Sustainable viticulture through NPBTs for biotic and abiotic stress management

Moffa L.[1,2], Gaetano G.[1,3], Gambino G.[4], Perrone I.[4], Pagliarani C.[4], Velasco R.[1], Chitarra W.[1,4], Nerva L.[1,4]
loredana.moffa@crea.gov.it
[1]Research Centre for Viticulture and Enology, Council for Agricultural Research and Economics (CREA-VE), Via XXVIII Aprile 26, 31015 Conegliano (Italy);
[2]University of Udine, Department of Agricultural, Food, Environmental and Animal Sciences (Di4A), Via delle Scienze 206, 33100, Udine (Italy);
[3]University of Milano, Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy (DiSAA), Via Celoria 2, 20133, Milano (Italy);
[4]Institute for Sustainable Plant Protection, National Research Council (IPSP-CNR), Strada delle Cacce 73, 10135 Torino (Italy).

New plant breeding techniques (NPBTs) aim to overcome traditional breeding limits for plant improvement to biotic and abiotic stresses satisfying the European Policies requirements that promote chemical input reduction and new environmental-friendly methods for a more sustainable agriculture.

We decide to apply genome editing (via CRISPR/Cas9) focusing on susceptibility genes to control powdery mildew: we chosen to knock-out two genes belonging to *MLO* (*Mildew Locus O*) family: *VvMLO7* and *VvMLO6*.

In parallel we used the same approach to cope with abiotic stresses, in specific drought, performing a knock-out of four genes, two belonging to *GST* (*Glutathione S-Transferase*) and two to *PME* (*Pectin Methyl Esterase*) gene families. Previous studies demonstrated a better drought tolerance in knock-out mutant for both these two gene families.

In parallel to genome editing, we also applied cisgenesis to move the resistance locus RPV31 (*Resistance to Plasmopara viticola*)(†) into economically important cultivars. This locus is formed by two different genes that were inserted (with native promoters and terminators) individually and in combination to evaluate their effects.

One of the drawbacks linked to classical *Agrobacterium tumefaciens* mediated transformation is the insertion of unrelated selectable marker genes (e.g., antibiotic resistance). These markers are required for transgenic plants selection, but undesirable to be retained in commercial plants due to possible toxicity or allergenicity to humans and animals, in addition to their potential hazards for the environment. To overcome these limits, we developed a "clean" transformation strategy using an inducible excision system based on a recombinase technology from the P1 bacteriophage. The Cre-lox system is controlled by a heat-shock inducible promoter that will be activated once the transformation event(s) will be confirmed. Embryogenic calli of Chardonnay, Glera, Microvine, Pinot Noir, Sangiovese, were used in stable transformation with *A. tumefaciens* GV3101 carrying the genome editing construct with the MLO-guideRNAs (two for each gene) and the cisgenic construct carrying the two RPV3-1 genes. Embryogenic calli of rootstocks 110 Richter and SO4 were transformed with genome editing construct carrying GST and PME guideRNAs in two independent transformations.

Regenerated embryos from all the transformation events are now under evaluation.

(†) In cooperation with the Institut of Applied Genomics, Udine.

Keywords: cisgenesis, genome editing, biotic and abiotic stress, Cre-lox, grapevine.