# Apple Breeding: Marker-Assisted Selection and Beyond

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**Abstract**

Apple breeding is very active all around the world with more than twenty scientific institutions involved and private companies and some 300 thousand seedlings screened each year. The story of apple breeding has followed the steps of any crop with the exception that the frequent occurrence of sport mutations and the discovery of chance seedlings has produced many varieties of the market that flanked those obtained through controlled crosses. The conventional breeding based on the cross of good parents and followed by phenotypic selection took the stage for long time. Studies on the heritability of traits can be found in the literature, but other genetic studies that would help the selection of cross parents, like the analysis of combining ability (CA), are rather rare. In the 1980s the molecular markers became popular among apple geneticists and helped to produce linkage maps and to assist breeders in the so called marker-assisted selection. In the very last years, thanks to the apple genome sequence that provided hundreds of thousand SNP (single nucleotide polymorphism) markers easily accommodated on DNA-chips, a new approach to breeding based on the genome-wide estimation of breeding value (GWEBV) of parents and offsprings, without any preliminary knowledge on marker-traits association appeared on the scene. This paper discusses the evolution of apple breeding by commenting the steps briefly outlined above.

**Keywords:** *Malus x domestica*, hybridization, genom, MAS, resistance

## Elma Islahı: Markör Destekli Seleksiyon ve Ötesi

**Özet**

Dünya genelinde kamu ve özel sektör tarafından yürütülen 20'den fazla aktif elma ıslah programı bulunmaktadır. Bu programlarda, her yıl yaklaşık 300000 çöğür değerlendirilmektedir. Elma ıslahı, diğer türlerde olduğu gibi yapılagelmekte olup, bazı istisnalar taşımaktadır. Doğal yollarla meydana gelen mutasyon ve tesadüf çöğürü orijinli çok sayıda elma çeşidi, pazarlama kanallarında kontrollü melezlemeler yoluyla elde edilen çeşitlerin ticari payını sınırlandırmaktadır. Üstün özellikler taşıyan ebeveynlerin melezlenmesi ve fenotipik seleksiyon aşamalarından oluşan klasik ıslah çalışmaları oldukça uzun zamana ihtiyaç duymaktadır. Literatürde istenilen bir özelliğe ait kalıtım derecesi ile ilgili çalışmalara ulaşabilmek mümkündür. Bununla birlikte, üstün özellikleri melezlere aktarabilecek "uyuşma yeteneği" gibi analizleri içeren genetik çalışmalar oldukça nadirdir. 1980'li yıllarda genetikçiler arasında popüler olan genetik markörler, genetik haritalar oluşturulmasına ve ıslahçıların markör destekli seleksiyon yapmasına olanak sağlamıştır. Son yıllarda elma genomundaki diziler sayesinde çok sayıda SNP markörü, DNA çipleri üzerinde kolaylıkla tanımlanabilmiştir. Böylece markör özellikleri tam olarak bilinmeden, ebeveyn ya da melezleri genom çapında ilişkilendirebilen yeni bir ıslah yaklaşımı söz konusu olmuştur. Bu çalışmada, dünden bugüne elma ıslahı yukarıda çerçevesi çizilen konular kapsamında değerlendirilmiştir.

**Anahtar Kelimeler:** *Malus x domestica*, melezleme, genom, markör destekli seleksiyon, dayanıklılık

## 1. Introduction

Apple breeding is the largest breeding activity of any fruit crop in the world. It involves more than twenty Institutions and private breeders in many Countries of the temperate zone with some 300 thousand seedlings screened each year. This activity is rather recent compared with that one carried out in annual crops, however it led already to the release of a number of new selections with interesting traits. Most of these new releases carry resistances to scab and several other diseases thus promising to mitigate the enormous environmental impact of the current apple industry.

In the following pages the paper charts shortly the story of breeding with some information on the new frontiers of the genomic selection that is revolutionizing the apple breeding strategies.

## 2. The breeding of the past centuries

Most apple varieties of the past including several of those most outstanding in the market came out as chance seedling from open pollination. This was the case for instance of 'Golden Delicious', that was for long time the main variety grown worldwide.

Royal Gala     Galaxy     Brookfield Gala     Obrogala     Gala Schniga

**Figure 1.** Several sport mutants of 'Royal Gala'

Other apple varieties came out from controlled crosses and were created by breeders who selected parents with the desired characters, crossed them, and waited for the results. This is what some geneticists refer to as a '*cross & pray*' approach in analogy with the better-known '*spray & pray*' approach used by some fruit scientists. The expression is rather rude but it is nonetheless not too far from reality; this is what apple breeders have done over the past two centuries, admittedly with some remarkable success. Conventional breeding in many instances was considered more a craft than a real science, since many of the underlying principles explaining interactions between genes have yet to be understood.

**3. The selection of sport mutants**

The frequent occurrence of sport mutations that is mutations that occur naturally in vegetatively propagated apple varieties was a factor that favored the scarce commitment of geneticists to apple breeding in the past. Skin color mutations, mutations in the vegetative habit and other less evident mutations in the apple fruit quality were largely exploited in the past and led to the release of what are called 'essentially derived' varieties (EDV). Long series of these ED varieties can be easily found in the Red delicious family, in Gala and Fuji families and with minor frequency in other varietal groups (Figure 1).

ED varieties are new varieties indeed, if the phenotypic differences hold steady with the time, but they are easily discovered with almost null investment and very often they replace the original genotype obtained in many cases with conspicuous financial and labor investments. This fact has raised many concern

about the intellectual property rights (IPRs) of original and essentially derived varieties and the question has not yet been solved by the plant variety patenting offices worldwide.

A discussion on IPRs is beyond of the object of this paper; nevertheless we wish to stress that the problem has implications on the breeding activity because it is clear that the appearance in the market of an ED variety mortifies the true breeding activities based on cross and selection and discourages the companies from investing.

The large presence of ED varieties among the varieties grown in the world together with the frequent use of recurrent 'good' parents in the controlled crosses of the past led to the restriction of the genetic base of apple, as it has been demonstrated by a brilliant paper on coancestry and funding clones of mod-
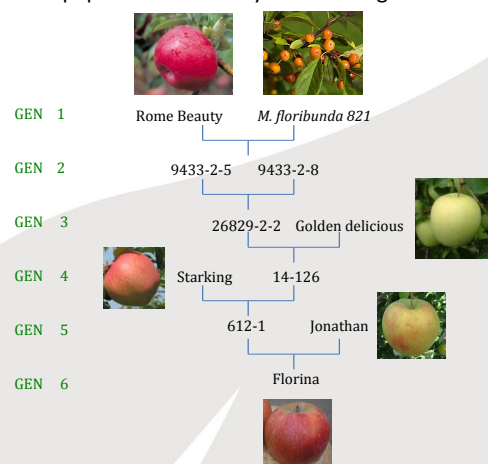


| GEN 1 | Rome Beauty | *M. floribunda 821* |
| GEN 2 | 9433-2-5 | 9433-2-8 |
| GEN 3 | 26829-2-2 | Golden delicious |
| GEN 4 | Starking | 14-126 |
| GEN 5 | 612-1 | Jonathan |
| GEN 6 | Florina | |

**Figure 2.** The classical track for the introgression of characters (in this case the scab resistance) from wild relatives. The initial interspecific cross was followed by an F2 and several pseudo back-crosses on different varieties of the market.

ern apple varieties published by Noiton and Alspach in 1996.

## 4. The current breeding strategies

Plant breeders since the discovery of genetic laws have struggled with polygenic traits and have relied on the quantitative approaches based on Fisher's
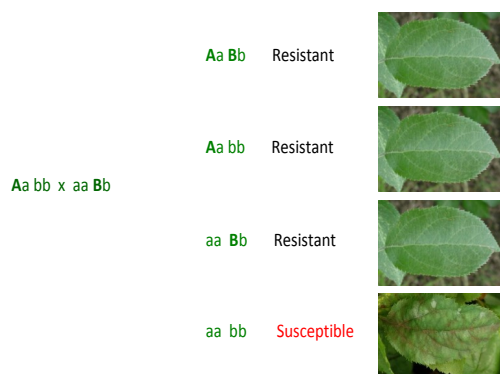


**Figure 3.** A cross between two genotypes carrying different genes of resistant to the same disease (Aabb and aaBb, the capital letters indicate the resistant dominant allele) produce offsprings that carry either or both resistance genes. The marker-based selection becomes bound when one wishes to screen the offsprings and select only those carrying both resistance genes donated by either parent.

theories for selection purposes. Even if quantitative genetics dates back to the 1920s, it is still yielding spectacular results; think of the green revolution and other remarkable achievements obtained in industrial and horticultural crops. That said, apple breeders, like many fruit crop breeders, have never seriously exploited approaches based on quantitative genetics, using as an excuse that woody species have long generation times and are mostly cross-pollinated (Figure 2). There is some truth in the fact that species like apple are not well suited to the creation of inbred lines. Concepts such as 'heritability of traits', 'combining ability', 'progeny test', 'gain from selection' are rarely mentioned in the apple breeding literature.

One of the few exceptions is the 20-years-old apple breeding program managed by Plant & Food (former HortResearch) of New Zealand, were a complex and articulated breeding strategy has been designed based on recurrent selection for general combining ability, that allowed to progeny test some 1,000 parents (Noiton and Shelbourne, 1992; Kumar et al., 2010). But this is a rare exception.

Recently, marker-assisted selection (MAS) has gained popularity as a powerful tool to efficiently breed plants. This approach is being used for the early selection of Mendelian traits under monogenic control, such as color, disease resistance and a few other characters. Breeders were able for the first time ever to base their selection on molecular markers rather than characters. These molecular markers are detectable at any stage of the plant's life and can thus be very useful in hastening the breeding process. This is particularly valuable in apple breeding as traits related to production become visible only after the plant has overcome the juvenility, which can last for as long as three to five years, according to the strategy adopted in growing the cross progeny.

An interesting case in which trait-associated markers can help the selection is when the breeder wishes to combine together two or more genes that show the same phenotype as it is often the case of different genes of resistance to the same disease. In such a case only the use of markers can guarantee the selection of progeny carrying both or all resistance genes of the parents (Figure 3).

## 5. The characters focused by breeders

Most if not all current apple breeding program include the resistance to diseases. Seventeen different genes of resistance to scab (*Venturia inaequalis*) are known and mapped (Bus et al., 2011) (Figure 4). Many of them have already been introgressed into elite cultivars, and in some cases parental genotypes with combined different resistance genes are available for breeders. Scab is not the only disease for which resistance genes have been found in apple. Powdery mildew (*Podosphaera leucotricha*), fire blight (*Erwinia amylovora*) are other examples of genes mapped. Most resistance genes have also tightly associated markers suitable for marker-based selection (Bus et al., 2011; Chagné et al., 2012; Jänsch et al., 2015).

Common goals of many modern apple breeding projects deal with the fruit quality. Crispness, firmness,

juiciness, equilibrate sugar/acid balance are all traits that are observed in the progeny and for which selection is commonly done. See for instance Kumar et al. 2013 for a list of traits of interest related to fruit quality.

Finally long storage and long shelf life are further attributes of apple fruit that are sought by breeders.

## 6. The breeding of the future

Markers for selection for quantitative traits are not so easy to manage. One can imagine that breeders could readily use a toolbox of dozens of markers, designed to select plants for improved sugar content, acidity, fruit size and other quality traits. Yet, venturing in this direction may not be the best way to improve fruit

cess. But what exactly is GWEBV?

Molecular techniques have made spectacular strides over the last few years and breeders can now obtain large arrays of molecular markers at a very low cost. Currently, SNPs are the markers of choice as they allow the whole genome of a species to be precisely analyzed. One must however make an intelligent use of the newly obtained information.

A genome-wide dense SNP array allows the simultaneous mapping of genes and QTLs controlling all traits targeted by a breeder (Goddard and Hayes, 2007; Heffner et al., 2009). Using this technique, the effect of SNPs on linkage disequilibrium, with all functional variants affecting the trait, can simultaneously be estimated making the approach suitable for quantita-
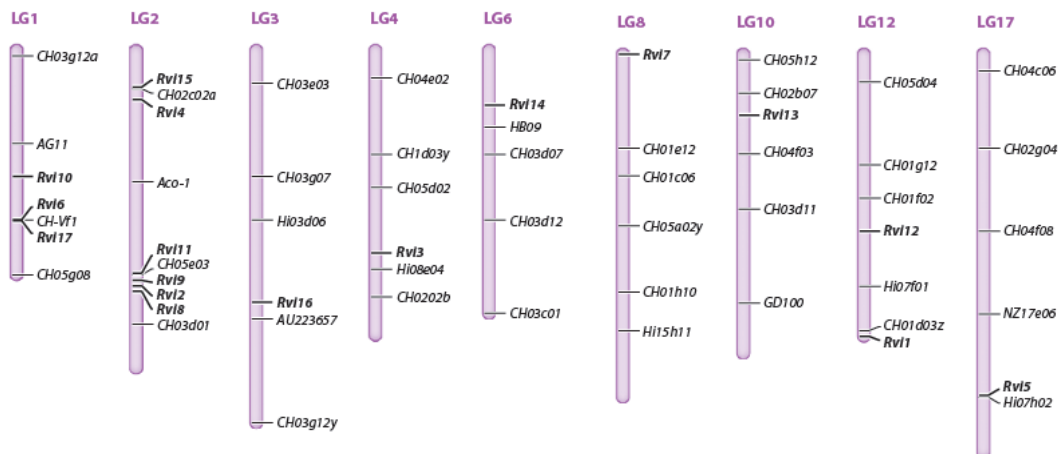


**Figure 4.** The genetic map of apple scab resistance genes (in bold) and the associated markers (after Bus et al., 2011)

trees: indeed this approach may be entirely inappropriate and could lead to inconsistent results. Since selectable markers are surrounded by many other interacting genes, which are often not considered, it might be naive, in spite of the apparent robustness of the technique, to blindly follow this route. One should candidly and in a forthright manner look at other solutions to efficiently breed fruits. Such a solution has already been explored by our colleagues in animal genetics. Indeed, GENOME-WIDE ESTIMATE OF BREEDING VALUE (GWEBV) of individuals could be a promising approach to ameliorate the breeding pro-

tive traits under polygenic control. What is amazing in this approach is that no knowledge of the association between markers, traits or genes is required!

Actually GWEBV may be a revolutionary concept in breeding. In fact, it might replace the need for the mapping of the genetic determinants of traits, and their associated markers, by the study of a so-called 'training population', a reduced set of individuals from the population within which the selection will be carried out. The idea is to obtain a signature of the best marker profile associated to the searched phenotype that is an ideal archetypal individual that pos-

sesses the traits of interest. Once this signature is obtained, the breeder can genotype any individual related to the original population, including the off-spring generations. In short, one can analyze the marker profile of an individual and derive its breeding value for the characters at stake (Figure 5).

This new breeding process therefore involves two phases. The first is the elaboration of a predictive model whereby individuals belonging to the training population are both genotyped using many molecular markers and phenotyped in replicated plots. The ge-nome scan of marker effect is then carried out using statistical approaches such as least-squares, BLUP (best linear unbiased prediction) or by Bayesian analy-sis. The basic mathematics behind all this are complex and belong to the realm of bioinformatics, but the concepts are now-a-days well mastered and software packages are available to perform the analysis. Even if a breeder does not understand completely the algo-rithms on which the process relies, he can be assisted by a bioinformatician to understand and use this ra-ther obscure 'black box'.

The final output of the analysis provides a model (a signature) that can be applied to the population to be bred, for which only a set molecular data needs to be collected. For people who wish to learn more about this approach, I would suggest consulting the seminal paper of Meuwissen et al. (2001). Until now only a few papers have been published on this concept, but many breeders working with husbandry animals like cows, chickens or pigs are quickly adopting such an approach for the evaluation of the breeding value of individuals, whether they are parental lines or off-spring. A few fruit breeding programs are now explor-ing this new breeding approach (Varshney et al., 2005). For instance, scientists from New Zealand and The Netherlands (Kumar et al., 2012a; b) are adopting this new strategy in their apple breeding programs.

Breeders of horticultural plants have lost their edge over time and for many reasons are lagging behind agronomic crops and animals breeders in the adop-tion of the recent molecular genetic tools. We are now in a position to make a giant leap-forward and to make a historical jump in this field. These new breed-ing strategies can enable the horticultural breeders to move once again at the cutting edge of molecular genetics. Let's follow them.

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