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Resistance to *Phytophthora fragariae* var. *fragariae* in strawberry: the *Rpf2* gene

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Abstract *Phytophthora fragariae* var. *fragariae* is the causal agent of red stele (red core) root rot in strawberry (*Fragaria* spp.). The inheritance of resistance to one isolate of this fungus was studied in 12 segregating populations of *F.* × *ananassa* derived from crosses between four resistant cultivars ('Climax', 'Redgauntlet', 'Siletz', and 'Sparkle') and three susceptible cultivars ('Blakemore', 'Glasa', and 'Senga' Sengana'). The analysis clearly supports the hypothesis of a single segregating dominant resistance gene. It is proposed that this gene be designated *Rpf2*.

Key words *Fragaria* × *ananassa* · Genetics · Inheritance · Red core · Red stele

Introduction

There is increasing evidence that cultivars of strawberry and races of *Phytophthora fragariae* var. *fragariae* (Wilcox et al. 1993) interact in a gene-for-gene (GFG) manner as described by Flor (1956) for flax and flax rust (*Melampsora lini*). A GFG model has been proposed for five pairs of interacting resistance factors of the host and avirulence factors of the pathogen based on the analysis of a large number of genotype-race combinations (Van de Weg 1989, 1997). Although within GFG systems resistance and avirulence factors are generally based on single genes (De Wit 1992; Thompson and Burdon 1992), their genetic basis has to be formally assessed by genetic analysis.

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The cultivated strawberry *Fragaria* × *ananassa* is an allo-octoploid (2n = 8x = 56) originating from a natural hybridisation event between the allo-octoploid species *F. chiloensis* and *F. virginiana*. While the genome structure of these species is still unresolved (see Galletta and Maas 1990), various structures have been suggested: AABBBBCC (Fedorova 1946) and AAA'A'BBBB (Senanayake and Bringhurst 1967). These allow di- and tetrasomic segregation to occur simultaneously within a single progeny. In the case of tetrasomy, easily interpretable segregation ratios may occur if the trait of interest is governed by a qualitative, dominant gene. Race-specific resistance has frequently been shown to be such a trait in other host-pathogen systems (Thompson and Burdon 1992).

In the investigation presented here inheritance of the race-specific resistance of the strawberry cultivars 'Climax', 'Redgauntlet', 'Sparkle', and 'Siletz' to the fungal isolate A7 is examined. On the basis of the GFG model (Van de Weg 1997) these cultivars were expected to possess a single resistance factor (2) for this isolate.

Material and methods

Plant material

Twelve strawberry (*Fragaria* × *ananassa*) progenies segregating for resistance factor 2 (Van de Weg 1997) were created by crossing four resistant genotypes ('Climax', 'Redgauntlet', 'Sparkle', and 'Siletz') with three universally susceptible genotypes ('Blakemore', 'Glasa', and 'Senga Sengana') (Table 1). In addition, 4 highly susceptible progenies were created; 3 by crossing among the susceptible genotypes mentioned above and 1 by crossing 'Senga Sengana' and Md683. Md683 carries resistance factor 1 which is not effective against the isolate used in the present study (Van de Weg 1997).

'Climax', 'Redgauntlet', and 'Sparkle' are interrelated and received their resistance from their common ancestor Aberdeen. They arose from the crosses TD8 × 'Aberdeen', N.J. 1051 × 'Climax', and 'Fairfax' × 'Aberdeen', respectively (Reid 1952; Scott et al. 1984).

Table 1 Percentages of healthy strawberry seedlings of (A) 12 R × S progenies descended from crosses between four resistant and three susceptible cultivars, and of (B) 4 S × S progenies of which the parents have no effective resistance, in eight tests for resistance (I–VIII) with isolate A7 of *Phytophthora fragariae* var. *fragariae*

Susceptible cultivar	Resistant cultivar and experiment							
	Climax		Redgauntlet		Sparkle		Siletz	
	I[a]	II	III	IV	V	VI	VII	VIII
A: R × S progenies								
Blakemore	60	46	46	44	46		49	54
Glasa	61	54	56	54	54	54	52	44
S. Sengana	61		53	49	56	49	50	58
Average	60	50	51	49	53	52	52	51
B: S × S progenies								
Blakemore × S. Sengana	27	–	3	7	8	6	7	7
Blakemore × Glasa	17	10	3	12	17	17	–	–
S. Sengana × Glasa	27	6	1	6	12	17	7	14
Md683 × S. Sengana	–	–	–	–	–	–	4	6
Average	24	8	2	8	12	12	6	9

[a] The S × S progenies of experiments I and II were tested simultaneously with the R × S progenies of 'Climax', those of III and IV, V and VI, and VII and VIII were tested simultaneously with the progenies of 'Redgauntlet', 'Sparkle', and 'Siletz', respectively

All pollinations were made in the glasshouse on emasculated flowers, which were enveloped with a parchment bag from emasculation on until the receptacles were clearly swelling. Resistant parents were used as the female, except in the crosses 'Glasa' × 'Climax' and 'Senga Sengana' × 'Climax'. Seeds were stratified for 8 weeks at 2°C, and sown in an autoclaved mixture of sand and peat based compost. Seedlings were raised under standard conditions.

The 12 progenies segregating for resistance will be referred to as the R × S progenies, regardless of the direction of the cross. The 4 highly susceptible progenies will be referred to as S × S progenies.

Disease test

Seedlings with two to five true leaves were lifted and inoculated with isolate A7 [race 1.3 (Van de Weg 1997)]. Experimental procedures and conditions were according to Van de Weg et al. (1996), with two modifications: inoculated seedlings were potted in smaller plastic pots (70 ml), and the period in which they stood in a shallow layer of water (2–7 mm) after inoculation was extended from 3 days to 6 weeks. The layer was replenished by watering on the soil of the plants.

Disease assessment

Six weeks after inoculation, seedlings were lifted and the soil was rinsed off the roots. Adventitious roots with any external or internal discoloration were examined microscopically to detect oospores (Van de Weg et al. 1996). Seedlings with more than three oospores were classified as susceptible, all others as healthy, being resistant or escapes from infection (see discussion).

Experimental design

The 3 R × S progenies from a single resistant parent (Table 1) were tested simultaneously in each of two replicative experiments. Since the research included four resistant parents (Table 1), a total of eight experiments was performed. Each of these experiments also included 3 S × S progenies (Table 2). Within an experiment, each of the 3 R × S and 3 S × S progenies were tested by 72 seedlings.

Table 2 Estimated percentages of resistant seedlings of 12 strawberry progenies when tested against isolate A7 of *Phytophthora fragariae* var. *fragariae*

Susceptible parent	Resistant parent				
	Climax	Redgauntlet	Sparkle	Siletz	Mean
Blakemore	44	41	39[a]	48	43
Glasa	49	52	48	44	48
S. Sengana	48[b]	47	46	51	48
Average	48	47	46	48	47

[a,b] Tested by 39 and 66 seedlings, respectively, instead of 144

Statistical analysis

In resistance tests involving *P. fragariae*, some susceptible plants often remain healthy due to their escaping infection (Draper et al. 1970; Maas 1970; Scheewe 1994; Van de Weg et al. 1997). Consequently, the proportion of healthy (H) seedlings depends on the proportion of genetically resistant seedlings (GR) and the proportion of susceptible seedlings which escaped from infection (E) according to the formula:

$$H = GR + (1 - GR) * E$$

which can also be written as

$$GR = (H - E)/(1 - E).$$

As H can be assessed in the R × S progenies and as E can be deduced from the S × S progenies, GR can be calculated.

Effects of the parentage on percentages of healthy and resistant seedlings were analysed by means of generalised linear models using a binomial distribution and a logit link function and tested at the 95% confidence level. The statistical package GENSTAT (Genstat 5 committee 1987) was used for analyses.

Results

Percentages of healthy seedlings of the R × S and S × S progenies are presented in Table 1. In the analysis of

the S × S progenies, no significant parental effects were found, indicating that all S × S progenies were equally susceptible, which enabled their data to be pooled within experiments. Experimental effects were highly significant ($P = 0.006$), with the percentages of healthy seedlings (escapes) ranging from 2% in experiment III up to 24% in experiment I (Table 1). In the analysis of the R × S progenies, percentages of healthy seedlings were around 50%, with the exception of those in experiment I, which were around 60%. The latter experiment also showed the highest percentage of escapes in the S × S progenies (Table 1).

Estimations for the percentages of resistant seedlings were obtained by adjusting the percentages of healthy R × S seedlings for escapes (using the formula given in 'statistical analysis'). These percentages were around 50% for all of the 12 R × S progenies (Table 2). In the analysis of these latter percentages, the resistant cultivars showed no significantly different parental effects. The susceptible cultivars also showed no different parental effects. Finally, differences among the 12 R × S progenies were also not significant, i.e., there was no significant interaction between resistant and susceptible parents. These results were expected for the segregation of a single major resistance allele.

Discussion

The 1:1 segregation ratio of the progenies provides Mendelian evidence for the presence of a single copy of a resistance gene in 'Climax', 'Redgauntlet', 'Sparkle', and 'Siletz'. Since the former 3 cultivars are interrelated and since no differences in resistance among them have been found when tested to a series of 12 mutually unrelated isolates (Van de Weg et al. 1997), it is concluded that they possess the same resistance gene. The respective allele must be dominant since 'Climax' upon selfing gives rise to both resistant and susceptible seedlings (Reid 1952), whereas recessiveness should have led to resistant plants only. It is proposed that this gene be designated *Rpf2* (see below). The resistance of 'Siletz' should also be due to *Rpf2* according to the GFG model (Van de Weg 1977).

Theoretically, 1:1 segregation ratios can also occur with certain combinations of complementary genes of a di- or oligogenic trait. For instance, two complementary genes should yield a 1:1 ratio if they exhibit independent and disomic inheritance, with one of them being homozygous and the other heterozygous for the resistance allele, and with a nulliplex susceptible genotype for both genes. It is highly unlikely that such specific conditions occurred by chance in all 4 resistant genotypes. Whereas this probability is already small for 4 randomly chosen resistant genotypes, it is minute for cultivars of subsequent generations originating from R × S crosses, like 'Climax' and 'Redgauntlet' (N.J. 1051 × 'Climax'). In this progeny one of the alleles of the homozygous gene should have come from the susceptible parent (which is N.J. 1051 for 'Redgauntlet'). Due to the low probability of this and on the basis of other alternative genetic models, *Rpf2* should be single gene based.

The data do not distinguish di- and tetraploid inheritance since the observed 1:1 ratio always occurs in simplex × nulliplex crosses, irrespective of ploidy level. Conclusions regarding the ploidy level at which *Rpf2* segregates can be drawn only by the examination of duplex times nulliplex crosses, or of the cosegregation of linked alleles (repulsion phase).

Percentages of resistant seedlings

Estimates of the percentages of resistant seedlings are affected by three types of error: the frequency at which susceptible seedlings escape from infection, the frequency at which resistant seedlings show some infection, and the frequency of inaccurate disease assessments.

Escapes

Frequencies of escapes were derived from the percentages of healthy seedlings of S × S crosses (Table 1). That such seedlings are really escapes was shown by a test in which all but 1 of the healthy S × S seedlings of experiment III (Table 2) became severely diseased after a second inoculation. As the 4 S × S progenies were equally susceptible to A7, inclusion of just a single S × S progeny as reference for susceptibility should suffice in future.

The frequency of escapes is affected by inoculum density (Maas 1970). In a preliminary experiment it increased from 10% to 50% by decreasing the inoculum density by 90% (Table 3). However, the estimated percentages of resistant seedlings were similar with both densities (Table 3), showing once more that adjustment for escapes of the percentages of healthy R × S seedlings leads to consistent segregation ratios.

Infection of resistant seedlings

In this investigation the distinction between susceptible and healthy seedlings was based on the presence or absence of a few oospores. This classification may lead to a slight underestimation of the actual percentage of resistant seedlings since *Rpf2* does not always fully exclude infection. In previous research (Van de Weg et al. 1997) 98.6% of the adventitious roots of the 4 resistant cultivars remained healthy, and 1.4% (7 out of 491) was slightly infected by A7. Here, seedlings generally had three or four adventitious roots at the

Table 3 Observed percentages of healthy and predicted percentages of resistant strawberry seedlings of 2 progenies, 1 segregating for resistance (R × S) and 1 entirely susceptible (S × S) to isolate A7 of *Phytophthora fragariae* var. *fragariae*, at two inoculum densities[a]

Progeny	% Healthy		% Resistant	
	Inoculum density		Inoculum density	
	Standard	Diluted[b]	Standard	Diluted
Redgauntlet × Glasa (R × S)	51	75	46	50
Senga Sengana × Glasa (S × S)	10	50		

[a] All treatments were tested simultaneously. Experimental procedures and conditions were as for the other experiments. Each progeny-density combination was tested using 96 seedlings
[b] Standard inoculum density diluted with distilled water in a 1:9 ratio by volume

time of observation. Assuming that here too each root had a 98.6% chance of remaining healthy, the chance that seedlings with three adventitious roots remained healthy should be $0.986^3 = 0.958$, which is 95.8%. In the case of four adventitious roots this would be $0.986^4 = 0.946$, which is 94.6%. Consequently, between 4.2% and 5.4% of the resistant seedlings will be misclassified as susceptible. This would reduce the percentages of resistant seedlings to be observed, which should be 47.9% (= 50% × 0.958) and 47.3% (= 50% × 0.946) in the case of a 1:1 segregation. These expected percentages are very similar to the observed ones (Table 1), confirming the degree to which resistant seedlings are correctly identified and the monogenicity of *Rpf2*. Apparently, the present approach of disease assessment suffices for screening populations segregating for *Rpf2*.

Disease assessment

Disease assessment included microscopic establishment of the presence of oospores, their appearance being the most dependable proof for the presence of the fungus (Bain and Demaree 1945). The necessity of microscopic observations for highly accurate disease assessments was supported by the correlation between these microscopic data and additional macroscopic observations based on the degree at which steles were reddened (data not shown). This reddening was assessed on a discrete scale of 2 (severely diseased) to 10 (no symptoms), following Converse and Scott (1962). The correlation fitted for 92.5% of the seedlings; of the deviating 7.5%, 2% had no oospores but showed red steles, 0.5% had oospores but showed no reddening, and 5% could not be rated macroscopically due to indistinct symptoms.

The macroscopic observations confirmed the dichotomy of the R × S progenies, as 46% of their seedlings rated 2, 51% rated 10, and 3% had intermediate ratings (data not shown).

Nomenclature

In strawberry, a convention on the denotation of genes still fails. Here, the symbol *Rpf* follows the gene nomenclature of Yoder (1986) and Søgaard and Von Wettstein-Knowles (1987) for genes in barley. The 'R' refers to a resistance gene of the host, while the 'p' and 'f' are the first letter of the generic (p) and varietal name (f), respectively, of the pathogen to which this gene confers resistance. The italicised symbol indicates a gene or locus, the nonitalicised symbol indicates the relative phenotype. The first letter is capitalised to indicate dominance of the resistance allele. The number refers to the resistance factor of the GFG model (Van de Weg 1977).

Genetics of other traits

Elucidation of the genetics of commercially interesting traits in the cultivated strawberry has for long been assumed hardly feasible due to its octoploidy (2n = 8x = 56). For some traits, however, the genetics has recently been elucidated. Arulsekar et al. (1981) showed that the isozymes phosphoglucoisomerase (PGI) and leucine amino peptidase (LAP) are governed by a single gene. Next, Van de Weg et al. (1989) found the resistance of various North American genotypes to *P. fragariae* var. *fragariae* to be inherited monogenically. Also, day-neutrality and sex (Ahmadi et al. 1990; Ahmadi and Bringhurst 1991), and resistance to *Colletotrichum acutatum* (Denoyes-Rothan personal communication) were found to be monogenic traits. The present report adds the resistance of *Rpf2* to this list. Results like these can be expected for monogenic traits for which one of the crossing parents has a single dominant allele, since this allele is passed on to half the gametes and should thus result in a 1:1 segregation ratio, independent of the ploidy level. Since the cultivated strawberry is an amphipolyploid, and not a true autopolyploid (Bringhurst 1990; Galletta and Maas 1990), such clear segregation ratios are likely to occur relatively frequently. These observed and the theoretically anticipated successes will hopefully encourage genetic research on other traits.

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