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NEW EVIDENCES ON THE GENETIC CONTROL OF THE STONY HARD TRAIT

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In the last decade the aim of stone fruit breeding programs has been the quality improvement. One of the most crucial problems is the firmness of the flesh that plays an important role in the shelf-life, storability and susceptibility to post-harvest diseases.

In this outline our group began to study the "*stony hard*"(hd/hd) trait in *Prunus persica*. Stony hard peaches have an extremely firm flesh and fruits can stay on tree up to twenty days after their ripening time. Yoshida first described this trait in 1976 as monogenic and recessive.

To identify a set of markers tightly linked to this trait we have constructed a genetic linkage map using a Yumyeong x O'Henry F_2 progeny (YxO'H). The map is composed of 98 loci (2 morphological, 79 AFLP, 17 SSR) and covers 609 cM. The loci are distributed in.15 linkage groups instead of the 8 expected for peach (2n=16). The "*stony hard*" trait resulted unlinked, probably due to the distorted segregation observed (9:7) compared to the expected (3:1).

At the same time to produce ESTs, we constructed a cDNA library from Yumyeong fruit mesocarp, ripening stage S4. We sequenced 864 clones and analysed 660 sequences; we obtained 61 contigs and 432 singlets. Yumyeong is a non-melting flesh peach, so, as expected, we didn't found ESTs related to endopolygalacturonases (enzymes involved in the softening of flesh) but, unexpectedly, we didn't find any ESTs related to the ACC oxidase (ACO). The ACC oxidase is the key enzyme in the biosynthetic pathway of Ethylene, the most important hormone involved in fruit ripening.

We suggest that the *stony hard* phenotype is due to the contemporary absence of ethylene and endopolygalacturonases (endoPG), so the trait would be not monogenic, but digenic. This can explain the type of segregation observed. In fact if we consider the independent segregation of the endoPG and ACC oxidase genes, 4 different phenotypic classes are expected [endoPG+/ACO+, endoPG+/ACO-, endoPG-/ACO+, endoPG-/ACO- (*stony hard*)]. However we can clearly distinguish only two phenotypes: the one of the endoPG+/ACO+ class (melting flesh) and the one that pools together the remaining three classes. Actually what we see is a segregation ratio of 9:7 versus the expected 9:3:3:1.

To validate our hypothesis a northern analysis will be performed, using an endoPG gene and the ACO gene as a probe, on: a Yumyeong fruit mesocarp at stage S4, a canning peach mesocarp (as a positive control for ethylene production and as a negative control for the endopolygalcturonase synthesis), a

melting flesh peach mesocarp (as a positive control for ethylene production and for the endopolygalacturonase synthesis).