Pedigree genotyping identifies all favourable alleles

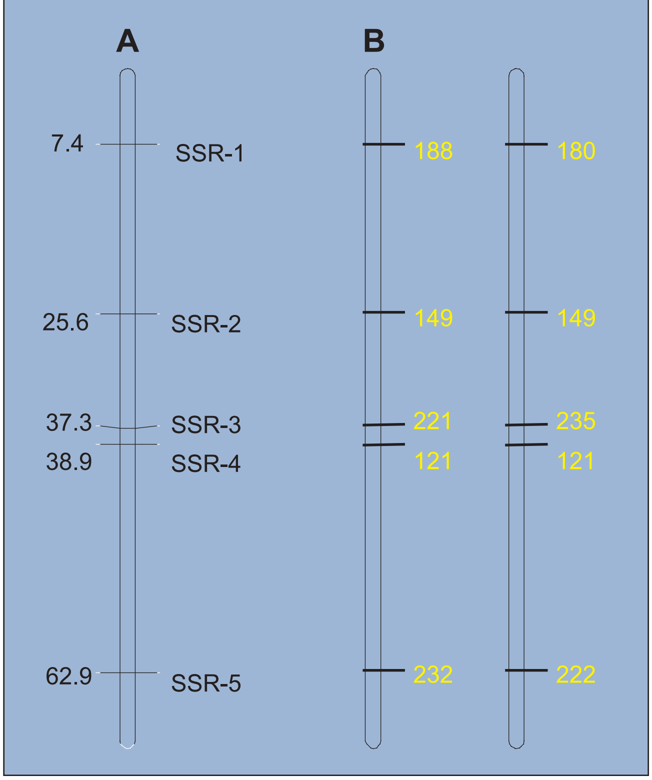
Pedigree Genotyping is a very cost-effective and short time-to-market approach to the mapping of genes in breeding material. In addition, the method is superior in detecting marker-allele associations and interactions between alleles. With Pedigree Genotyping, all favourable alleles of a breeding programme can be identified, not just the one or two that would segregate in a special 'mapping population'.

Eric van de Weg, Hans Jansen, Marco Bink, Roeland Voorrips

To date, molecular markers have been identified for many loci governing important horticultural traits. These markers have usually been identified in a single, specific cross. Consequently, only one or two favourable alleles of a locus are identified while a breeding programme usually includes various favourable alleles. If a breeder focuses his selection on these alleles, he would unnecessarily throw away many favourable genotypes. This reduces the efficiency of his programme. In addition, the genetic diversity of his material is unnecessarily narrowed down.

A new approach called 'Pedigree Genotyping' makes it possible to find markers for all favourable alleles present in a breeding programme. The costs for this approach are low compared to traditional marker research. Its benefits are obtained by the use of data from the ongoing breeding programme.

Figure 1A. Molecular marker map of chromosome 10 of apple. B: Allelic composition of the two individual homologous chromosome of Golden Delicious for the five SSR markers of Figure 1A.



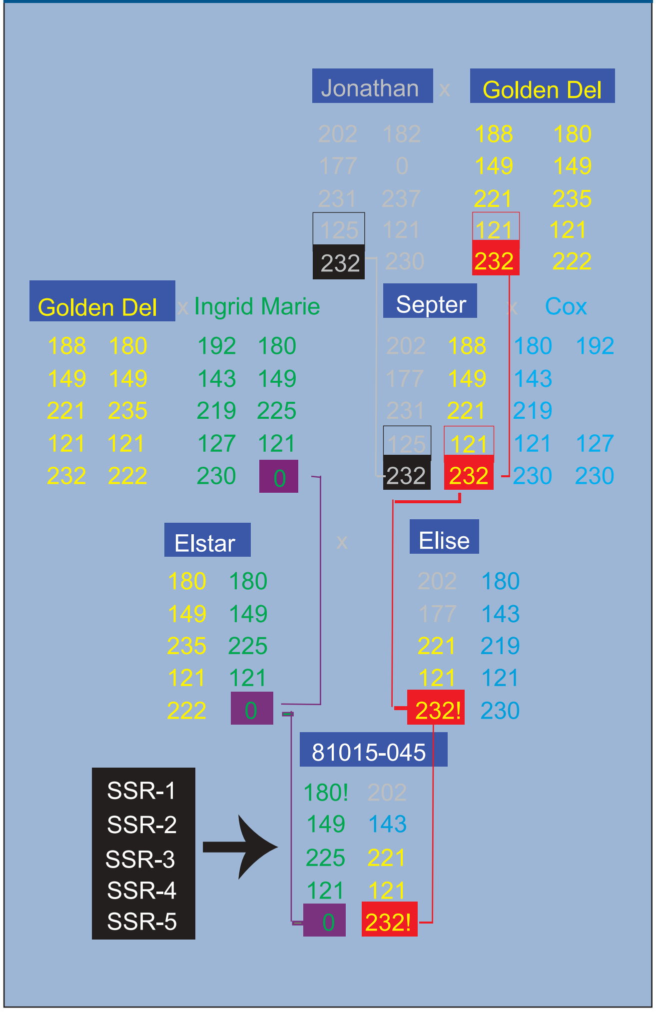
Pedigree genotyping

This principle is illustrated here for fruit firmness in apple. Apple is a diploid, outcrossing, vegetative propagated species. Chromosome 10 is interesting in relation to fruit firmness. *Figure 1A* shows a linkage map of this chromosome containing five SSR markers. SSR markers are co-dominant often having different marker alleles at both (homologous) chromosomes (*Figure 1B*). This makes them very suitable to follow their inheritance through a breeding program.

Figure 2 shows the pedigree of selection 81015-045, which is based on four different founder cultivars: Golden Delicious, Jonathan, Cox and Ingrid Marie. This pedigree is genotyped with the five SSR markers. We can now follow the transmission of these markers from one generation to the next, putting Pedigree Genotyping to work.

An example: 81015-045 has two alleles for SSR-5: '232' and '0'. Using the pedigree we can show that these two alleles are derived from ancestors Golden Delicious and Ingrid Marie, respectively. This is called an "Identity by Descent" (IBD) analysis. The identity of an allele of a modern selection can now be expressed in terms of alleles of founding cultivars. These founder alleles are used as factors in statistical analysis.

Figure 2. Pedigree of selection 81015-045 and the allelic composition for each genotype for the five SSR markers of Figure 1.



Marker-allele associations

One major locus for fruit firmness is located close to marker SSR-5. Some cultivars and related breeding selections, including those of *Figure 2*, were phenotyped and genotyped. Firmness was measured by penetrometer; values around 8 are desired, while 4 corresponds to apples that can be squeezed by hand. Results are presented in *Figure 3*. The '232' allele of Golden Delicious (GD) and Wagnerapfel (Wa) appear to be associated with good firmness. The average firmness of genotypes having this allele was around 8.3. This favourable linkage seems to be absent for the '232' marker of Jonathan (Jo), which has an average value of 6.6 and is thus associated with soft fruit. The same SSR-allele can thus be associated with different phenotypic effects depending on the origin of the marker. Therefore, it is important that the origin of the allele is taken into account in a statistical analysis in which traits are related to marker alleles.

Interactions between alleles

Sometimes a trait is not determined by the alleles separately, but by their interaction. Specific combinations are then much more favourable than expected based on their separate values. Such combinations are exploited in F1-hybrid cultivars or in vegetative propagated crops, and are automatically identified by Pedigree Genotyping.

Starting points

Pedigree Genotyping can start from ground zero, when no marker-locus association is known for the trait of interest. It can also start of from an already known locus, as in our example of firmness. Starting from a known locus, new alleles for this locus are identified.

When no locus is known, Pedigree Genotyping can identify loci for a trait once sufficient numbers of genotypes have been evaluated. Compared to a single cross, a larger number of genotypes is required because of the larger number of alleles that have to be accounted for. However, once incorporated in ongoing breeding programmes, the numbers of individuals will steadily grow over the years, and may soon exceed the size of any single cross.

Cost-effective

Pedigree Genotyping searches for new marker-trait associations while breeding thereby making this approach cost effective. It avoids the costs of growing and phenotyping of specially designed 'scientific' progenies. Besides, SSR markers are cost effective for genotyping. The SSR markers of our example can be simultaneously tested. A chromosome can thus be genotyped by a single PCR reaction and a single lane of a gel. Once a genomic region of interest is identified, testing additional markers could increase its marker density improving resolution and leading to more tightly linked markers.

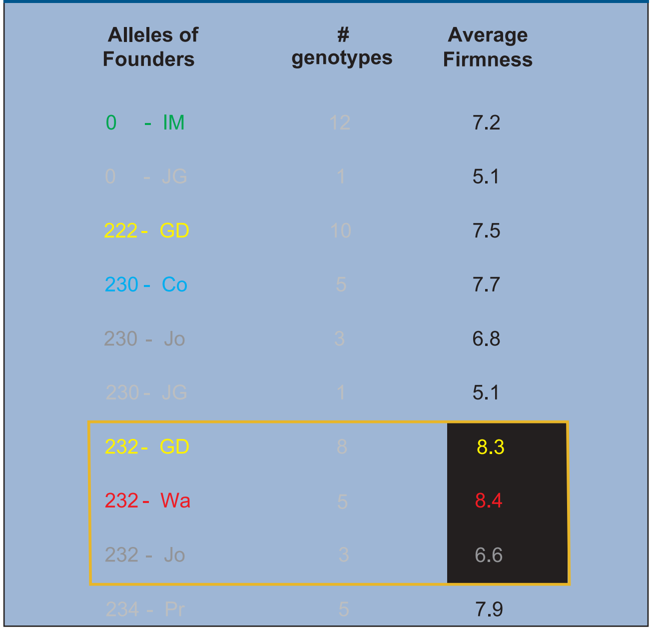
Requirements

Pedigree Genotyping requires genetically related breeding material, a set of multi-allelic markers (like SSRs) that cover the genome segment of interest, software to calculate the genetic value of different marker alleles, and on the longer term a database to store all phenotypic and genotypic data. To date, the availability of co-dominant markers varies per species. Plant Research International and Biometris are developing the required software.

Crops to go for

Pedigree Genotyping offers great perspectives for any crop in any breeding system. The greatest advantages are obtained if genotypes from past breeding programmes and their phenotypes are available or easy to produce (e.g. vegetative propagated crops, or inbred lines and their F1's, or phenotyped genotypes available by DNA samples), and (2) when it takes a long time period to construct and evaluate mapping populations, or (3) when many loci

Figure 3. Founder alleles of SSR-5 (distinguished by size and colour), and the average firmness of genotypes in which they occur.



Alleles of Founders	# genotypes	Average Firmness
0 - IM	12	7.2
0 - JG	1	5.1
222 - GD	10	7.5
230 - Co	5	7.7
230 - Jo	3	6.8
230 - JG	1	5.1
232 - GD	8	8.3
232 - Wa	5	8.4
232 - Jo	3	6.6
234 - Pr	5	7.9

are already known from special mapping populations, and (4) when the trait of interest is oligogenic.

Advantages

Pedigree Genotyping is a powerful approach towards marker-assisted breeding. Its advantages in a nutshell:

- markers are found for most alleles that are relevant to the breeder since they are part of his own breeding material,
- alleles that show interactions are identified,
- Pedigree Genotyping can be fully performed on existing pedigrees thus reducing costs and time-to-market.

Pedigree Genotyping will change the way breeders work with their material. Within a Pedigree Genotyping context, the breeding material is not only a source of new varieties but also a source of information. The value of this information will grow as more molecular data and phenotypic characterisations accumulate over generations. This requires a long-term view of its value. But after all, a long-term view is what breeders are famous for.

The authors are researchers at Plant Research International BV & Biometris, P.O. Box 16, 6700 AA Wageningen, The Netherlands, W.E.van-deweg@wur.nl