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Characterization of putative pear-apple hybrids

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Introduction

Putative pear-apple hybrids generated at University of Bologna (Unibo) were characterized at Edmund Mach Foundation by single sequence repeat (SSR) markers, metabolomics analysis and cellular DNA content to determine which hybrids were true.

Malus and *Pyrus* are closely related, with highly co-linear genomes. However, the two genera are characterized by many specific differences, including disease resistances, secondary metabolites, fruit texture, flavour and shape. Hence, intergeneric hybrids between apple and pear provide a germplasm resource not only for genomic, transcriptomic and metabolic profiling studies, but also for applying advanced breeding strategies.

Materials and methods

Leaves were sampled from three putative pear-apple hybrid plants: one putative pear-apple hybrid from 'Abate' x 'Fuji' and two from 'Decana' x 'Murray' developed at Unibo. DNA was extracted using the DNasy Plant Mini Kit® (Qiagen). Seventeen published SSR markers[1,2,3,4,5] mapping to different chromosomes were selected to check the degree of relationship between the hybrids and their parents. The SSRs data were analysed using GeneMarker, GenAlex and Whichparents software.

Fresh leaf samples (100 mg) were accurately weighed into a 15 mL plastic tube and extracted with 80% methanol in a ratio of 1/40 (w/v) for 24 h at 4 °C in the dark, after which the resulting supernatant of each sample was filtered into 500 µL vials for metabolomic analysis.

DNA content was measured according to Plant Cytometry Services (Schijndel, The Netherlands)[6].

Results

SSR analysis confirmed that the 'Abate' x 'Fuji' progeny was a true hybrid, but the two from 'Decana' x 'Murray' were not. As result of population assignment, the lower log-likelihood value for the apple parents ('Fuji' and 'Murray') on the X axis indicates population 1 as the most likely for apple; a lower log-likelihood value for pear parents ('Abate' and 'Decana') on the Y axis indicates population 2 as the most likely for pear. The 'Abate' x 'Fuji' hybrid was located between the two parents (Figure 1A). Moreover, the result of Whichparents analysis highlighted the presence of null alleles at five loci, probably due to the inability of apple markers to determine the pear allele presence. The genetic result agreed with the metabolomics analysis, which confirmed that the putative hybrid 'Abate' x 'Fuji' accumulated genus-specific secondary metabolite phloridzin from *Malus* and arbutin from *Pyrus*. The two putative pear-apple hybrids from 'Decana' x 'Murray' accumulated only arbutin (Figure 2).

Significant differences (p=0.0001) in absolute DNA content of the *Malus* and *Pyrus* genotypes, as well as for the putative hybrids were found by flow cytometry. The DNA content of apples 'Gala' and 'Murray' was on average 1.51 pg/2C in comparison with pear cultivars at 1.12 pg/2C. The DNA content of 'Zwintscher's hybrid'[6] and the 'Abate' x 'Fuji' hybrid was 1.30 pg/2C, which is intermediate between the DNA content of the *Malus* and *Pyrus* parents. The two other putative hybrids ('Decana' x 'Murray' 1, 'Decana' x 'Murray' 2) have a DNA content closer to pear (Table 1).

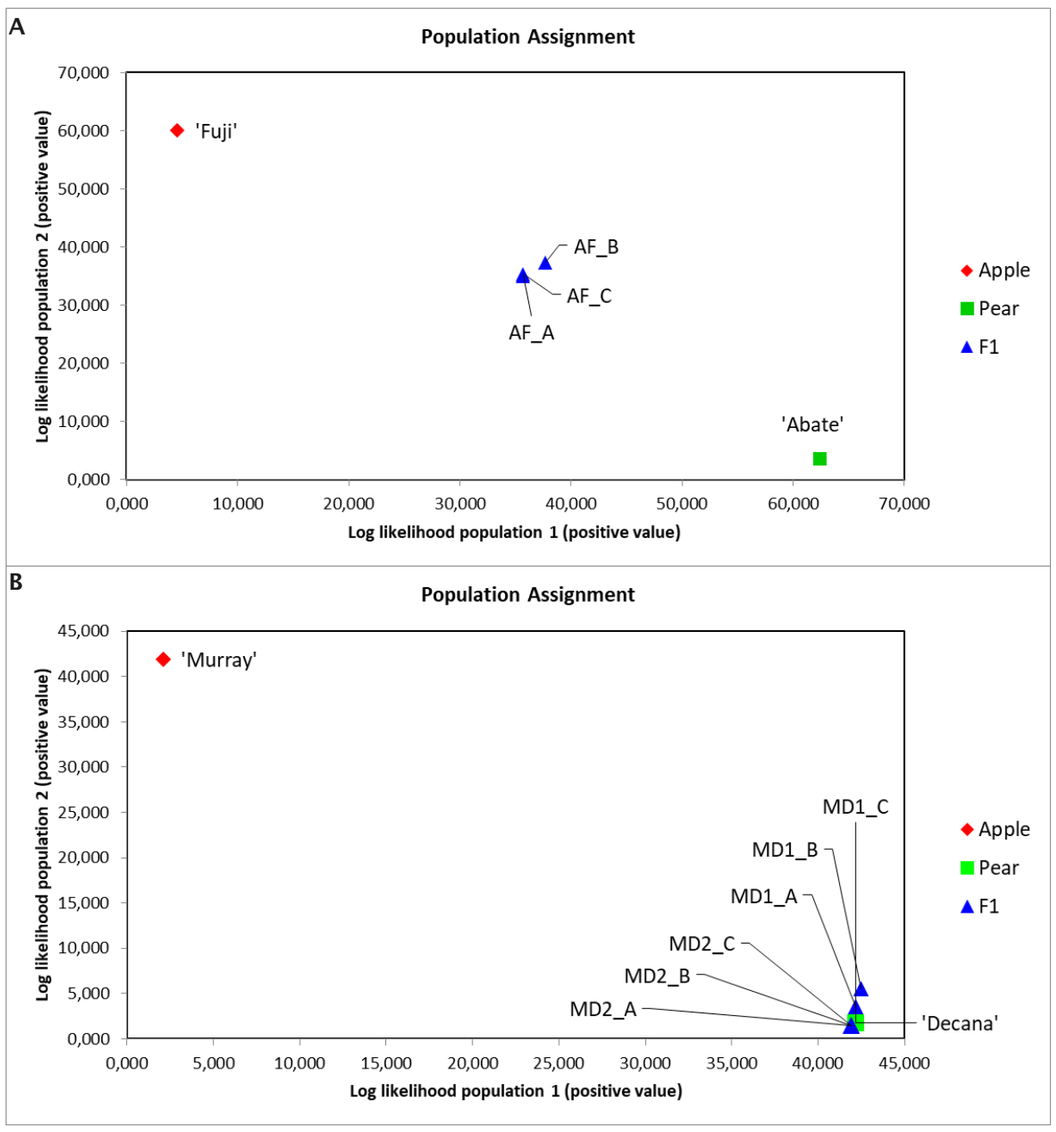

Figure 1: Population assignment of putative hybrids as deduced from the SSR marker analysis. Chart represents the positive log-likelihood of assignment of each sample by GenAlex. The lower log-likelihood value for apple parents ('Fuji' and 'Murray') X axes indicates population 1 as the most likely for apple; a lower log-likelihood value for pear parents ('Abate' and 'Decana') on Y axes indicates population 2 as the most likely for pear. The three replicates of the putative hybrid from 'Abate' x 'Fuji' (AF) were located between the two parent groups (A), and the three replicates of two putative pear-apple hybrids from 'Decana' x 'Murray' (DM1, DM2) were located near pear (B).

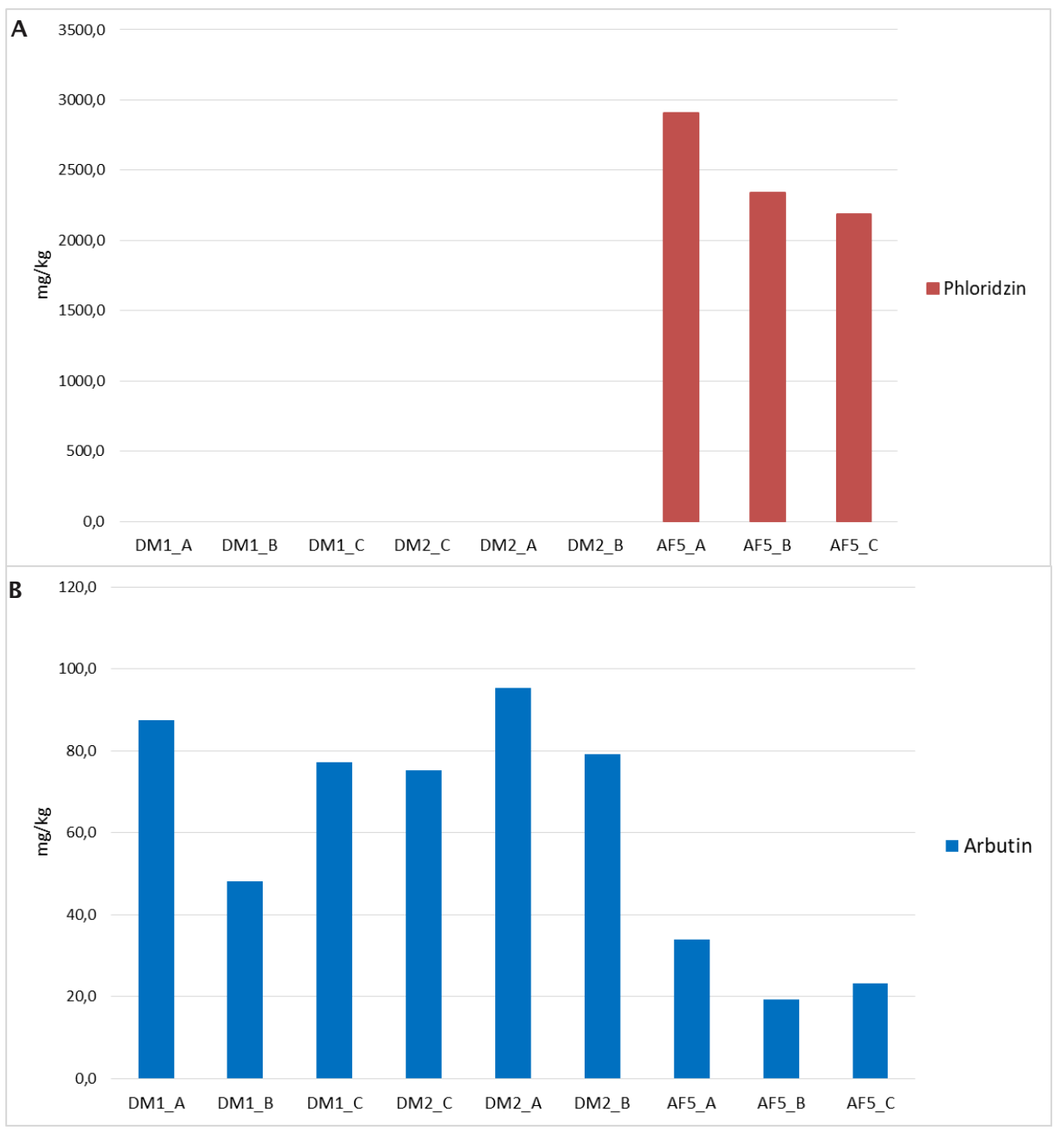

Figure 2: Metabolomics analysis. The three replicates of the putative hybrid from 'Abate' x 'Fuji' (AF) accumulated both genus-specific secondary metabolites phloridzin from *Malus* (A) and arbutin from *Pyrus* (B), while the three replicates of two putative pear-apple hybrids from 'Decana' x 'Murray' (DM1, DM2) accumulated only arbutin.

Cultivar	Mean DNA content [pg] ± SD
'Zwintzscher's Hybrid'	1.30 ± 0.01
'Murray' (apple)	1.54 ± 0.01
'Gala' (apple) in vitro	1.49 ± 0.01
'André Desport' (pear)	1,12 ± 0.01
'Abate' x 'Fuji'	1.29 ± 0.01
'Decana' x 'Murray' 1	1.19 ± 0.01
'Decana' x 'Murray' 2	1.20 ± 0.01

Table 1: Cellular DNA content.

Discussion and Conclusions

Our results suggest that the putative hybrid from 'Abate' x 'Fuji' is a true hybrid, as is 'Zwintscher's hybrid'[6].

Our next step is to map recombination events during the hybridization of pear and apple more precisely, using the 20K apple SNP array[7] to delineate their chromosomal structure.

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