT 66 | 2·41m | 2·77k | IBPT 90 | 6·22bcd | 7·33ab |
| IBPT 69 | 4·89fgh | 5·96cde | IBPT 92 | 5·20efg | 5·90cde |

Means with different letters are significantly different at $P = 0{\cdot}05$ level (Newman–Keuls test). The values represent the mean of host reaction rate, scale range 1 to 9, from three replications

and F10 pure lines tested in experiments I and II is shown in Table 1. Analyses of variance for the 16 parents, the 48 randomized (F10) pure lines and the whole set of 64 genotypes showed the occurrence of a highly significant genotype effect. Parental genotype 'DC$^2$-30-N2' showed the highest level of partial resistance, and the two pure lines, 'IBPT-66' and 'IBPT-84', had the highest values for resistance to bacterial leaf streak in both experiments, whereas 'IBPT-89' was characterized by the highest susceptibility (Table 1).

There was thus a large genetic variability amongst the 64 genotypes tested for resistance to *X. campestris* pv. *cerealis*. These results suggest that the genotype is a major determinant of resistance to bacterial leaf streak. Genotype susceptibility and resistance to bacterial leaf streak has been also reported in wheat by Hagborg (1974), Duveiller (1990), Demir & Ustun (1992) and in triticale by Cunfer & Scolari (1982).

A summary of the data from experiments I and II is shown in Table 2. The difference between the mean of the disease scores of pure lines (PL) and that of the parents (P) was not significant in the first experiment but was significant in the second trial, indicating that the 48 randomly selected pure lines were probably not completely representative of the whole population.

Resistance (expressed as the lowest disease score) of the best parent (BP) was significantly superior to that of the best pure line (BPL) in the second experiment. This could be due to the limited number (48) of pure lines studied and the consequent failure to accumulate favourable resistance alleles. Increasing the number of pure lines is likely to result in the identification of genotypes that might prove to be as resistant as the best parents.

**Table 2** Genetic gain for partial resistance to bacterial leaf streak in hexaploid wheat

| Genotypes | Experiment I | Experiment II |
|---|---|---|
| Parents (P) | 4·09 | 3·76 |
| Pure lines (PL) | 4·99 | 5·12 |
| PL-P | 0·90 NS | 1·36* |
| Best Parent (BP) | 1·93 | 1·58 |
| Best Pure Line (BPL) | 2·41 | 2·77 |
| BPL-BP | 0·48 NS | 1·19* |
| LSD (0·05) | 1·01 | 1·01 |

*Significant at $P = 0{\cdot}05$ level; NS, not significant; LSD, least significant difference.

**Table 3** Heritability of resistance to bacterial leaf streak in hexaploid winter wheat

| Genotypes | Experiment I | Experiment II |
|---|---|---|
| $\delta^2$G-Parents (P) | 1·10 | 1·36 |
| $h^2$ | 72·80 | 78·61 |
| $\delta^2$G-Pure Lines (L) | 0·96 | 1·01 |
| $h^2$ | 72·11 | 73·18 |
| $\delta^2$G-(P + L) | 1·19 | 1·42 |
| $h^2$ | 73·68 | 78·02 |

$\delta^2$G, Genetic variance; $h^2$, heritability (%).

The heritability values of various cultivars show that the parents (P), the F10 pure lines (PL) as well as parents and pure lines (P + PL) were characterized by nearly the same heritability (Table 3). Heritability estimates were relatively high (70%); thus, it should be relatively easy to breed for resistance.

The genetics of resistance to bacterial diseases in wheat has been hardly investigated so far. Under artificial disease pressure in the field, resistance to *X. campestris* pv. *undulosa* in bread wheat appeared to be conditioned by five genes which differed in strength of resistance expression (Duveiller *et al*., 1993). As far as partial resistance to bacterial leaf streak is concerned, diallel analysis of five genotypes and their 20 F1 hybrids also showed highly significant general and specific combining abilities, proving the importance of genetic effects in controlling the disease (Alizadeh *et al*., 1994).

Analysis of variance for the eight genotypes of the third experiment (parents and F10 pure lines), representing a large variability for resistance and susceptibility, inoculated with the (IBLS20) strain of *X. campestris* pv. *cerealis* in the previous experiments, and four other strains showed a high significant genotype effect with all of strains studied (Table 4). Significant correlations between strains ($r$: 0·87–0·971) show that a genotype resistant or susceptible to one strain will have similar reactions with other strains. Milus & Chalkly (1994) also showed a high variability of the resistance of 19 wheat cultivars to 81 bacterial strains, and each cultivar had a similar susceptibility phenotype.

Finally, we may conclude that partial resistance to bacterial leaf streak in hexaploid wheat is genotype-dependent and highly heritable.

**Table 4** Genetic variability for resistance of eight wheat genotypes to five strains of *Xanthomonas campestris* pv. *cerealis*

| | Experiment II | Experiment III | | | |
|---|---|---|---|---|---|
| Varieties | IBLS 20 | IBLS 22 | IBLS 25 | IBLS 29 | IBLS 41 |
| C61 | 1·60e | 4·00f | 2·00hi | 5·00e | 2·00hi |
| $Dc^2$30N2 | 1·58e | 3·00g | 1·75i | 2·25h | 3·00g |
| Clement 2 | 5·24ab | 9·00a | 9·00a | 8·00b | 9·00a |
| C45 | 5·58a | 8·00b | 9·00a | 6·50c | 8·00b |
| IBPT 66 | 2·77k | 3·90f | 3·00g | 3·95f | 4·00f |
| IBPT 84 | 3·51jk | 4·00f | 4·00f | 5·00e | 6·00d |
| IBPT 89 | 7·67a | 9·00a | 8·00b | 7·75b | 9·00a |
| IBPT 90 | 7·33ab | 9·00a | 9·00a | 9·00a | 9·00a |

Means with different letters are significantly different at $P = 0{\cdot}05$ level (Newman–Keuls test).
The values represent the mean of host reaction rate, scale range 1 to 9 from three replications.
Correlations (ddl = 6): r (IBLS 20 and IBLS 22) = 0·93; r (IBLS 20 and IBLS 25) = 0·914; r (IBLS 20 and IBLS 41) = 0·943; r (IBLS 20 and IBLS 29) = 0·893; r (IBLS 22 and IBLS 25) = 0·971; r (IBLS 22 and IBLS 29) = 0·939; r (IBLS 22 and IBLS 41) = 0·943; r (IBLS 25 and IBLS 29) = 0·901; r (IBLS 25 and IBLS 41) = 0·962; r (IBLS 29 and IBLS 41) = 0·874; All 'r' values are significant at $P = 0{\cdot}01$ level.

Bycross-ing different genotypes, resistance to this disease should be transferred to susceptible ones.

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