Study the Reactions of Powdery Mildew (*Podosphaera pannosa*) in Resistant and Susceptible Rose Genotypes and Their Segregating Progeny

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Abstract

Rose is one of the most important ornamental plant in floriculture. Powdery mildew *Podosphaera pannosa* (Wallr.: Fr.) de Bary is one of the most important disease in garden and greenhouse grown roses. The relation between fungal development and plant resistance was investigated microscopically on young greenhouse leaves of two rose genotypes with different levels of resistance and on 90 progeny derived from a cross between these two genotypes and also on a selective interesting progeny. Two monoconidial pathotypes of powdery mildew including R-E and R-P with differing in virulence on roses were used for studying the plant defence mechanisms. The parent plants of this population 'Yesterday' and *R. wichurana* differ in resistance towards to two monoconidial powdery mildew isolates. *R. wichurana* shows partial resistance to the pathotypes R-E and R-P; 'Yesterday' is resistant to pathotype R-E but susceptibility to R-P. Segregation for resistance to the two powdery mildew isolates was studied in the offspring by a bio-assay and by a microscopy study of specific resistance mechanisms. Microscopic observations discriminated in more detail resistance mechanisms in the rose plants at different developmental stages of the fungus. Induced plant reactions, hydrogen peroxide production and cross sections through infected leaves were examined. The variation in development of the fungus on these rose genotypes depended on the relative presence of normal or abnormal haustoria, induced cell reactions, papilla formation or physical barriers.

***Keywords*: disease resistance, *Podosphaera pannosa, R. wichurana*, segregation, powdery mildew**

Introduction

Roses are the most important ornamental crop in the floriculture industry (3). Powdery mildew, caused by *Podosphaera pannosa* (Wallr.:Fr.), is the most commonly occurring disease on roses cultivated in greenhouses (6) and is also important on field grown roses. For powdery mildew several pathotypes have been described on roses (5; 4). In roses morphological barriers, papillae formation, induced single cell and multicell reactions with or without cell collapse and the formation of abnormal haustoria upon powdery mildew infection have been shown by Dewitte et al. (2007) together with rose genotype dependent reactions.
The aim of the current study was to gain insight into the segregation of resistance and the underlying mechanisms in roses. For this purpose, a diploid segregating population was made. Resistance in parent plants and offspring was tested with 2 well characterized powdery mildew pathotypes in order to study pathotype specific resistance. The pathotypes used have been described by Leus et al. (2006). R-E is virulent on rose, while, R-P is virulent on both *Prunus avium* and rose.

Materials and methods

A diploid (2n=2x=14) rose population, derived from a cross between the cultivar 'Yesterday' (seed donor) and the species *R. wichurana* Crep. (pollen donor) was used for the inoculation tests and resistance of the segregation progeny was studied. The population consisted of 90 individual genotypes planted in the field. The plants were more than 2 years old at the start of the experiments. Experimental inoculations were made by an inoculation tower with 2 pathotypes of *Podosphaera pannosa*, R-P and R-E. The two monoconidial isolates were chosen from the powdery mildew collection described by Leus et al. (2006). We aimed to inoculate with about 60 conidia/cm². The number of conidia/cm² was counted

microscopically after tower inoculation on a control dish with the agar medium but without leaves. Between 30 to 80 conidia/cm² were accepted as final amount. The pathotypes were multiplied by dusting conidia on in vitro rose plantlets of the susceptible rose cultivar 'Gomery'. The percentage of infected leaflet surface was scored microscopically (with a binocular microscope, Leica Wild MZ8, magnification: 15x-32x) in steps of 10% for every leaflet of a leaf according to Leus et al. (2003). Scores of the infected leaflets were used to calculate the disease index (DI) for every inoculation test (Liu et al. 1996). From the different repetitions the mean DI was calculated for every genotype. In each repetition of inoculation, leaves without infection were excluded from computation of the DI. The range of DIs obtained was divided in 5 equal classes from 1 (resistant) to 5 (susceptible) and a class was attributed to every genotype. Statistical analysis was performed using STATISTICA 8 software (StatSoft, Tulsa, OK, USA). The leaves were carefully checked to be free of contamination. Therefore for each inoculation experiment we kept some non-inoculated leaves as a control in the growth chamber under the same condition as the inoculated leaves.

All experiments were performed with both pathotypes separately. For microscopic evaluation, 7 leaves (49 leaflets) were observed per genotype–isolate combination using a light microscope (Leica DMIRB; magnifications: 100x-200x).

Results

Based on the scores of the parents and F1 plants inoculated using the inoculation tower, resistance classes (1=resistant to 5=susceptible) were assigned to every genotype for the pathotypes R-E and R-P separately. Parent *R. wichurana* was scored class 2 for pathotype R-P and class 1 for pathotype R-E. Parent 'Yesterday' showed to be very susceptible (scoring class 5) to pathotype R-P and was profoundly resistant to pathotype R-E with no observed fungal development. In total 64 (71%) and 76 (84%) of the F1 genotypes belonged to class 2 or 3 for pathotype R-P and R-E respectively (Figure 1). Of 90 genotypes, 40 individuals obtained the same class for both pathotypes. Some genotypes belonged to different classesof resistance when inoculated with different pathotypes. Nine genotypes had more than 1 class difference between both pathotypes. Only 2 F1 genotypes showed more resistance than the parent *R. wichurana* for R-P. Only a weak correlation was observed between the classes attributed to the two pathotypes on the individual genotypes (Spearman's rho = 0.33; $p < 0.05$). There was no significant difference between the total mean DIs for the F1 population when both pathotypes were compared (Mann-Whitney U test; $p < 0.05$).

Sporulation was scored at 10 dai (days after inoculation) with, 35 leaflets evaluated per plant genotype and per pathotype. Normal abundant sporulation (score 3 or 4) was only observed for R-P on 'Yesterday' that developed and covered almost 20% to 100% of the leaflet surface while on *R. wichurana,* the development of conidia was limited to score 2 to 4 that covered only 10-35% of the leaflet surface. Pathotype R-E could only infect and sporulate on *R. wichurana* (5-25% of the leaflet surface), resulting in a score of 1 to 2.

Discussion

The 2 pathotypes of *P. pannosa* used in this study were characterized by Leus et al. (2006). While the pathotype R-E is only virulent on roses, R-P can infect roses and *Prunus*. In our study, differences between the two pathotypes used were confirmed as differential reactions in the parents *R. wichurana* and 'Yesterday'.

For macroscopic evaluation of the parents and F1 population inoculations were performed with an inoculation tower, resulting in a relatively homogeneous distribution of the conidia over the leaf material as previously shown by Linde and Debener (2003) and Leus et al. (2003). The homogeneous distribution of the conidia is essential for the evaluation of plant

disease with a quantitative inheritance. It is a key factor for genetic studies to measure the contribution of minor genes for resistance (7).

Different resistance classes were found for the parents by tower inoculation. Variable microscopic reactions were observed in both parents to both fungal isolates. Microscopic evaluation revealed more detailed information on resistance mechanisms in the parent plants. Remarkably immediately after germination of the R-E conidia on 'Yesterday', fungal development was arrested and no secondary mycelium development was recorded by microscopic evaluation. Usually 1 cell reaction (hypersensitive response or HR) was observed under the arrested conidia. To pathotype R-P in spite of high cell reactions, this parent plant showed high susceptibility (class 5). In a former study by Leus et al. (2006) where they tested R-E and R-P, no fungal development was observed for both pathotypes on *R. wichurana*. Leus et al. (2006) used in vitro plantlets with the aim of distinguishing fungal pathotypes. In the study of Dewitte et al. (2007) very few conidia of R-E developed secondary mycelium on *R. wichurana* and no sporulation occurred. At 48 hours after inoculation only 1.7% of the conidia showed cell reactions beneath the germ tube on *R. wichurana*. Dewitte et al. (2007) found that in *R. wichurana* inoculated with R-E, resistance mainly happens based on papilla formation and formation of abnormal haustoria. Cell collapse, characterized as necrotizing cells, was often seen when the fungus started to produce secondary mycelium. The results in the study presented here contrast with those obtained by Dewitte et al. (2007) but this can be explained by the difference in leaf age.

After the tower test only 4 genotypes showed a higher resistance (class 1) to R-E compared to the resistance in the parents. In the study presented here many genotypes showed raised susceptibility to one or both pathotypes. we concluded that the specific resistance segregated in the progeny. In some genotypes there were a lot of conidia that did not form secondary mycelium similar what observed in parent 'Yesterday'. The segregation in the offspring points to monogenic resistance or as it is called race-specific resistance to R-E. In such cases, a resistance gene should be exist in the host which confers a specific interaction with an avirulence gene of the pathogen (3).

For the R-P isolate, the formation of secondary mycelium was much lower on most genotypes compared to both parents. Only 1 plant developed more secondary mycelium, although the number of cellular reactions was higher. Furthermore, the number of cellular reactions depends on the expansion of the fungus. In conclusion, our results show that resistance reactions to powdery mildew in roses do not only result in different resistance mechanisms depending on the rose genotype but are also pathotype dependent.

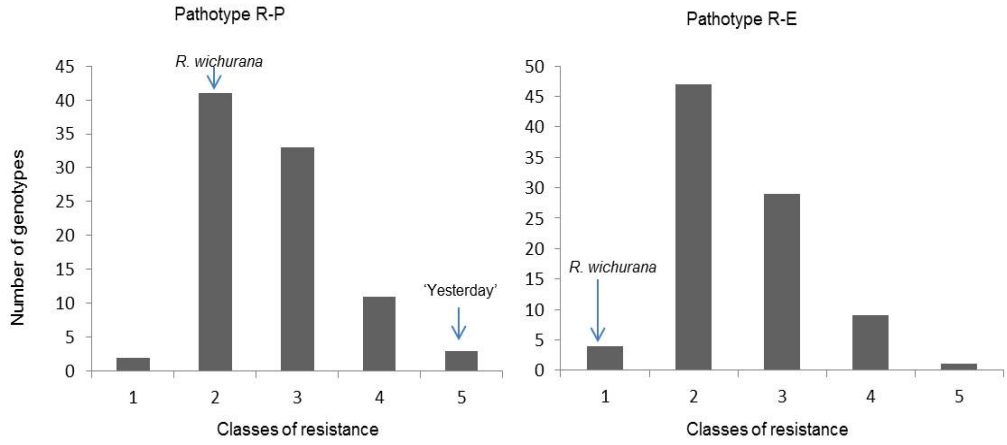

Figure 1. Classes of resistance obtained based on mean DI as scored for each individual F1 rose genotype (90 genotypes) from the tower inoculation for both powdery mildew pathotypes. Conidial density: 60 conidia/cm² (on average) with at least five repeated inoculations. Class: 1 = very resistant, 2 = partial resistant, 3 = medium resistant, 4 = susceptible, 5 = very susceptible (Liu et al. 1996). Pathotype R-E did not develop secondary mycelium on parent 'Yesterday' (only can germinate)

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