Challenges in apple breeding

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Abstract

Current challenges in apple breeding relate to broaden the genetic basis and the development of varieties resilient to biotic and abiotic stresses that are competitive on the marketplace. Therefore, breeding programs rely on efficient methods to speed up breeding and cope with the increasing number of features to select for. In this context, molecular tools hold promise as they allow for early multi-trait analyses. In organic apple production, there are some specific requirements for apple cultivars connected with plant protection products, growing conditions and management such as fruit thinning, influencing breeding objectives and selection.

Keywords: apple breeding, fruit quality, storage, resilience

Introduction

<u>*Fruit quality and storage*</u>

Fruit quality is especially challenging to select for in apple breeding (Kellerhals et al., 2019). Crunchy texture, juiciness, balanced sugar/acid ratio and optical features such as attractive red fruit colour, regular shape and size are currently highly required features. These mainstream fruit quality attributes are also requested by consumers purchasing organic apples. However, there is also a niche market for apple specialities and special use apples. During our collaboration with FiBL at Frick for the evaluation of advanced selections and cultivars, in several genotypes an increased frequency of fruit russeting was observed under organic compared to integrated management. Another challenge for organic and integrated production with low input are storage diseases and physiological disorders. Until now, requirements for storage diseases and disorders have often been considered less important when a new selection or variety has an excellent eating quality.

Selection for fruit quality features is time-consuming because it can only be performed when the first fruits are present. Therefore, marker-assisted breeding for fruit quality holds promise. Currently, marker-assisted selection (MAS) is easier to implement for mono- or oligogenic traits such as a specific scab resistance, while this method is less adapted for complex polygenic traits such as fruit quality. However, there is scope that in the near future traits such as fruit crispness and firmness can be analysed with molecular tools more accurately (di Guardo *et al.,* 2017, Chagné *et al.*, 2019). Chagné *et al.* (2019) showed that all 28 SNP (single nucleotide polymorphism), used for fruit crispness and firmness, are scorable and polymorphic. The assays of Chagné *et al*. included 15 SNPs across five loci (MdPG1, MdACO1, MdACS1 and QTLs derived from 'Braeburn' (LG15-BB) and (LG16-BB)) and 13 assays around the LAR1 and Ma1 loci. Some of those loci have also been considered in the Agroscope apple breeding program which is currently moving from SSR marker analyses for disease resistances and specific fruit quality traits towards SNP based approaches.

In this paper, the focus is mainly on classical approaches to evaluate fruit quality features in a breeding program and on current limits and challenges related to these features.

<u>*Selection process*</u>

Agroscope apple breeding is carried out over several selection steps. After the initial screening of the seedling progenies in the glasshouse at the four leaf stage for scab resistance, the next selection step is based on growth habit and mildew tolerance of the one

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year old seedlings in a container plot. About 10% of the original seedlings number reaches the next step, called level 1 (one tree on rootstock M27 with interstem 'Schneiderapfel'). Promising selections are chosen for the following level A, with four trees on rootstock M9. From there, the best ones are selected for level B with a 3 x 5 trees replicate trial on rootstock M9. Level C consists in row plantation on rootstock M9 or alternative rootstocks such as B9 or G11. Level B is currently running according to organic fruit growing standards, whereas level 1, A and C are under a reduced integrated production (IP) management.

Resilience towards biotic and abiotic stresses

To achieve resilience towards biotic and abiotic stresses, new directions are being considered in the breeding program. The range of pests and diseases is increasing dramatically due to climate change and international exchanges of people and goods. Diseases such as *Marssonina coronaria* (Schärer *et al*., 2018), *Elsinoe pyri* (Scheper *et al*., 2013) and pests such as *Halyomorpha halys* are examples. *Halyomorpha halys*, native to Eastern Asia, has become as a serious orchard pest in the United States, Asia and Europe. With more than 120 different host plants, it is highly polyphagous and spreading rapidly worldwide, notably through human-mediated activities (Haye *et al.,* 2015). Shanovich *et al*. (2019) described a method to evaluate cultivar susceptibility, which might be of interest for apple breeding programs. The challenge in breeding programs will be to consider an optimal number of aspects while using the fastest and most accurate methods as possible for selection, such as molecular selection. Abiotic stresses are concerning drought tolerance, sunburn, frost tolerance and other aspects of adaptation to climatic change. Up to now, they were less frequently considered in breeding.

Broad genetic basis

Although the apple tree is an outcrosser retaining a high genetic polymorphism, the genetic basis of modern cultivars is narrow, which could be a concern while breeding for new traits. An enlargement of the genetic basis is required and traditional heirloom varieties are a potentially useful pool (Kellerhals *et al.,* 2018). With the drastic changes caused by climate change and the continuous demand for low input cultivation of apple, it might be necessary to incorporate also plant material from outside the cultivated gene pool (Peace *et al*., 2019). Diversity management is crucial to screen for allelic diversity that will be useful today or in the future to improve the specific traits targeted by breeders. Thus, it is necessary to establish, and maintain reference populations (Jung, oral communication) or so-called core collections (Lassois *et al*., 2016) and to genotype and phenotype those to enlarge publically accessible databases. Data from such databases can be used by scientists and breeders to find interesting genotypes and to create prediction tools. Ideally, the phenotyping of this diverse material should be done in several well-characterized environments in order to quantify genotype by environment effects. The current apple REFPOP put in place based on the EU project FruitBreedomics serves this goal. This population is representative of old and modern apple gene pools, planted in six European countries, including Agroscope at Wädenswil, Switzerland. It is phenotyped and genotyped across these countries with the same methods (Jung *et al.* in prep).

Material and Methods

A level C trial with different advanced apple selections and cultivars carrying scab resistance (*Rvi6*), and fire blight tolerance on the rootstock B9 with interstem 'Golden Delicious' was established at Güttingen near the Lake of Constance in spring 2011, with 52 trees per cultivar in row plantation. Plant protection was applied according to IP guidelines. Fruit size and colour were analysed by a GREEFA fruit sorting machine. A level B trial under organic management was planted in spring 2015 at Wädenswil and analysed according to the protocols of levels B and C. Next to this plot a plantation of 30 preselected heirloom varieties, including the standard cvrs. 'Empire' and 'Boskoop', was planted in spring 2017. Fruits from the third leaf trees (crop 2019) were stored at 1°C in a normal cold store for 2 month and then evaluated for different fruit features on a 1 to 9 scale (texture, flavor, juiciness, etc.) and an overall score was given. Storage trials are regularly performed with advanced selections of level B and C under controlled atmosphere (CA) storage conditions (1°C and 3°C, 1.5% CO_2, 1.5 % O_2, 92% relative humidity). Measurements are being made after about 120 days and 200 days under CA storage visually for physiological disorders and storage diseases, and with the automated fruit analyser "Pimprenelle" (Setop Giraud-Technology, Cavaillon, France). Measurements of internal quality (sugar, acidity, firmness) are performed immediately after removal from cold store and again after one week at room temperature (20°C) in order to evaluate shelf life capacity.

One year old seedlings (n = 289) of the cross combination 'ACW 17314' ('Goldrush' x 'Crimson Crisp') crossed with the Swiss heirloom variety 'Seemer' (original population of 760 offspring seedlings) were visually selected in the container field for their growth habit and tolerance against powdery mildew, after screening with an artificial apple scab inoculation in the glasshouse. Further, the selected seedlings (n = 289) were analyzed for a chosen SNP marker set (Table 1) by the company LGC Genomics Ltd. (UK).

Results

Fruit size and fruit colour are important quality features on the market. High amounts of over colour and appropriate fruit size contribute to a high pack-out, which is required for commercial success. Figure 1 displays fruit size calibration and Figure 2 fruit over colour percentage for fruits of the cvrs. 'Lucy', 'Ladina' and 'Ariane' in the Güttingen trial. The critical factor in all tree cultivars, but especially for 'Ariane' is a tendency to small fruits. The amount of over colour is high in all three cultivars, allowing for a high pack-out of purchase-ready fruit.

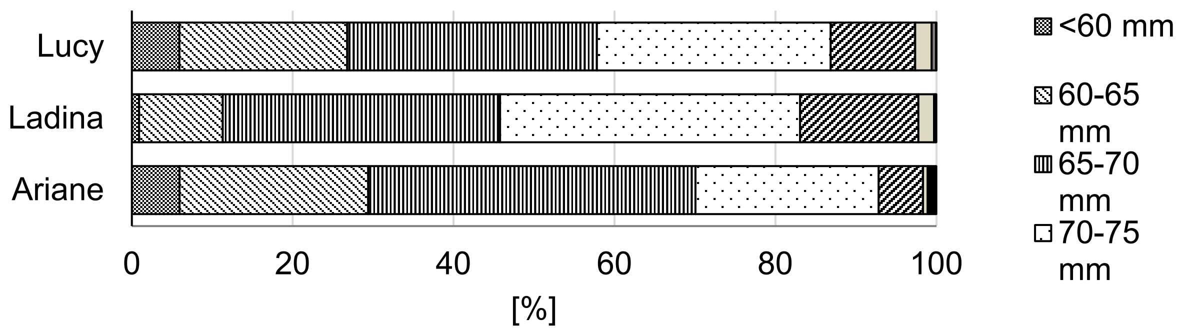

Figure 1: Fruit size calibration (average of crops 2016, 2017, 2019), 52 trees, Güttingen

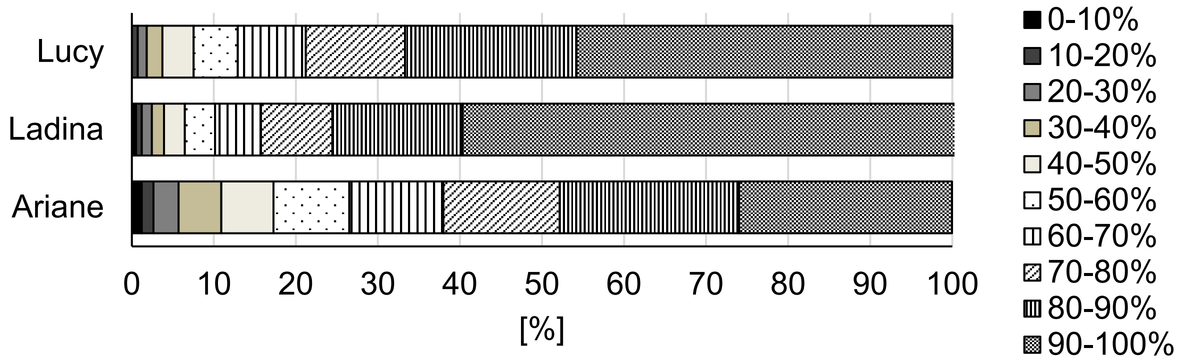

Figure 2: Fruit colour calibration (average of crops 2016, 2017, 2019), 52 trees, Güttingen

Regular yielding is especially important in organic production as the tools for fruit thinning are restricted. Regular bearing results in higher fruit quality, less physiological disorders, better pack-out and a better overall economic result. Figure 3 highlights yielding features of three different apple cultivars showing clear differences in yield regularity.

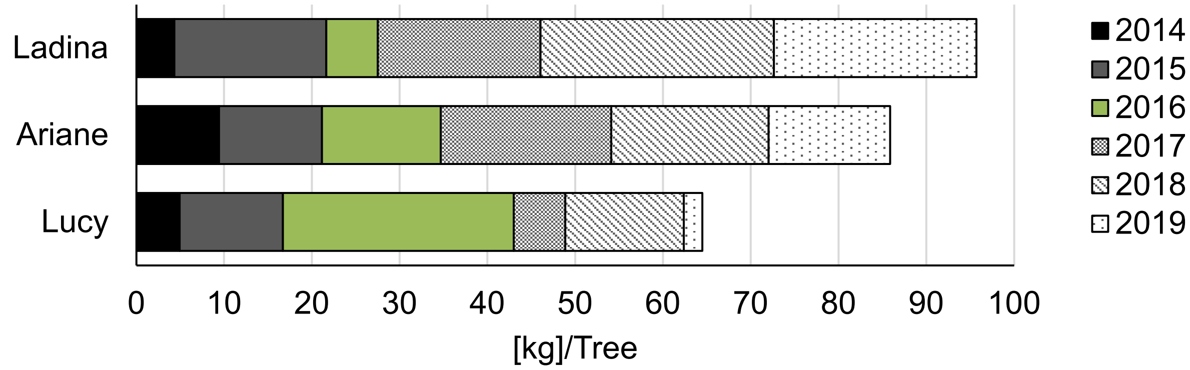

Figure 3: Cumulated yield from 2014 to 2019 for the cvrs. ‘Ladina’, ‘Ariane’ and ‘Lucy’ (Güttingen, IP management)

Figure 4 shows measurements of the internal fruit quality parameters acidity, firmness and soluble solids at harvest for four advanced selections and ‘Ariane’ as standard for two seasons. All the new selections and the standard have a well-balanced sugar-acid ratio and the fruit firmness required by the consumer. However, these features need to be evaluated also upon storage.

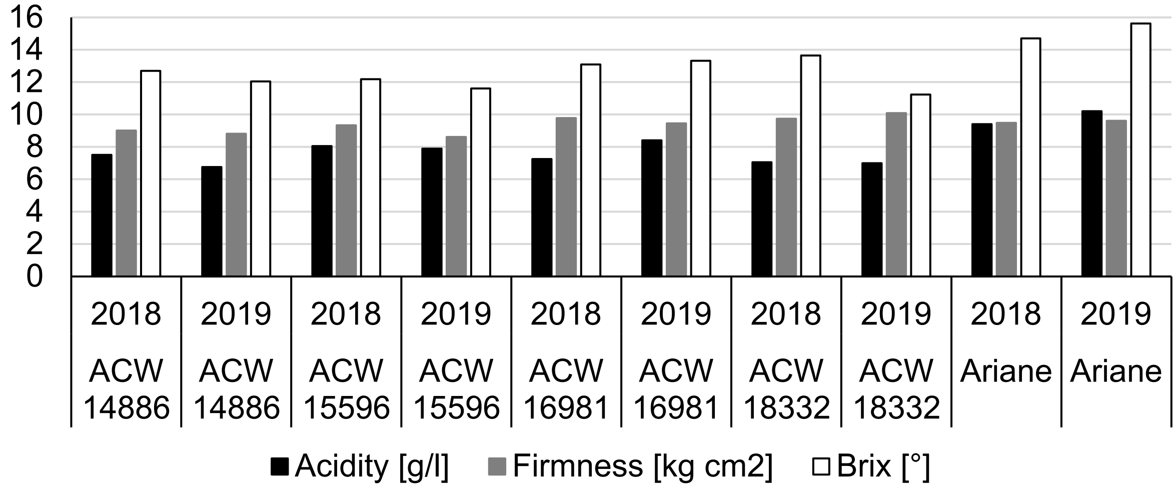

Figure 4: Fruit quality features measured by the analyser “Pimprenelle” for four advanced selections and the standard cv. ‘Ariane’ at harvest in the level B orchard at Wädenswil with organic management.

The evolution of fruit quality characteristics during storage is crucial for the market success. Figure 5 displays the evolution of acidity (g Malic acid/l), soluble solids (°Brix) and firmness (kg/cm^2) for the advanced selection ‘ACW 18332’ over almost 200 days of CA storage.

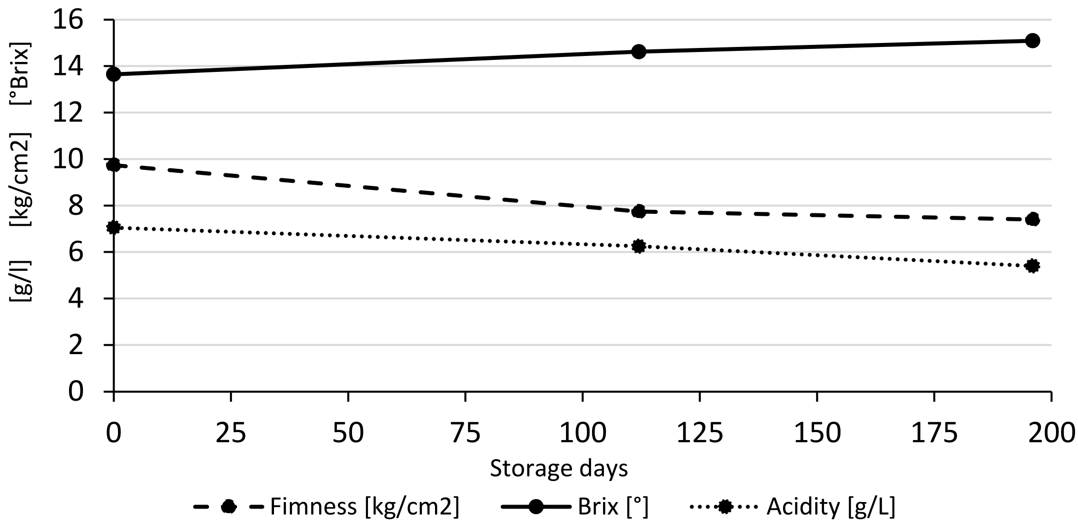

Figure 5: Evaluation of firmness, Brix, and acidity for the selection 'ACW 18332' in the 2018/19 CA storage trial at 1°C, 1.5% CO_2, 1.5 % O_2, 92% relative humidity.

The evaluation of traditional heirloom varieties for eating quality after two month of storage revealed that none of them achieved a very high score and that the average perception tends to be lower than with current commercial cultivars (Figure 6). However, some accessions reached a score comparable to the standards 'Boskoop' and 'Empire' and they should be observed more closely in the coming years.

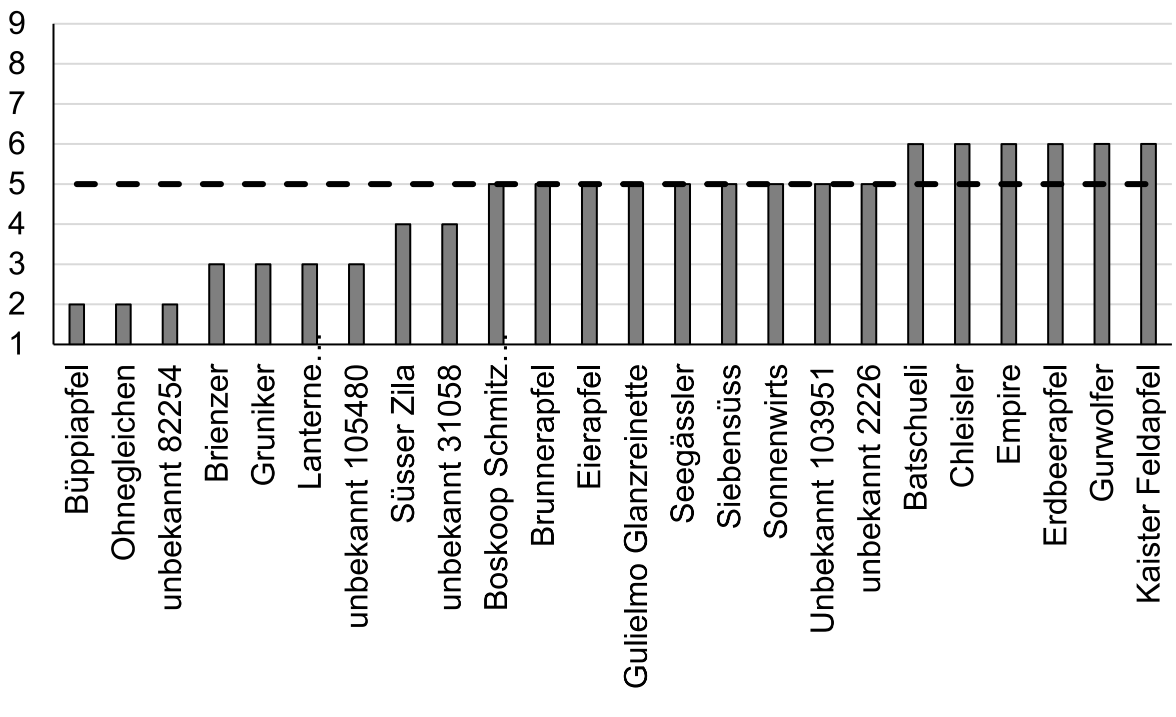

Figure 6: Evaluation of potentially interesting heirloom varieties for overall fruit quality with fruit from 2019 after 2 months of cold store at 1°C. Organic plot, 3rd leaf, 2 x 5 trees per accession (1 = very poor, 5 = average, 9 = excellent)

Table 1: Molecular analysis with SNP markers for the ‘ACW 17314’ x ‘Seemer’ progeny (n = 289)

Traits	SNP	References
Rvi6 (Apple scab resistance *Vf*)	MS8_Y124	Jänsch *et al*. 2015
DP-fl (Rosy apple aphid resistance)	Dpfl_SNP_585	Bianco *et al*. 2014
Md-ACS1 (Ethylene production)	Md-ACS1SNPa	Baumgartner *et al*. 2016
Md-PG1 (Polygalacturonase production)	Md-PG1SNP1	Baumgartner *et al*. 2016
MYB10 (Red skin color)	MYB10_ss475879531	Chagné *et al*., 2016

Parents:
ACW 17314 (‘Goldrush’ x ‘Honeycrisp’) (*Rvi6, Dp-fl*, Md-PG1) x ‘Seemer’ = ‘Brunnerapfel’

Traits	***Rvi6***	***Dp-fl***	**Md-ACS1**	**Md-PG1**	**MYB10** (low red)	**MYB10** (medium red)
% offspring plants carrying the SNP	77.2	58.5	30.8	48.8	95.1	3.8

SNP genotyping with plants from the ‘ACW 17314’ x ‘Seemer’ progeny (n=289) confirmed the presence of the scab resistance *Rvi6* markers in 77.2% of the progeny plants previously selected for scab symptoms in the glasshouse (Table 1). Furthermore, 58.5 % of the plants carry markers for a rosy apple aphid resistance. More than one third of the offspring shows promising fruit storage potential and firmness. Finally, almost 4% of the seedlings are expected to produce medium red coloured apples according to MYB10 SNP results. Thus, here we show that MAS allows selecting efficiently for a combination of traits, which are relevant for both fruit quality and tree resilience.

Discussion

Challenges for fruit breeding consist in changing requirements of the market and the consumer, climate change inducing new biotic and abiotic stresses and a relatively narrow genetic basis in current commercial cultivars. To keep the selection process in manageable dimensions, easy to handle and comparatively cheap selection methods are required. For example for analyzing fire blight tolerance of breeding material, current methods are quite laborious, time consuming and therefore expensive (Kellerhals *et al.,* 2018). It would be useful to select as early and complete as possible with molecular tools for fruit quality features as well as traits related to resilience to biotic and abiotic stresses.

Genomic Selection (GS) is supposed to considerably improve breeding efficiency in a range of crops. While for example some major alleles for fruit texture can be already targeted with the above-mentioned marker-assisted selection method, GS would allow further exploiting the phenotypic variability underlined by minor effect alleles. In this frame, the complexity of fruit texture needs to be addressed and its diversity must be well represented in the genetic material. This was the focus of a recent work in collaboration between Agroscope and the Fundazione Edmund Mach (Italy) where the GS approach was used in a large apple population for predicting fruit acoustic and mechanical features (Roth *et al.,* 2019). The high accuracies values found in that study highlighted the large potential of this method for fruit texture.

Although our trials have shown, that the potential of heirloom varieties to improve fruit eating quality might be limited, they need to be further tested for stress-related traits, such as quantitative disease resistance, which remain to be identified.

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