MARKER-ASSISTED SELECTION IN POME FRUIT BREEDING[1]

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Summary
The development of markers-assisted selection strategies is one of the main priority in pome fruit breeding. Many different types of DNA analyses have been used to identify markers linked to both monogenic and multigenic traits in apple (*Malus* × *domestica* Borkh.) while still a few reports are available on European (*Pyrus communis* L.) and Asian (*Pyrus pyrifolia* Nakai) pears. The state of the art in the application of molecular markers in pome fruit breeding is reviewed and updated.

Keywords
Apple, pear, linkage, QTLs, marker-assisted selection

Introduction

Pome fruit breeding is a very difficult task mainly because of the lenghty juvenile phase and the very high level of heterozygosity of each apple and pear genotype. The first consequence is that most of the valuable traits present in one parent cannot be completely inherited as a whole due to their heterozygosity. Therefore the development of new techniques for the early selection of seedlings carrying valuable traits has become a priority in pome fruit breeding. The development of molecular markers linked to important agronomic traits has already made it possible to improve and to speed up some selection procedures.

Markers linked to monogenic traits

Most of the markers identified so far are linked to monogenic traits, i.e. mainly forms of resistance to the main pathogens and pests. Most of the markers identified so far are linked to the *Vf* gene for scab resistance, derived from *Malus floribunda* 821 (Table 1). Starting from this position, a map-based gene cloning approach was used to identify putative resistance genes at the *Vf* locus and at least one of these genes, called *HcrVf2*, is able to confer resistance to transgenic apple plants (Belfanti et al, 2003). Other sources of monogenic scab resistance have been reported and recently a few markers have been identified for the *Va*, *Vb*, *Vbj*, *Vm* and *Vr* genes (Table 2).
Another important disease of apple is powdery mildew (*Podosphaera leucotricha*) for which different resistance sources (*Pl1*, *Pl2*, *Plw* and *Pld* genes) have been identified in apple germplasm. The early evaluation of mildew susceptibility in segregating progenies is very difficult therefore reliable markers for these genes would be of great interest. The available markers for the *Pl1*, *Pl2*, *Plw* and *Pld* genes are still under testing because sometimes the correlation between molecular and two phenotypic dataset proved to be poor (Table 2).
The *Sd1* gene conferring resistance to two rosy leaf curling aphid (*Dysaphis devecta*) biotypes was finely mapped on linkage group 12 of apple and from the closest markers a map-based gene cloning was also started. Some markers have been developed for the *Eriosoma lanigerum* resistance genes but their reliability is still to be demonstrated (table 2).
There are also a few markers in apple available for the selection of agronomic and fruit quality traits as the columnar *Co* gene, the *Ma* gene controlling fruit acidity and the red or yellow skin colour (Table 2).

[1] From Tartarini and Sansavini 2003.

In Japanese pear only two examples of phenotype-linked markers have been reported for the black spot susceptibility and scab resistance genes.

Molecular maps and qtls

To date, at least 5 apple molecular maps are available (Table 3). Earlier maps were mainly based on RAPD or RFLP markers, thus being of limited use or difficult to transfer in crosses other than the one from which they were built. Recently, a very detailed map of apple based on more than one hundred SSRs has been published (Liebhard et al., 2002) and can be used as a reference map in any apple progeny. Analogous limits can be reported in pear but recently, it has been demonstrated that apple SSRs can be efficiently used both in European and Asian pear (Yamamoto et al. 2003) showing fairly good degree of sinteny among pome fruit species.

The identification of QTLs in fruit trees is rather difficult as the two parentals are highly heterozygous and the segregation analysis of each quantitave trait requires large progenies to increase its reliability. The first example of QTL mapping in apple led to the the identification of two main loci involved in fruit firmness and other QTLs were also identified for sensory assessments of fruit texture.

Various QTLs related to different multigenic sources of scab resistance (i.e. Discovery, TN10-8, Durello di Forlì) have been found with respect to various inocula (Durel et al., 2003).

Functional Markers

RFLP, RAPD, SSR and AFLP markers are only genetically linked to the trait of interest and no functional relationship can be inferred. Recently more attention has been focused on identifying differences in specific DNA sequences putatively involved in the expression of given traits. These differencethat might be related to the gene function and to the phenotype have been called functional markers.

The availability of many different sequences in DNA databases increased the possibility to produce functional markers in any species simply by a simple PCR approach. To date, about 1400 apple and pear gene sequences have been published in the DNA database but many more information are available in other species from the plant genome sequencing projects. The map co-localisation of putative functional markers and a given trait (or QTL) will makes it possible to infer their possible involvement in the expression of various complex traits. Three main examples have successfully been used in both apple and pear by using this PCR-based approach.

Gametophytic self-incompatibility is controlled by a single locus and it has been demonstrated that a basic glycoprotein with RNase activity is involved in blocking incompatible pollen tubes in variuos species. Specific primer pairs designed in the *S*-RNase conserved regions have been used to identify analogous sequences in both apple and pear and these *S*-PCR fragments can be used to map the trait (linkage group 17 of apple) and to determine the allelic composition of various cultivars.

A PCR approach was also used to identify the ACC-synthase and oxidase gene fragments in various apple and pear cultivars. The differences observed in the allele sequences suggested their possible relationship with the amount of fruit ethylene produced.

The sequence homologies between groups of cloned resistance genes suggested to investigate disease resistance by using heterologous PCR primers from conserved ipervariable regions. In some species, specific RGA sequences were mapped in genome positions, including resistance loci. Apple and pear RGAs showed good similarities to both RGA and resistance gene sequences found in DNA data banks but to date no clear associations between a specific RGA sequence and a resistance trait have been found.

MAS in pome fruit breeding

Most of the available apple and pear markers can be used in marker-assisted selection but MAS efficiency can widely varying depending on the estimated genetic distance between the marker and the linked gene. Of course, the use of two markers flanking the gene of interest is more advisable, in particular, if the distance between the gene and each marker is not very close. Molecular-based selection in pome fruit breeding progenies can be particularly important for traits that are difficult to evaluate (e.g. mildew resistance in apple) or delayed in time by juvenility (e.g. fruit traits) but today the broadest molecular screening can only be efficiently performed for the *Vf* scab-resistance gene.

The availability of a number of markers linked to the *Vf* gene made it possible to optimize MAS and to investigate in detail its advantages with respect to phenotypic selection methods. MAS is estimated to be more precise or less time-consuming than the available phenotypic selection protocols and can also make it possible to distinguish heterozygous and homozygous genotypes since two reliable flanking codominant markers are available. Therefore, "positive" MAS selection in favor to a specific allele can even be very informative even for easy-to-score phenotypic traits, as the *Vf* scab-resistance.

Another MAS advantage is the possibility of performing an efficient "negative" selection against the "donor" chromosomal regions in the vicinity of the introgressed gene and this type of selection cannot be performed by using a standard phenotypic selection. Iin fact it has been demonstrated that most of the advanced apple *Vf*-selections chosen only on a phenotypic basis are still carrying a large portion of the *floribunda* genome in the *Vf*-chromosome even after 5-6 generations. Of course the elimination of "wild" chromosomal regions in pseudo-test cross progenies can also be speeded up at the whole-genome level using a map with few but well-distributed SSR markers.

Availability of molecular markers linked to different resistance genes against the same pathogen and their map position can also be used to estimate the possible relationship among various, apparently unrelated resistance sources. In fact, it has been demonstrated that markers linked to a specific gene (i.e. *Vf or Vm; Sd1*) are not present in selection carrying other resistance genes. This marker-specificity can be used to easily select plants carrying multiple resistances against the same pathogen.

Conclusions

In the last decade a great deal of research throughout the world have been dedicated to improve selection strategies in pome fruit species by using the available molecular techniques. While the identification of markers linked to a trait is quite straightforward, its reliability is correlated both to the reproducibility of molecular techniques (e.g. RFLPs and SSRs are more reliable between labs than RAPDs) and, even more, to the quality of phenotypic data used for its development.

The great usefulness of MAS in improving and speeding up the selection process in pome fruit breeding was demonstrated with the available literature at least for monogenic traits. Unfortunately, most of the valuable agronomic traits in pome fruits are controlled by more than one gene and since only a few QTL examples are available, it is not possible to give any detailed account about the efficiency of marker assisted selection (MAS) for complex traits. The recent availability of a wide set of codominant SSR markers will soon open up the potential of investigating in detail the genetics of many polygenic traits. The potential of functional markers was also demonstrated to some extent in apple and pear and current functional genomics research will represent a further evolution of knowledge in molecular genetics.

By now a lot of work have still to be done to turn theory into the practical application of MAS in pome fruit breeding.

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Table 1
Main molecular markers linked to the *Vf* gene for scab-resistance genes in apple [(a)]

Marker type	Marker name [(b)]	Distance (cM)	Progeny size
RAPD	$OPM18_{900}$	10.6	59
RAPD	$OPU01_{400}$	19.7	59
RAPD	$OPD20_{500}$	19	158
RAPD	$OPA15_{900}$	-	-
CAPS	$OPM18_{900}$	1.9	600
SCAR	$OPU01_{400}$	4	
RAPD	$OPH01_{1100}$	10	160
RAPD	$OPR16_{400}$	13 / 14	98 / 160
RAPD	$OPAM19_{2200}$	0.9	109
RAPD	$OPAL07_{560}$	0.9	
RAPD	$OPC09_{900}$	8.8	
RAPD	$OPAB19_{1430}$	13.4	
RAPD	$OPC08_{1100}$	15.5	
RAPD	$OPK16_{1300}$	4.3	138
RAPD	$OPAR4_{1400}$	3.6	138
RAPD	$OPAG12_{800}$	9.9	155
RFLP	MC112a	7.7	
RFLP	pB610a	7.8	
RFLP	MC110a	8	
RAPD	$OPAG05_{1900}$	8	
RAPD	$S5_{2500}$	1.3	73
RAPD	$B505_{1700}$	7.8	
RAPD	$B398_{480}$	10.8	
RAPD	$K16_{1300}$	15.9	
SCAR	$OPAM19_{526}$	0.9	600
SCAR	$OPAL07_{466}$	0.9	
AFLP	EA2G11-1	0	430
AFLP	EA12MG16-1	0	
AFLP	EA11MG4-1	0	
AFLP	ET2MC8-1	0	
AFLP	ET3MG10-1	0	
AFLP	ET8MG1-1	0	
AFLP	ET8MG7-1	0	
AFLP	EA9MC15-1	0.2	
AFLP	EA4MG1-1	0.2	
AFLP	EA16MG2-1	0.2	
AFLP	ET4MC14-1	0.2	
AFLP	ET8MG16-1	0.2	
AFLP	ET3MG10-2	0.2	
AFLP	ET10MG8-1	0.2	
AFLP	ET9MC3-1	0.4	
AFLP	EA5MG3-1	1.1	
AFLP	EA8MC13-1	1.5	
AFLP	EA13MC16-1	2.2	

[(a)] From Tartarini and Sansavini (2003)

[(b)] Distances refer to those reported for the first identification since many markers have been used in many labs and in different progenies

Table 2
Molecular markers linked to monogenic traits in apple[(a)]

Trait	Gene	Marker name [(b)]	Distance (cM)	Progeny size
a) Resistance to:				
Scab	*Vm*	$OPB12_{687}$	6-8	180
Mildew	*Pl1*	$OPAT20_{450}$	4	64
		$OPD2_{1000}$	5	64
	Pl2	$OPAT20_{900}$	4	61
		$OPN18_{1000}$	-	96
		$OPAJ4_{750}$	-	96
Dysaphis devecta	*Sd1*	MC029b	2.2	135
		MC064a	1.5	135
		2B12a	1.5	134
		$OPC08_{1700}$	14.7	129
		$OPT09_{1200}$	18.9	127
		2B12 SCAR	1.5	134
		E6/M6R2	1.5	130
		E6/M6R1	1.5	130
		E6/M8R1	-	130
Eriosoma lanigerum	*Er1*	OPC20 SCAR	8	135
		GS327 SCAR	11.5	135
		GS327 SCAR	26.1	398
		OPO05 SCAR	25	398
	Er3	OPO05 SCAR	0.8	362
		OPO05 SCAR	2.5	120
b) Others:				
Columnar habit	*Co*	OA11-1025	6-15	-
		B347z-890	1.8	172
		OA11z-570	4.7	172
		B318y-440	3.2	172
		S34y-810	8	172
		OA11-1005	8	172
Fruit color	*Rf*	BC226	1.7 [(c)]	178
Fruit acidity	*Ma*	OPT16-1000	0	151

[(a)] From Tartarini and Sansavini (2003)
[(b)] Distances refer to those reported for the first identification since many markers have been used in many labs and in different progenies
[(c)] pooled estimate from 4 progenies

Table 3
Main characteristics of apple linkage maps[(a)]

Cross	Progeny size	Number of LG F/M [(b)]	Size F/M [(b)] (cM)	Main types of mapped markers
Rome Beauty × White Angel	56	21/24	-/950	RAPD (90%)
Wijcik × NY75441-67	114	20/16	798/692	RAPD (>90%)
Wijcik × NY75441-58	172	18/18	858/898	RAPD (>90%)
Prima × Fiesta	152	17/17	842/984	RAPD (46%) and RFLP (43%)
Fiesta × Discovery	112	17/17	914/1015	RAPD (51%) and SSR (49%)

[(a)] from Tartarini and Sansavini (2003)
[(b)] F/M = female/male