Local European Cultivars as Sources of Durable Scab Resistance in Apple

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Abstract

The V_f resistance gene has been widely used in apple breeding programmes to control *Venturia inaequalis*, the causal agent of scab, the major apple disease. Since the appearance of new strains that are able to overcome this major gene, research in Europe has been focused on durable resistance. The objective of one task of the European DARE project was to find cultivars which show a broad spectrum of resistance to the different scab races. This collaborative work involved 6 partners who tested 36 cultivars with various local scab inocula collected in the participating countries and with 8 monoconidial strains belonging to known races or isolated and characterized in the frame of the DARE project. Tests were performed each year from 1998 to 2001. Symptoms were assessed using macroscopic scoring scales. Some microscopic observations were performed; these resulted in a better knowledge of the host/pathogen interaction. Very diverse and complex resistance behaviours were found: the cultivars which showed the widest range of resistance were mostly local cultivars and some newly selected hybrids combining major genes and partial resistance. It would be worthwhile to include these individuals as parents in apple breeding programmes to improve the durability of scab resistance. Some methodologies and strategies to reach this goal will be proposed.

INTRODUCTION

Most of the apple breeding programmes in the world aim to release new cultivars combining high fruit quality, regular cropping and resistance to pests and diseases particularly scab (due to *Venturia inaequalis*) the most important apple disease. The V_f gene is the main source of scab resistance included in apple breeding programmes (Laurens, 1999), but it has been overcome by new strains of *V. inaequalis* (Parisi et al., 1993; Roberts and Crute, 1994) that are now mostly widespread in Northern Europe (Parisi et al., 2004). As most of the genotypes carrying V_f are susceptible to at least one known scab strain, breeders aim now to enlarge the set of parents to include new sources of durable resistance in their programmes. This paper deals with a main objective of the DARE project: the phenotypic characterization of the resistance status of a large range of apple cultivars. Pathological screening tests have been performed both in the glasshouse and in the field. We report here the results of a wide network of glasshouse tests performed in six sites: BAZ- Dresden (Germany), CRA-Gembloux (Belgium), DCA-University of Bologna (Italy), ETH-Zürich (Switzerland), INRA-Angers (France) and PRI-Wageningen (The Netherlands).

The main aims of this task were to study the behaviour of cultivars selected for their polygenic resistance to scab and also the emergence of *V. inaequalis* strains virulent to a wide host range, with special attention to virulence to the V_f gene. A special emphasis

was put at the beginning of the project on methodological aspects: glasshouse conditions, development stage of the trees, inoculation methods, symptom assessments.

MATERIALS AND METHODS

From 1998 to 2001, 21 scab tests were performed by the six partners on a common set of 36 cultivars. Glasshouse conditions were fixed to get an optimum development of scab and also to allow a good plant growth as well. Many meetings and visits were organized to ensure common assessment at the different sites.

Plant Material

The set of 36 cultivars was selected at the beginning of the project in agreement with all the partners (Angers meeting, 1998). Budwoods of each cultivar came from a single origin; they were distributed to all the partners who grafted them on the MM106 rootstock which produced vigorous trees. The tested cultivars were:

- Six "control" cultivars including three susceptible ones, *Malus floribunda* 821 and two V_f resistant cultivars. This set allows the detection and characterization of the presence of the races 6 and 7.
- 22 local cultivars selected by the different partners for showing very few symptoms of susceptibility in the field in each country; and so, assumed to carry a high level of partial resistance.
- Eight new selected hybrids carrying both a major resistance gene and partial resistant genetic background. This paper doesn't report any of the results from these hybrids.

Inocula

The cultivars were tested with two kinds of inocula:

- Six local "breeding" inocula, obtained after collecting scabbed leaves in the field of each institute BAZ, CRA, DCA-BO, ETH, INRA and PRI. They can be considered as mixtures of strains representative of the inoculum status at each site. Each institute performed scab tests with its own inoculum on one, two or three years.
- Eight monoconidial strains: three reference strains belonging to known races, characterized and maintained by the Pathology station at INRA Angers (Bénaouf and Parisi, 2000): 104 (race 1), 302 and 1066 (race 7), and five strains isolated and characterized in the frame of the project (Parisi et al., 2004) and tested by PRI (EU-NL-19, EU-NL-05, EU-NL-24) and INRA (EU-B-04, EU-D-42, EU-NL-24).

Concentration of inocula varied between $1.5\ 10^5$ and $5\ 10^5$ conidia/ml in the different tests. The level of infection was monitored on some trees of susceptible cultivars included in each experiment.

Macroscopic Assessments

Symptom scoring was performed, leaf/leaf, on at least 5 trees carrying one to two growing shoots per cultivar. The most important problems of growth were recorded on the cultivar 'Rote Sternrenette' on which most of the assessments were made on non-growing shoots.

Two main scoring scales were used:

1. Classes of Symptoms. Chevalier and collaborators proposed a scale to assess scab resistance/susceptibility symptoms based on seedlings segregating for major genes (Chevalier et al., 1991). In the present study, working with some cultivars assumed to carry partial resistance, a larger range of symptoms was observed. During a common meeting, all the partners assigned the different observed resistance/susceptible features to the 5 classes, "1", "2", "3a", "3b" and "4", proposed by Chevalier. An additional class has also been added to characterize the typical stellate necrotic resistance symptoms due to the Vg gene.

2. Severity. (Croxall et al., 1952 modified by Parisi et al., 1993), which estimated the amount of sporulating leaf area.

On the basis of the assessments made on all the replicated trees, each cultivar has

been classified into one of three different groups: susceptible (high sporulation severity and/or high incidence), resistant (no sporulation on any of the leaves); the intermediate group includes cultivars which show few sporulation either with low incidence or low severity.

Microscopic Assessments

At INRA Angers, some assessments were also performed both in light and in scanning electron microscopy (SEM). They gave informative data on various key points of the interaction between *V. inaequalis* and some apple cultivars: aspect and abundance of the subcuticular stroma, amount of spore germination, conidiogenesis intensity and quality, plant reaction… Microscopic observations gave useful complementary information to better explain macroscopic data (Chevalier et al., 2004).

RESULTS

Results displayed in Table 1, can be interpreted in two ways: i) inoculum characterization on a common set of cultivars and ii) cultivar characterization against various inocula.

Inoculum Characterization

Results on monoconidial strains (Table 1) can partially be compared with those obtained by Parisi et al. (2004) on a range of differential hosts - 'Gala', 'Golden Delicious', 'Prima', *Malus floribunda* 821, 'Fiesta', 'TN10-8', 'Discovery', 'Durello di Forli' - inoculated with strains EU-B-04, EU-D-42, EU-NL-05, EU-NL-19 and EU-NL-24. Few discrepancies can be found between the two experiments: 'Golden Delicious'/EU-NL-05; 'Discovery'/EU-D-42 and 'TN10-8'/EU-NL-19; some other slight quantitative variations can also be found. Except for the 'Golden Delicious'/EU-NL-05 interaction, the overall amount of sporulation is higher in Parisi's tests whose tests have been performed in controlled growth chambers which gave better condition for scab development.

Our tests gave additional results on the various cultivar/strain interactions:

- the monoconidial strain EU-B-04 behaved as the strain 104 (race 1) inoculated in 1998: only 3 cultivars ('Fiesta', 'President Roulin', 'La Paix') reacted very differently with the 2 inocula.
- the strain EU-D-42 gave, more or less, the same reactions on the 5 control cultivars as the strain 302, the reference strain for race 6 in this trial. However in the local cultivars many discrepancies can be pointed out between the reactions observed with these 2 strains. These results show that within race 6 type strains, many "subraces" can be found.
- the strain EU-NL-19 showed very different patterns amongst the local cultivars: it was the only tested inoculum which attacked 'Z190'; it also sporulated slightly on 'Alkmene'.
- the strain EU-NL-24 was tested in two locations: at INRA and PRI. Some discrepancies occurred: no sporulation on *M. floribunda* 821 at PRI; 'Lombarts Calville' highly susceptible at INRA but resistant at PRI. For 'Alkmene' and 'Renetta Grigia di Torriana', the differences were much more quantitative: 'Alkmene' sligthly susceptible at Angers and highly at PRI which was the opposite for 'Renetta Grigia di Torriana'.

Data from the local inoculum tests in the 6 sites were also very informative: results showed a large variability in aggressiveness but also in virulence. The FAW test showed very low level of susceptibility: only 'Gala' and 'Golden Delicious' exhibit some significant sporulating severity. Some differences in virulence were recorded between years in each of the sites. This could be due to climatic effects or to different composition of the mixture of strains used in the different years. DCA-BO and PRI results seemed to be more consistent from one year to the other than those from CRA and BAZ. The BAZ inoculum has got a particularly complex virulence pattern: it is the result of the mixture of many strains of different races including race 7. PRI inoculum attacked 'Prima' in 1999 but not in 1998. Therefore, it could contain conidia from race 6 strains.

Cultivar Behaviour

1. Susceptible Control Cultivars. ‘Gala’ was highly susceptible to all the tested inocula. ‘Golden Delicious’ was also highly susceptible except to the monoconidial strains EU-D-42 (race 6) and 1066 (race 7) tested at Angers. A dubious feature appeared at PRI when ‘Golden Delicious’ was tested with EU-NL-05, assumed to be a race 7 strain (Parisi et al., 2004): significant sporulation was recorded. Fiesta was susceptible to a lot of inocula but with a weaker severity of sporulation compared with the 2 previous susceptible controls.

2. *Vf* Cultivars. ‘Prima’ was resistant to most of the inocula. It was only susceptible to the two strains of race 6 (strains 302 and EU-D-42), to the strain EU-NL-24 and to the local PRI isolate inoculated in 1999 (but not in 1998). **‘**Priscilla’ was susceptible to race 7 isolate and to one of the two race 6 strains but resistant to EU-NL-24 and to the race 1 inocula. It was also resistant to all the local inocula. *Malus floribunda* 821 was susceptible to the race 7 strain and to the German inoculum tested in Dresden for 3 years. It showed some weak susceptibility with EU-NL-24 and EU-NL-05.

3. Local Cultivars. Very interesting and variable features can be found in the list of the 22 local cultivars (Table 1). ‘Herrnhut’ and ‘Egremont Russet’ were highly susceptible to almost all the inocula; on the other hand, only one cultivar, ‘Dülmener Rosenapfel’ was resistant to all the tested inocula. Ten cultivars showed very interesting resistance behaviours: ‘TN10-8’, ‘Colapuis’, ‘Ruban’, ‘Z190’, ‘Lombarts Calville’, ‘Alkmene’, ‘Discovery’, ‘Durello di Forli’, ‘Firiki’ and ‘President Roulin’ (Table 2). They showed good resistance levels against a large range of inocula. Some of them exhibited typical symptom features whatever the strains: very strong and specific reactions of crinkling and necrosis for ‘Alkmene’ and ‘Z190’; and for ‘Ruban’, there was high incidence but with very low severity of some symptoms and some very small sporulating spots on some leaves.

DISCUSSION AND CONCLUSIONS

This collaborative work has been very powerful to screen cultivars with a wide range of scab inocula. First of all, significant progress has been made developing methods to test the scab partial resistance in the glasshouse. The two macroscopic descriptors – class of symptoms and sporulation severity- recorded on each leaf and on each growing shoot gave a large amount of data which gives confidence on the accuracy of the results on the behaviour of each cultivar in relation to different strains of *V. inaequalis*. It has also been proved that five trees, each with at least one growing shoot is the minimum replication number required to detect quantitative variation of partial resistance between cultivars (data not shown). However, these results should be confirmed by data assessed from leaves and fruits on adult trees. Preliminary results from such trials in the frame of the DARE project, showed that susceptibility/resistance behaviour expressed in the field can be slightly different than those observed in the glasshouse or growth chamber. In particular, it seems that for some cultivars, the scab resistance behaviour on fruits is different than that assessed on leaves (Parisi, pers. commun.).

On the basis of glasshouse tests, our data show that from the set of 22 cultivars selected on the basis of their good level of resistance in the field, 11 showed resistance to a large spectrum of inocula in glasshouse. These results are very encouraging for further breeding works. We could imagine that a high number of durable resistant genotypes could be found if a larger set of cultivars were assessed. This is certainly the most valuable way to enlarge the pool of sources of scab resistance within apple cultivars. Getting a good phenotypic variability doesn’t always mean efficient variability for breeding. Thus, a preliminary working step would be to develop new genetic studies to check what are the main genetic resistance factors present in each genotype. This was one of the main commitments of the DARE project based on advanced molecular studies on few partial resistant cultivars such as ‘Discovery’, ‘Durello di Forli’ and ‘TN10-8’; various regions involved in the resistance have been located on the genetic maps of these parents (Durel et al., 2004; Tartarini et al., 2004). These studies showed that the resistance of such cultivars is complex and supposed to involve various genetic mechanisms of

resistance: quantitative (QTLs) but also qualitative (major genes) with different locations within the apple genome.

Adapted recurrent selection strategies as those tested in New Zealand (Noiton et al., 1992; Oraguzie et al., 2004) would certainly be the best strategies to keep the genetic variability and at the same time to widen the genetic basis of the durable resistance of the progenies as well. However, the methods to screen for durable resistance within the progenies have also to be considered carefully. The aim is to select individuals which are resistant to a broad spectrum of virulent strains. Two complementary strategies could be adopted: i) the most classical way is to develop pathological tests on progenies using many virulent strains representative of *V. inaequalis* known diversity. The DARE project allowed to characterize these strains, to get them available and to set up the screening methodology; the only point now is to validate a routine test to check out thousands of seedlings against many strains annually; ii) Marker Assisted Selection (MAS) but today very few easy-to-use and efficient markers are available for this purpose. Research is already in progress to improve MAS in apple breeding but further studies are needed.

The DARE project progressed the assessment of phenotypic and genetic variability of scab resistance and improved the understanding of the genetic architecture of resistance. The practical use of durable resistance in apple breeding programmes needs to develop new adapted strategies which could improve the selection process. Furthermore, we have to keep in mind that the new durable resistant cultivars will also have to combine high fruit quality, long storage ability and shelf life. The new European project HiDRAS (Gianfranceschi and Soglio, 2004) develops new research on the genetics of these complex traits in order to improve the selection methodology in the future.

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Tables

Table 2. Classification of local cultivars according to their resistance spectrum. Synthesis made from the results of DARE glasshouse tests.

Highly susceptible	Susceptible to a large range of inocula	Susceptible to some inocula	Resistant to a large range of inocula	Resistant to all the tested inocula
Herrnhut	Decio	Rote Sternrenette	TN10-8	Dülmener
Egremont	Renetta Grigia di	Obereider Glanzrenette	President Roulin	Rosenapfel
Russet	Torriana	Orangenapfel	Colapuis	
	Charles Ross		Ruban	
	SchneiderApfel		Firiki	
	Reinette Clochard		Z190	
	La Paix		Discovery	
			Alkmene	
			Durello di Forli	
			Lombarts Calville	

Table 1. Synthesis of the results of the scab screening tests performed in glasshouse from 1998 to 2001.

	Local inocula												monoconidial strains								
	BAZ			CRA			DCA		PRI		FAW	INRA	INRA						PRI		
													race 1		race 6		race 7				
													104	B04	302	D42	1066	NL24	NL24	NL05	NL19
	98	99	00	98	99	00	98	99	98	99+00	99+0 0	98	98	00	98	00	98	01	00	00	01
Golden Delicious		na								(2)											
Gala										na											
Fiesta					na					(1)											
Prima				na	na		na														
Priscilla				na	na				(2)												
Malus floribunda 821				na	na					na					(2)						
TN10-8						na			na	‘											
Reinette Clochard				na	na				na												
Colapuis						na			na												
Ruban						na			(2)												
Z190				na		na				na											
Lombarts Calville						na				(2)											
Rote Sternrenette						na	na	(2)		na	na	na	(1)		(2)		na				
Obereider Glanzreitte				na		na	(1)		na						(2)						
Schweizer Orangenapfel				na	na					na											
Schneiderapfel					na				na												
Alkmene				na	na					na											
Dülmener Rosenapfel				(2)	na	na				na				na				(2)			
Herrnhut				na		na			(2)	(2)								(1)			
Charles Ross						na	(2)			na								(1)			
Egremont Russet						na			(2)												
Discovery		(1)		na	na																
Decio				na		na			(2)												
Durello di Forli						na				na											
Renetta Grigia di Torria				na		na	na		(2)												
Firiki				na	na																
Président Roulin				na		na				na											
La Paix						na			(1)	na											

Legend: ( ) : number of shoots taken into account (if<3); na : non available data; susceptibility: high severity and/or high incidence; no sporulation; low severity and/or low incidence (<3)