Resistance Breeding in Apple at Dresden-Pillnitz

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Abstract

Resistance breeding in apple has a long tradition at the Institute of Fruit Breeding now Julius Kuehn-institute in Dresden-Pillnitz. The breeding was aimed at the production of multiple resistance cultivars to allow a more sustainable and environmentally friendly production of apple. In the last decades a series of resistant cultivars (Re®-cultivars) bred in Dresden-Pillnitz has been released, 'Recolor' and 'Rekarda' in 2006. The main topic in the resistance breeding programme was scab resistance and the donor of scab resistance in most cultivars was Malus x floribunda 821. Due to the development of strains that are able to overcome resistance genes inherited by M. x floribunda 821 and due to the fact that single resistance genes can be broken easily, pyramiding of resistance genes is necessary. Besides scab, fire blight and powdery mildew are the main disease for which a pyramiding of genes is aspired in Pillnitz. Biotechnical approaches are necessary for the early detection of pyramided resistance genes in breeding clones. This paper will give an overview of the resistance breeding of apple in Pillnitz and the methods used.

Keywords: breeding, resistance, marker assisted selection

Introduction

Apple is a perennial crop and the long period apple trees are exposed to the environment promotes development and mutation of pathogens. To defeat the pathogens and to produce high quality apples an enormous effort is required. Mainly pathogens are combated with plant protection agents. Depending on the production system chemical or organic compounds are used. Resistance breeding aims at the development of plants defeating pathogens by themselves. The most dominant and harmful pathogens in apple production are fire blight (*Erwinia amylovora*), powdery mildew (*Podosphaera leucotricha*) and scab (*Venturia inaequalis*). In recent years the disease apple proliferation caused by the phytoplasm *Candidatus* Phytoplasma *mali* (Seemüller & Schneider, 2004) has become important.

Breeding of resistant apple in Dresden-Pillnitz has a long tradition and was aimed at the production of cultivars with high quality and multiple resistances to abiotic and biotic stresses. In a process lasting decades cultivars were developed with combined resistances to fire blight, scab and powdery mildew. Most of the Pillnitzer cultivars carry the *Vf* resistance gene from *M. x floribunda 821* but cultivars like 'Regia' or 'Realka' carry the *Vh4* gene from the 'Russian seedling' and the scab resistance of 'Reglindis' is based on the polygenic resistance from 'Antonovka'. Resistances based on single genes can be easily overcome by pathogens as happened for *Vf*. To avoid easily breaking of resistant genes and to ensure a more durable resistance the pyramiding of resistance genes is acquired.

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Resistance breeding in Dresden-Pillnitz is now focussed on pyramiding of resistance genes for scab as well as for powdery mildew and the identification, analysis and introduction of new resistance mechanisms for fire blight. Pyramiding of resistance genes needs new selection strategies. Classical screening methods like artificial inoculation of seedlings with scab are combined with molecular marker techniques.

This paper will present information about the success in scab resistance breeding, the strategies used to pyramid resistance genes and results of our work on fire blight.

Material and Methods

Crosses

Crosses were performed in the field. Flowers were bagged to prevent outbreeding and pollinated with pollen using a brush. Mature fruits were harvested and seeds released. Seeds were sown out in trays in the greenhouse.

Pathogen inoculation and resistance screening

Seedlings derived from crosses made for scab resistance were artificially inoculated with scab in the greenhouse. Inoculum was gained from leaves with scab collected in the orchard. At least scab races 1, 6 and 7 are present in the Pillnitz orchard. In recent years an increase of races 6 and 7 can be assumed. Plants were phenotyped around four weeks after inoculation and susceptible seedlings discarded in general.

Resistant clones were grown in plots in the orchard where no fungicides were applied. Natural incidence of scab and powdery mildew was assessed.

Resistance to fire blight of cultivars or breeding clones was estimated by artificial shoot inoculation in the greenhouse as described by Peil et al. (2007). Mapping of fire blight resistance is described in Peil et al. (2007).

Marker assisted selection

Marker analyses were performed as described by Király et al. 2007.

Results and Discussion

Table 1 summarizes the results of the scab resistance breeding from 1972 to 1998 in Dresden-Pillnitz. From around 52.000 seeds produced by protected crossing of parents 26.000 seedlings were not susceptible by artificial inoculation with scab in the greenhouse.

Table 1: Efficacy of scab resistant breeding from 1972 - 1998

Plot	Number of seedlings	2. Selection		3. Selection		Cultivars released	
		No. of seedlings	%	No. of seedlings	%	Number	%
D1	1155	48	4.16	37	3.20	1	0.087
C9	575	43	7.48	4	0.69	0	0
B11	10404	159	1.53	17	0.16	0	0
C4	6599	215	3.26	8	0.12	2	0.030
E6*	2992	37	1.24	14	0.47	0	0
E8	2814	27	0.96	12	0.43	0	0
E10*	1519	25	1.65			0	0
∑	26058	554	2.12	92	0.37	3	0.006

*Selection in this plot is not finished until now

Resistant seedlings were grafted on to rootstocks and evaluated in the field. Promising clones were propagated and evaluated in a second selection step. Performance of advanced clones was determined in the third selection step. Until now only three cultivars, 'Rebella', 'Recolor' and 'Rekarda' were released from 52.000 seeds. Both 'Rebella' and 'Rekarda' result from the same cross, Golden Delicious' x Remo', and carry the *Vf* gene from *M.* x *floribunda* 821, 'Recolor' is a descendant from 'Regine' (*Vf*) and 'Reglindis', who inherited its scab resistance from 'Antonovka'.

An output of three cultivars from around 50.000 seeds means that 17.000 seeds were needed to get one cultivar. Only one out of around 9.000 scab resistant seedlings showed the appropriate quality to become a cultivar. This proportion underlines the enormous effort which is necessary to develop a new cultivar.

Although 15 scab resistant Re-cultivars have been released between 1990 and 2006, only three of them derive from crosses made in Dresden-Pillnitz. The other twelve cultivars derived from crosses made in Müncheberg, The material or Müncheberg was transferred in the 1970 's to Dresden-Pillnitz were further selection was done. Most of the Re-cultivars are multiple resistant (Fischer 2000).

The next step in scab resistance breeding is pyramiding of genes. Several crosses were performed to combine *Vf* and *Vh4* (inherited from 'Russian Seedling'). As donor for *Vh4* the Pillnitz cultivar 'Regia' was used in general. Since only *Vh4* resistant clones can be detected by artificial inoculation of seedlings with scab in the greenhouse (races 6 and 7, overcoming the resistance inherited by *M*. x *floribunda* 821 were present in the inoculum), the application of molecular markers is necessary to identify clones with *Vf* and *Vh4*. Thirty nine breeding clones from eleven *Vh4* x *Vf* crosses were selected due to their pomological characteristics. Although these clones do not have sufficient quality traits necessary for cultivars, they might be useful as donors for pyramided resistance genes. Therefore, their genotype was analysed with molecular markers AL07 and AD13 for *Vh4* and *Vf.* (Tab. 2)

Table 2: Scab resistance genes in breeding clones detected with molecular markers

Cross					Number of selected clones with gene combination			
Mother cultivar	Resist. gene	Father cultivar	Resist. gene	Number o selected clones	*Vh4Vf*	*Vh4vf*	*Vh4Vf*	*vh4vf*
Rebella	*Vf*	Regia	*Vh4*	2	2			
Regia	*Vh4*	Enterprise	*Vf*	7	4	1	2	
Regia	*Vh4*	Reanda	*Vf*	5	4		1	
Regia	*Vh4*	Rebella	*Vf*	3	2		1	
Regia	*Vh4*	Recolor	*Vf (Va)*	1		1		
Regia	*Vh4*	Renora	*Vf*	5		3	1	1
Regia	*Vh4*	Topaz	*Vf*	7	1	2	4	
Regia	*Vh4*	Pi-AS 43,98	*Vf*	2	1	1		
Regia	*Vh4*	Pi-As 17,183	*Vf*	1	1			
Regia	*Vh4*	Pi-AS 41,170	*Vf*	3	1	2		
Regia	*Vh4*	Pi As 30,92	*Vf*	3	1			2
∑				39	17	10	9	3

Seventeen breeding clones showed the respective molecular markers for both *Vf* and *Vh4* and ten clones carried only *Vh4*. Due to the fact that scab races 6 and 7 are present in the orchard in Pillnitz and the inoculum for artificial inoculation was collected in this orchard, no seedling of the crosses mentioned in Table 1 without Vh4 would have passed the scab screening in the greenhouse. Nevertheless nine breeding clones only showed the fragment indicating *Vf* and three clones showed neither the *Vh4* nor the *Vf* marker. The populations were produced in different years and the screening of scab resistance in the greenhouse was done always in the year after crossing. An explanation for clones carrying only *Vf* surviving the screening might be due to the low distribution of scab races 6 and 7 in the orchard some years ago. The genetic distance of molecular maker AD13 and *Vh4* (Boudichevskaja et al. 2006) can explain the selection of three breeding clones without any fragment indicating *Vf* or *Vh4*. A recombination between *Vh4* and AD13 would result in Vh4 resistant plants with the molecular marker. Another important feature of the crosses is that 'Reanda' as well as 'Enterprise' are resistant to fire blight. Genotypes with *Vf* and *Vh4* will be tested in an artificial shoot inoculation.

Besides pyramiding of scab resistance genes, crosses have been done to pyramid powdery mildew resistance genes. Breeding clones carrying *Pl1* and *Pl2* and clones with three genes, *Pl1*, *Pl2* and *Plmis* (Fig. 1), were detected with molecular markers. A pre-screening of seedlings with powdery mildew by artificial inoculation like in scab is not feasible; adult resistance in trees to powdery mildew is different from resistance of young seedlings. Clones with Pl1 and Pl2 were used in crosses with genotypes *Vf Vh4* to combine all four resistance genes. Table 3 shows the respective genotypes of a part of the population analysed with molecular markers.

Table 3: Number of seedlings with different resistance gene combination identified with molecular markers. Seedlings result from a cross *vf Vf vh4 Vh4* x *pl1 Pl1 pl2 Pl2*

	Number of seedlings with the marker fragments				
Parental genotype	*pl1 pl2*	*pl1 Pl2*	*Pl1 pl2*	*Pl1 Pl2*	∑
vf vh4	-	4	7	3	14
vf Vh4	3	5	3	2	13
Vf vh4	29	3	8	9	49
Vf Vh4	10	1	6	1	18
∑	42	13	24	15	94

Only one out of 94 seedlings tested showed the respective fragments for all four resistance genes. Assuming an independent inheritance one out of 16 seedlings should display all four markers together. In the present progeny the segregation of resistance genes seems to be distorted as *pl2*, *Vf* and *vh4* genotypes are over represented. The *Pl1 Pl2 Vf Vh4* genotype will be used in crosses with high quality cultivars.

Some of the scab resistant Pillnitzer cultivars are highly resistant to the bacterial pathogen *E. amylovora*. A high correlation of the results from artificial shoot inoculation and percentage of tree losses caused by natural fire blight infection were observed for the highly resistant cultivars (Peil et al. 2004). Screening of clones for resistance to fire blight is generally done by artificial shoot inoculation. To get reliable results a high number of replicates are required, making the screening time and cost expensive. An inoculation of seedlings with fire blight and the maintaining of non-infected seedlings is not allowed in Germany, due to the quarantine status of the disease.

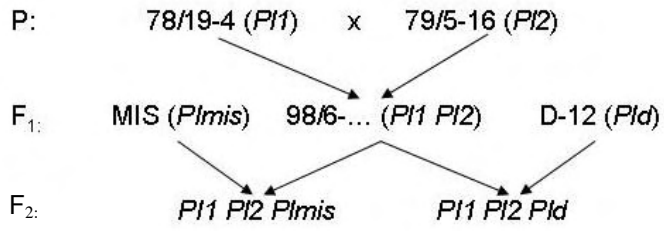

Figure 1: Breeding scheme used to pyramid powdery mildew resistance genes
MIS: mildew immune seedling; D-12 (mildew resistant seedling)

Molecular markers linked to fire blight resistance could enhance and speed up the breeding process. A progeny segregating for fire blight resistance was established at the Julius Kuehn-Institute and a major QTL for fire blight resistance explaining up to 80 % of the phenotypic variance was detected on linkage group 3 and confirmed (Figure 2, Peil et al. 2007, 2008).

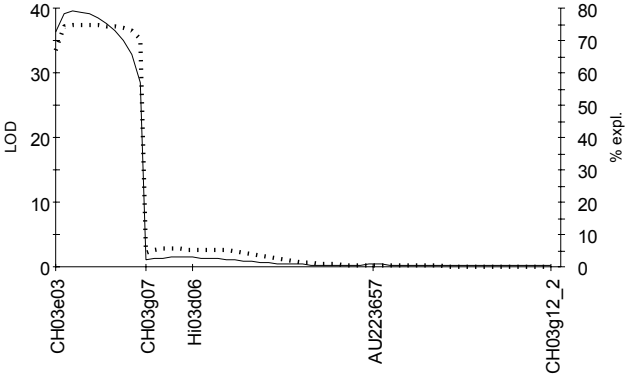

Figure 2: Interval mapping of LG 3 using MQM analysis. Population 'Idared' x R5, average of trait necrosis determined 2005 and 2006
LOD (logarithm of the odds) score: dashed line, % explaining: filled line

The microsatellite marker CH03e03 can be used for marker assisted selection but is only useful in progenies derived from *M.* x *robusta* 5. Other QTLs for fire blight resistance have been mapped (Calenge et al. 2005, Khan et al. 2006) and recently a paper presenting molecular markers for fire blight resistance was presented (Khan et al. 2007).

Apple breeding for fire blight resistance at the Julius Kuehn-Institute includes the identification of new resistance sources and the establishment of pre-breeding material. For that purpose the genetic resources, apple cultivars and wild species, available at the Julius Kuehn-Institute have been screened for fire blight resistance. Until now 135 accessions of 20 wild species and species hybrids were analysed. The results show that a big variability in between accessions of a wild species exists.

Highly resistant cultivars were found in the species *M. baccata, M. fusca* and the hybrids *M.* x *floribunda*, *M.* x *prunifolia* and *M.* x *robusta.* None of 30 accessions of the European wild species *M. sylvestris* was resistant.

Besides wild species, fire blight resistant cultivars like 'Enterprise', 'Reanda' or 'Rewena' have been used to introduce fire blight resistance. The development of molecular markers useful not only in specific populations is necessary to screen for fire blight resistance in a very early stage.

Breeding clones with pyramided resistance genes, for scab as well as for powdery mildew, have been developed at the Julius Kuehn-Institute. More effort is required to improve the inner and outer quality of advanced breeding clones. Competitive resistant cultivars are needed to promote a sustainable and ecological production of apple.

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