

Broaden the genetic basis in apple breeding by using genetic resources

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Abstract

Broadening the genetic basis in apple breeding by using apple genetic resources as parents in crosses is a promising approach to achieve more diversity in fruit quality as well as disease and pest resistance. Projects in Switzerland with Agroscope and Poma Culta, with scientific support of FiBL, as breeding partners and 'FRUCTUS' involved in the evaluation of fruit genetic resources allow for a more exhaustive incorporation of traditional varieties in apple breeding.

Keywords: Apple breeding, *Venturia inaequalis*, *Erwinina amylovora*, *Marssonina coronaria*, apple genetic resources

Introduction

In recent years there was an increasing concern among breeders and especially organic growers in respect of the narrow genetic basis of cultivated apple varieties. It is obvious that the genetic basis of current cultivars as well as in disease resistant cultivars is narrow (Banner, 2010).

Agroscope, FiBL and Poma Culta initiated in 2016 a new project (acronym 'NAGBA') with the aim to revive apple genetic resources preserved in Switzerland through breeding for organic production. The basis of this project were former and parallel activities related to the inventory, collection and description of apple genetic resources. The large genetic variation present in apple genetic resources was by far unexploited. In Switzerland, thanks to the activity of pioneer pomologists such as Karl Stoll in the German and Roger Corbaz in the French speaking part, the awareness related to the value of fruit genetic resources arose some decades ago. They started in the 1970ies to collect traditional varieties and founded in 1985 the association 'FRUCTUS'. Additionally, the foundation 'ProSpecieRara', and different local organizations actively collected heirloom varieties and established ex situ collections. A tremendous support of those NGO activities came since the end of the 1990es with the National Plan of Action (NAP-PGREL) that allowed the governmental support of projects related to the conservation, description and use of PGRFA (plant genetic resources for food and agriculture) based on the Global Plan of Action and the Convention on Biodiversity.

An inventory of the still existing apple genetic resources was established between the years 2000 and 2005 and the decentralized collections were completed. Since 2002, fruit genetic resources preserved in those collections were phenotypic and genotypic characterized whilst funded by the Swiss Federal Office for Agriculture (FOAG). The projects mainly run through the association 'FRUCTUS' at Agroscope in Wädenswil. The obtained data were introduced in the National Database (www.bdn.ch) where they are available for the public. The Swiss apple genetic resources conserved in the National Network of Swiss Fruit Genetic Resources Collections were genotyped with microsatellite markers. The obtained data set was used to detect genetic similarities between individual accessions, thereby identifying duplicates within the collections. Additionally, genetic structure, relationships between

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genetic diversity and original geographical sampling locations and correlations between phenotypic traits and genotype were analyzed (Bühlmann *et al.*, 2015).

Besides molecular and pomological characterization, additional features such as resistance to apple scab (*Venturia inaequalis*), powdery mildew (*Podosphaera leucotricha*), fire blight (*Erwinia amylovora*) and *Marssonina coronaria* were evaluated in the pool of Swiss apple genetic resources (Gassmann *et al.*, 2017). This allowed a first selection of top 100 accessions out of a total of 630 which were tested mainly for scab and powdery mildew incidence in an orchard not sprayed with fungicides but also for other features as described by Kellerhals *et al.* (2016).

Since longer time, skilled pomologists, based on their experience, declared some traditional apple varieties as promising potential parents for breeding. Occasionally, such genitors were included in the Agroscope apple breeding programme. Based on the projects to describe the fruit genetic resources, a broader range of potentially interesting candidates is being established. The NAGBA project, coordinated by FiBL includes Agroscope and Poma Culta as breeding partners. Poma Culta carries out a biodynamic apple breeding programme. Both, Agroscope and Poma Culta perform crosses during flowering time in their orchards. Moreover, new orchards with the top 30 accessions of heirloom varieties were established at both breeding sites under organic growing conditions in order to evaluate their performance.

A major concern among organic apple growers is the durability of disease resistances. In recent years, varieties carrying solely one major resistance such as *Rvi6* (*Vf*) are vulnerable to resistance breakdown. In the NAGBA project the use of parents carrying polygenic resistance is favored.

Material and Methods

Scab resistance screening

An initial screening for scab resistance on the young progeny seedlings at the 4 leaf stage in the Agroscope breeding programme is performed in the glasshouse using an artificial inoculation of scab conidia according to Kellerhals *et al.* (2014). A lower scab conidia concentration of 43'000 conidia/ml was applied when dealing with polygenic resistance, in the case of monogenic resistance usually the scale of Chevalier *et al.*, 1991 and a concentration of about 400'000 conidia/ml is applied. However, in the NAGBA project all progenies were inoculated with the lower dosage and scored according to the quantitative scale developed by Lefrancq *et al.* (2004) with the following seven severity classes: 0 = no symptom; 1 = 1 to < 5%; 2 = 5 to < 10%; 3 = 10 to < 25%; 4 = 25 to < 50%; 5 = 50 to < 75% and 6 = 75 to 100%. The parent 'ACW 20280' is the only parent carrying *Rvi6* resistance. The screening for scab resistance at Poma Culta was performed at the 4 leaf stage in a plastic tunnel using a standard FiBL inoculum with a concentration of 55'000 conidia/ml. For symptom scoring the scale of Lefrancq (2004) was also applied.

Fire blight resistance screening

The top 30 accessions out of the above mentioned top 100 according to hitherto evaluation were analysed for fire blight susceptibility in the glasshouse test with artificial shoot inoculation according to Baumgartner *et al.* (2015). Plant material preparation and inoculation, in a cabin of the quarantine glasshouse at Agroscope in Wädenswil, was conducted as described by Khan *et al.* (2006). Five to 12 replicate trees of each selection, showing active shoot growth and a minimal shoot length of 10 cm, were punctured with a syringe at the shoot tip with an *E. amylovora* solution (Swiss strain ACW 610 *Rif.*; 10⁹ cfu/ml). Cultivars 'Gala Galaxy' and 'Enterprise' were included as susceptible and resistant controls,

respectively. Shoot length and length of necrotic lesion (cm) was measured 7, 14, and 21 DAI (days after inoculation). The susceptibility of the selections and control cultivars was expressed in percentage lesion length (PLL) of the total shoot length and afterwards displayed as percentage relative to the susceptible cultivar 'Gala Galaxy' (Figure 2).

Marssonina coronaria resistance screening

Seven replicates of one year old potted trees of 39 accessions (top 30 and reference varieties) were grafted on M9-T337 rootstock. They were tested in two inoculation series according to Vorley *et al.* (2014). For inoculation the cultivars were placed together in the climate chamber at FiBL on wagons each with 16 plants. Once inoculated, the wagons were wrapped with plastic to maintain a high level of humidity and to encourage successful infection. After 72 hours of incubation the plastic wrap was removed. The plants were inoculated twice. For the 1st inoculation 150 ml of inoculum per wagon was sprayed on both leaf surfaces, for the 2nd inoculation 200 ml of inoculum was used. Afterwards the plants were transferred to a plastic tunnel where symptom development was observed over a period of 2 months. The following symptoms were scored:

- first symptom development
- leaf spots
- leaf yellowing and drop

Molecular analyses

For the analysis of parental varieties and progeny plants with molecular markers (SSRs) leaf samples were taken, properly labeled, deep frozen in a paper envelope and analyzed in the lab of the company 'Ecogenics'. Marker analysis was accomplished following the protocol of Frey *et al.* (2004) using multiplex PCRs with fluorescently labeled primers. Data analysis was done with GeneMapper™ Software (Applied Biosystems).

Results

Scab resistance screening

Figure 1 shows the results of the glasshouse scab screening for the different severity classes and for additional phenotypic resistance symptoms such as pin point pits (PPP), stellate chlorosis (SC) and stellate necrosis (SN). The observation of such symptoms could be an indicator for hitherto unknown resistance factors present in the parents and transmitted to the progeny. Plants showing more than 25 % scab severity (class 4 to 6) are discarded. In the case of the progenies 'ACW 16981' ('Milwa' x 'La Flamboyante') x 'Schorenapfel' and 'ACW 14617' ('Champagner Reinetten' x 'A 810-390') x 'süßer Zila' a high proportion of potentially field resistant plants was detected (classes 1 to 3).

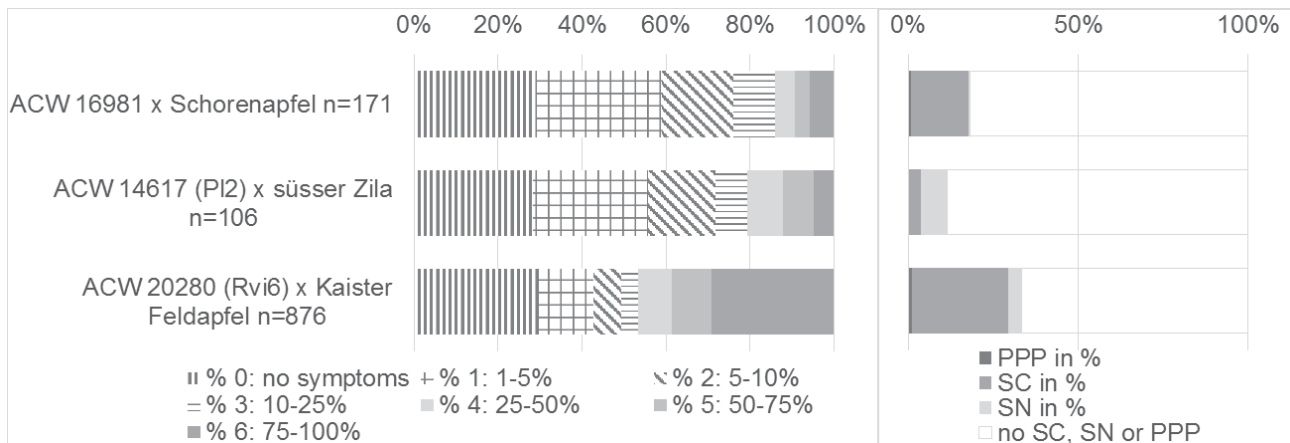


Figure 1: Segregation of the progeny plants in the scab glasshouse screening according to the scale of Lefrancq *et al.* (2004) with addition of the following defense reaction symptoms observed: pinpoint pits (PPP), stellate chlorosis (SC) and stellate necrosis (SN), n = number of seedlings.

Fire blight resistance screening

The top 30 accessions were analysed over three years for fire blight susceptibility in the glasshouse test with artificial shoot inoculation. However, not all accessions were tested over three years, some only one or two years, others three times. Figure 2 shows, that with repeated evaluation in different years a relatively large variation of the score is possible. However, the control cultivars ‘Enterprise’ and ‘Gala Galaxy’ remained in their expected range and it was possible to define accessions with low, medium and high disease severity. The varieties ‘Midonette’ and ‘Schorenapfel’, showing a comparable resistance level to the control cv. ‘Enterprise’, are therefore promising potential breeding parents.

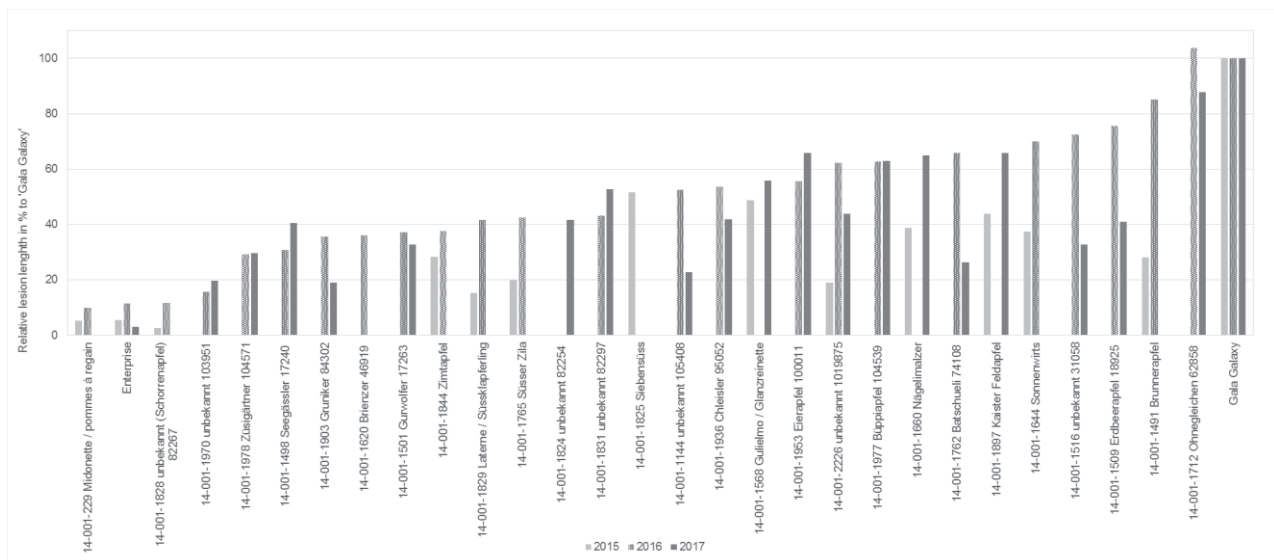


Figure 2: Mean lesion length 21 DAI in % to ‘Gala Galaxy’ of 30 top apple genetic resources accessions tested with the glasshouse shoot inoculation test. Cvrs. ‘Enterprise’ and ‘Gala Galaxy’ as tolerant and susceptible controls, respectively.

Marssonina coronaria resistance screening

Considerable differences in the susceptibility were observed in this preliminary screening. Several accessions showed good resistance to *Marssonina coronaria*. Among them were ‘Brunnerapfel’, ‘Sonnenwirts’, ‘Süßer Zila’, ‘Zimtapfel’ and ‘Züsigärtner’ (data not shown).

For 2018 the most susceptible and most tolerant genotypes will be planted in an open air plot. The plants will be artificially inoculated and disease development observed.

Molecular analyses

Results of molecular analyses of parents for a restricted number of disease resistance and fruit quality related markers are shown in Table 1. Only few major disease resistance genes are present as the focus was put on polygenic resistance. For considered fruit quality related QTL's, the presence of *Md-ACS1* and *Md-PG1* is rather limited. Based on the markers present in the parents, phenotypically preselected progeny plants were analysed for relevant markers (results not shown) to render the selection of promising plants more efficient.

Table 1: Results of marker analyses of the parents used in the Agroscope part of the NAGBA breeding project.

Accession (Parents)	Markers present for
ACW 14617 (Champagner Reinette x A 810-390)	<i>PI2</i>
ACW 16981 (La Flamboyante x Milwa)	<i>Md-ACS1</i>
ACW 20280 (Ariane x Topaz)	<i>Rvi6</i> , <i>Md-ACS1</i>
Kaister Feldapfel	-
Schorenapfel	<i>Md-PG1</i>
Süsser Zila	<i>Md-PG1</i>

PI2: powdery mildew resistance (*Podosphaera leucotricha*)

Rvi6: scab resistance (*Vf*, *Venturia inaequalis*)

Md-ACS1: fruit quality: storage, ripening (ethylene biosynthesis)

Md-PG1: fruit quality: firmness, crunchiness (cell wall degradation)

Discussion

The evaluation of traditional heirloom apple varieties in Switzerland allows for their integration as breeding partners in order to broaden the genetic basis. Data on fruit and tree characteristics are an important basis for choosing suitable parents. Scab screening of the progeny seedlings in the glasshouse and confirmation in the outdoor seedling nursery where no fungicides are applied allows the selection of desired resistant plants.

Molecular markers for selection in apple breeding were mainly developed for major disease resistance genes. To detect polygenic QTL effects, performant tools still need to be developed and implemented. Also fruit quality traits are often quantitatively inherited. Marker-assisted breeding should significantly increase breeding efficiency as undesirable genotypes can be discarded at the early seedling stage. Efficient early selection is particularly advantageous in perennial crops like apple because fruit quality traits can be evaluated only after the long juvenile period. In the NAGBA project and Agroscope breeding programme, markers related to the ethylene biosynthesis and therefore storability and firmness of the fruit (*Md-ACS1*, *Md-ACO1*) as well as related to texture and mealiness (*Md-PG1*) are considered. Fruit ethylene production genotypes for *Md-ACS1* and *Md-ACO1* were determined for 60 apple cultivars and 35 advanced breeding selections by Zhu and Barritt (2008). Earlier studies showed that genotypes homozygous for the *ACS1-2/2* allele produce less ethylene and have firmer fruit at harvest and after storage than *ACS1-1/2* and *ACS1-1/1* genotypes. Variations in apple fruit texture depend on degradation occurring in fruit primary cell wall and middle lamella. This process is regulated by an enzymatic network generally encoded by large gene families, among which polygalacturonase is devoted to the depolymerization of pectin. In apple, *Md-PG1*, a key gene belonging to the

polygalacturonase gene family, was mapped on chromosome 10 and co-localized within the statistical interval of a major QTL associated to several fruit texture sub-phenotypes (Longhi *et al.*, 2013). Preliminary comparison of fruit quality traits using phenotyping and genotyping data leads to the conclusion that there is not a strong correlation between presence or absence of markers and their related phenotypic trait expression (D. Suter, Agroscope, oral communication). It might be therefore still too early to broadly apply those markers for fruit quality traits in an efficient and economical way in marker-assisted breeding.

Another obstacle for the success of a wider range of new varieties with a broad genetic background including heirloom accessions are the market rules. The fruit trade should be prepared to commercialize also varieties from small scale production. However, public private partnership as it is performed in the NAGBA project for organic fruit growing seems to be a promising approach to achieve progress by respecting the required guidelines and exploiting modern breeding approaches.

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