

## FROM A MAJOR QTL FOR MONOTERPENE CONTENT IN GRAPE BERRIES TO FUNCTIONAL VALIDATION OF POSITIONAL CANDIDATE GENE *VvDXS*

GRANDO M.S., EMANUELLI F., COSTANTINI L., BATTILANA J.

Department of Genomics and Biology of Fruit Crops, IASMA Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38010 San Michele all'Adige, Trento (Italy)

*QTL mapping, association study, enzyme activity, transgenic lines*

All the QTLs mapped for grapevine up to now were based on bi-parental populations which represent only a small slice of the overall variation of the corresponding trait, and no QTL cloning was reported, although projects are underway. The availability of the grapevine genome sequence and new sequence data allow to compare the genome structure in different grapes but also to decipher genetic differences in specific genomic regions where QTLs for important traits are known to be located.

In our experiment, a structural gene of the plastidial methyl-erythritol-phosphate pathway, 1-deoxy-D-xylulose 5-phosphate synthase (*VvDXS*), was suggested as a candidate gene for grape aroma having been localized within a major QTL for linalool, nerol, and geraniol concentrations in berries. Examining nucleotide diversity and linkage disequilibrium within the *VvDXS* gene in grapevines with different genetic backgrounds, and testing for association between individual polymorphisms and berry flavour, a putative causal SNP responsible for the substitution of a lysine with an asparagine was identified. Network analysis revealed a major star-shaped cluster of reconstructed haplotypes unique to muscat-flavoured varieties. Moreover, muscat-like aromatic mutants displayed unique non-synonymous mutations near the mutated site of muscat genotypes.

The significance of these nucleotide differences was investigated by comparing the monoterpene profiles with the expression of *VvDXS* alleles throughout berry development in Moscato Bianco, a cultivar heterozygous for the SNP mutation. Although correlation was detected between the *VvDXS* transcript profile and the accumulation of free monoterpene odorants, the modulation of *VvDXS* expression during berry development appears to be independent of nucleotide variation in the coding sequence. In order to assess how the non-synonymous mutation may enhance berry flavour, an *in vitro* characterization of enzyme isoforms was performed followed by *in vivo* overexpression of each *VvDXS* allele in tobacco. The results showed that the amino acid non-neutral substitution influences the enzyme kinetics by increasing the catalytic efficiency and also dramatically affects monoterpene levels in transgenic lines. These findings confirm a functional effect of the *VvDXS* gene polymorphism and pave the way for metabolic engineering of terpenoid content in grapevine.