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Genetic analysis of resistance to blackspot (*Diplocarpon rosae*) in tetraploid roses

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Abstract *Diplocarpon rosae* is the causal agent of rose blackspot, one of the most severe diseases of field-grown roses. The genetics of resistance to this pathogen was investigated in crosses between tetraploid rose genotypes. The hybrid breeding line 91/100-5, which exhibits a broad resistance to all isolates tested so far, was selfed to produce an F_2 population, backcrossed to the susceptible tetraploid variety 'Caramba' and crossed to the susceptible varieties 'Heckenzauber', 'Pariser Charme' and 'Elina'. Infection experiments resulted in segregation ratios consistent with the presence of a single dominant resistance locus in the duplex configuration in the hybrid 91/100-5. This suggests, together with previous data on the race structure of the fungus, a "gene-for-gene" type of interaction in the pathosystem *Diplocarpon/Rosa*. We propose to designate this gene *Rdr1*, which is the first resistance gene described in the genus *Rosa*. The advantages and limitations of such an interaction type for future rose breeding programmes and for marker-assisted selection strategies are discussed.

Key words *Diplocarpon rosae* · Blackspot · *Rosa* · Resistance · Genetic analysis

Introduction

Genetic analysis of the inheritance of disease resistance in plants is one of the key steps in the analysis of plant pathogen interactions. It will provide knowledge crucial to the development of strategies for conventional resistance breeding as well as for projects having the aim of localising and isolating plant resistance genes (Crute and Pink 1996; Briggs and Johal 1994).

Rose blackspot caused by the host-specific facultative fungal pathogen *Diplocarpon rosae* Wolf is considered to be the most severe disease of field-grown roses worldwide (Horst 1983). Infected plants very often develop heavy chlorosis at the site of infection and lose their foliage very early in the vegetation period. Repeated infection cycles severely weaken the susceptible plants, which show reduced growth and eventually die. The only possibilities to control blackspot in the field are the application of fungicides and/or breeding for resistance. As the application of pesticides faces growing concerns from consumers and public authorities, improved breeding strategies for the production of blackspot resistant rose varieties will be a necessity in the near future.

Numerous investigations on the biology of rose blackspot (Aronescu 1934; Frick 1943; Reddy et al. 1992) and the occurrence of resistance in wild and cultivated roses (Knight and Wheeler 1978a, b; Palmer et al. 1966; Jenkins 1955; Wiggers et al. 1997) have demonstrated the presence of resistance, mainly in wild rose species. However, to date no detailed information about the inheritance of any resistance to blackspot has been reported.

In previous projects we were able to demonstrate the occurrence of at least five physiological races of *Diplocarpon rosae* by the inoculation of ten plant genotypes with 15 single conidial isolates (Debener et al. submitted). We further demonstrated the occurrence of differential interactions between rose genotypes and blackspot races and the occurrence of rose genotypes resistant to all fungal isolates and conidial mixtures tested in our lab. Here we present data on the genetic analysis of the resistance present in the tetraploid breeding line 91/100-5, which displays a broad resistance to all races and which is currently used as a donor to introduce this resistance into the

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genetic background of cultivated roses in model breeding programmes.

The future goal of our project is the analysis of molecular markers closely linked to resistance genes against blackspot and the development of improved breeding strategies based on marker-assisted selection for blackspot resistance in roses.

Materials and methods

Plant material

All of the rose genotypes used in the present study are part of the genotype collection of the Institute for Ornamental Plant Breeding in Ahrensburg. The susceptible varieties 'Caramba', 'Heckenzauber', 'Elina' and 'Pariser Charme' as well as the genotypes 88/124-46 and 91/100-5 are maintained in vitro as shoot cultures (Davies 1980), in the greenhouse and in the field. The F_2 progeny from 91/100-5 is maintained in the field, whereas the BC and the F_1 progenies are maintained in the greenhouse. The origin of the resistant tetraploid breeding line 91/100-5 from 88/124-46 has been described in Drewes-Alvarez (1992) and is depicted in Fig. 1. The diploid hybrid 88/124-46 had been derived from a cross between a tetraploid garden rose and a diploid *Rosa multiflora* followed by four cycles of open pollination. The diploid status of 88/124-46 as well as the tetraploid status of CT 40 and 91/100-5 was determined cytologically by Drewes-Alvarez (1992). The resistant hybrid 91/100-5 served as a parent for five different crosses (Table 1). As the parent line CT40 as well as the progenitor 88/124-46 (Fig. 1) were both resistant, whereas the present 'Caramba' is susceptible it was concluded that resistance to blackspot in 91/100-5 was derived from 88/124-46 and probably originated in *Rosa multiflora* (Drewes-Alvarez 1992; Debener et al. submitted).

Crosses

The different crosses performed for the present study are listed in Table 1. They were performed in the greenhouse at ambient temperature above 20°C and additional light between January 1994 and April 1994. Pollen was harvested from flowers 1 or 2 days before opening by removing all anthers and dehiscing them at room temperature overnight in dessiccators containing dry silica gel. The pollen was either used immediately for pollinations or stored dry at

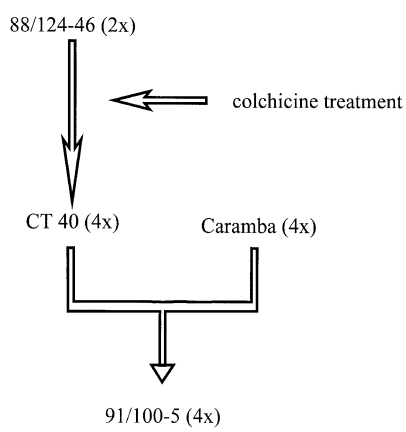

Fig. 1 Origin of the resistant breeding line 91/100-5

Table 1 Crosses performed for the analysis of the inheritance of disease resistance to the blackspot isolate Dort E4

Cross	Type of cross	Seed parent	Pollen parent (rose class)[a]
93/2	F_2	91/100-5	93/100-5
95/1	Backcross	91/100-5	Caramba (Hybrid Tea)
95/2	F_1	91/100-5	Pariser Charme (Hybrid Tea)
95/3	F_1	91/100-5	Heckenzauber (Floribunda)
95/4	F_1	91/100-5	Elina (Hybrid Tea)

[a] The rose classes are listed according to Cairns (1993)

− 80°C. The mother plants were emasculated as soon as their petal color became fully visible, and the flowers were pollinated immediately or the day after with small paint brushes. The hips were harvested in autumn, and the seeds were washed and sown into non-fertilized soil immediately. Germination was enhanced by keeping the trays moist at 4°C for about 8 weeks. After the first seedlings emerged, the trays were moved to a greenhouse compartment kept at 20°C. When the seedlings had developed their first true leaves, they were picked individually into pots with fertilised soil and kept in the greenhouse.

Fungal strains and infection assay

The single conidial isolate Dort E4 (Debener et al. submitted) belongs to the previously identified race five, which infects all tested rose varieties. It was multiplied on excised leaves of the generally susceptible variety 'Pariser Charme' by pipetting or spraying suspensions of 10^5 conidia/ml in tap water onto the leaves and incubating the leaves in moist plastic containers for 2 days at 20°C in an incubator. The leaves were subsequently incubated at room temperature until new conidia were formed. Inoculum was produced by washing off newly formed conidia with sterile tap water and adjusting the concentration to 10^5 conidia/ml.

The inoculation was accomplished by cutting off three to five leaves at their base, washing them briefly in tap water, placing them in moist, translucent plastic containers and pipetting 8 to 20 10-µl droplets of conidial suspension onto the upper surface of the leaflets. Any additional injury of the leaves apart from cutting was avoided. The leaves were incubated as for inoculum multiplication. The scoring of the interaction phenotype of the tested plants (resistant or susceptible) was done 10 and 14 days after inoculation. Those plant genotypes on which mycelial growth beyond the area of inoculation and the formation of acervuli could be observed were considered to be susceptible. Those genotypes where no mycelial growth beyond the inoculation area could be observed were considered to be resistant.

The analysis of segregation ratios was done by the χ^2 test (Bortz et al. 1990).

Results

Previous infection assays performed in connection with experiments on the identification of fungal races of *Diplocarpon rosae* had shown that the hybrid 91/100-5 was resistant to all tested blackspot isolates from all five races. Furthermore, it was also resistant to broad mixtures of conidia from several geographic locations

Table 2 Phenotypic ratios of resistant and susceptible plants in five different crosses after inoculation with the blackspot isolate Dort E4

Cross (type of cross)	*n*	Resistant	Susceptible	Ratio	χ^2	*P*
93/2 (F_2)	251	248	3	35:1	2.33	0.1–0.2
95/1 (BC)	68	59	9	5:1	0.58	0.3–0.5
95/2 (F_1)	98	83	15	5:1	0.13	0.7–0.8
95/3 (F_1)	119	98	21	5:1	0.08	0.7–0.8
95/4 (F_1)	59	51	8	5:1	0.41	0.5–0.7

(Debener et al., submitted). Therefore, line 91/100-5 was selfed, backcrossed to the susceptible parent variety 'Caramba' and crossed to susceptible varieties 'Pariser Charme', 'Heckenzauber' and 'Elina' (Tables 1 and 2).

To investigate the inheritance of resistance to blackspot in these populations, we carried out infection experiments with the race five isolate Dort E4. This isolate was chosen because isolates belonging to race five infected all commercial rose varieties tested and because Dort E4 is one of the most aggressive isolates in our collection (Drewes-Alvarez 1992; Debener et al. submitted).

Repeated inoculations of varieties 'Pariser Charme', 'Caramba', 'Heckenzauber' and 'Elina' with Dort E4 resulted in successful infections at more than 90% of the inoculation sites, whereas not a single infection event was observed for the line 91/100-5. After the inoculation of 251 field-grown plants of the progeny from cross 93/2 with the isolate Dort E4, 3 susceptible and 248 resistant plants could be identified (Table 2). The observed segregation pattern fits a ratio of 35 resistant to 1 susceptible plants at the 5% level of confidence, which is the expected ratio for a single dominant locus in the duplex configuration (RRrr) for a random chromosome assortment in F_2 populations (Lindstrom 1936). The observed ratio does not fit the theoretically possible ratio of 21:1 for random chromatid assortment ($\chi^2 = 6.49$, $P = 0.025$–0.01).

Infection experiments with the same isolate were performed with progenies from the crosses 95/1, 95/2, 95/3 and 95/4 in three, two, three and one independent experiments, respectively (Table 2). In all cases the observed segregation ratios fit a 5:1 segregation of resistant to susceptible plants, which is also in agreement with the presence of a single dominant locus in the duplex configuration in 91/100-5. Similar to the results for the F_2 population, the fit of the observed segregation ratios to a theoretically possible random chromatid segregation ratio of 11:3 (resistant:susceptible) was much weaker with χ^2 values of 2.74 (91/100-5 × 'Caramba'), 2.18 (91/100-5 × 'Pariser Charme'), 1.01 (91/100-5 × 'Heckenzauber') and 1.50 (91/100-5 × 'Elina'), respectively.

No difference in the segregation ratios between the backcross population and the different F_1 populations could be observed, indicating that the different susceptible genetic backgrounds did not influence the resistance reaction.

Discussion

The segregation ratios of 35:1 in the F_2 progeny and 5:1 (resistant:susceptible) in the BC and F_1 progenies provide evidence for the presence of a single dominant resistance gene in the duplex configuration (RRrr) in the rose genotype 91/100-5. The allele conferring resistance in 91/100-5 must be dominant because this hybrid, which originates from a cross between the resistant line CT40 and the susceptible variety 'Caramba', is resistant (Drewes-Alvarez 1992) and the major classes in both segregation types (35:1 and 5:1) in the progenies from 91/100-5 comprise the resistant plants. A recessive resistance gene should lead only to susceptible progeny in the F_2 and, depending on the configuration of the recessive resistance allele in the susceptible parents for the BC and F_1 populations, either 0:1 (nulliplex, RRRR and simplex, RRRr), 1:5 (duplex, RRrr) or 1:1 (triplex, Rrrr) ratios of resistant versus susceptible progeny, respectively. In the case that 91/100-5 would carry two unlinked dominant and independently acting resistance genes, ratios of 35:1 ($R^1R^1r^1r^1/R^2R^2r^2r^2$), 11:1 ($R^1R^1r^1r^1/R^2r^2r^2r^2$) or 3:1 ($R^1r^1r^1r^1/R^2r^2r^2r^2$) of resistant versus susceptible plants would be expected in the backcross and F_1 populations. In an F_2 population only 1 out of 1296 plants would be expected to be susceptible. When a dominant gene would occur in the triplex configuration (*RRRr*) only resistant progeny would be expected in F_2, backcross and F_1 populations.

We propose to name the resistance gene analysed in the present study *Rdr1*, following the gene nomenclature of Yoder (1986) and as used for example, by Van de Weg (1997) for a resistance gene in strawberry. In accordance to this system the capital first letter R refers to a dominant resistance gene in the host, whereas d and r are the first letters of the generic (*D*iplocarpon) and species (*r*osae) name of the pathogen. *Rdr1* is the first resistance gene described in roses.

All of the observed segregation ratios fit much closer to those expected for a random assortment of chromosomes than for a theoretically possible random assortment of chromatids (Lindstrom 1936). The fact that this difference could be observed in all crosses indicates that random assortment of chromosomes is the mode of inheritance in roses. Furthermore, no differences in the type of segregation between the four different

susceptible genetic backgrounds ('Caramba', 'Pariser Charme', 'Heckenzauber' and 'Elina') used as crossing partners of 91/100-5 could be detected. This indicates that the genetic background of the susceptible parents does not influence the expression of blackspot resistance from 91/100-5 in the populations studied so far.

The detection of a single dominant resistance locus in 91/100-5 together with the occurrence of physiological races in the pathogen (Debener et al. submitted) suggests a "gene-for-gene" type of interaction for this particular host/pathogen combination. This is in accordance with other well-known pathosystems involving obligate or facultative biotrophic plant pathogens (Crute and Pink 1996; Briggs and Johal 1994; DeWit 1992) in which mostly single dominant loci for resistance on the host side interact with single dominant loci for avirulence on the pathogen side.

However, final proof of a "gene-for-gene" interaction must also involve the genetic definition of pathogen avirulence genes, which is beyond the scope of the present study. The fact that blackspot resistance in 91/100-5 is based on a single dominant gene facilitates the introgression of this resistance into the genetic background of modern rose cultivars, which are mostly lacking any effective resistance to blackspot. Compared to quantitative traits, relatively few generations of backcrosses to cultivated roses are needed to dilute the unwanted genetic background of the wild donor species and to obtain hybrid plants which fit the ornamental requirements of modern rose cultivars. However, the disadvantage of utilising monogenic resistance genes in breeding programmes lies in the relatively high probability that new pathogenic races of the fungus will emerge that are able to overcome the action of this particular resistance gene. Besides searching for new sources of blackspot resistance, we are currently developing strategies for marker-assisted selection for resistance breeding in roses (Malek and Debener in preparation). The BC and F_1 populations described in the present study are used as model populations to search for molecular markers closely linked to the resistance gene *Rdr1*. In the near future markers for resistance genes from different sources (e.g. wild species) may be used for an efficient pyramiding of monogenic resistance genes in rose breeding programmes.

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