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APPLICATION OF NEW BREEDING TECHNIQUES TO IMPROVE IMPORTANT AGRONOMICAL TRAITS IN PRUNUS SPECIES

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Recent technological advances in genomics and bioinformatics are a powerful tool for the improvement of plants of agronomic interest. In this scenario, particular attention is paid to New Breeding Techniques (NBT) which, by exploiting the new acquiring knowledges on the structure and functioning of plant genomes as well as on relevant biological mechanisms, allow the introduction of specific mutations in predefined gene sequences. Of particular interest is the CRISPR-Cas9 approach which allows the selection of plants in which only the trait of interest is surgically modified, without the permanent introduction of exogenous DNA into the genome. In the *Prunus* species, some of the most important challenges that researchers are called to face are: i) the introduction of resistance to biotic stresses (*i.e.* Sharka); ii) the alteration of plant architecture for better light interception and cultivation operations and iii) the improvement of productivity through the reduction of juvenile phase. In our study, we focused on genes known to be involved in each of the processes and we developed specific strategies based on the design of one or two guides which target specific exonic regions with the aim to achieve gene disruption. For the host susceptibility genes involved in recessive resistance to Sharka disease, a single guide strategy is being pursued for the eIFiso4E gene: two guides were designed, one on exon 1 and the other on exon 3; each of the guide was individually cloned into 3 plasmids harboring the hCAS9 cassette with BlpR or NptII as the selection gene or NptII fused to the DsRed reporter gene, according to the Golden Braid cloning methodology. PpeTAC1 is the gene responsible for the *habitus* due to its role on defining the branches angle, therefore its silencing leads to an

increase crop yields through a more efficient interception of the light. To silence PpeTAC1, two sgRNAs were designed with the aim to obtain a deletion of 400 bp and cloned into a vector harboring the hCAS9 cassette and NptII marker gene. Lastly, to obtain modulation of the flower biology, the strategy involves the knock-out of the PpeTFL gene in *Prunus,* trough the excision of a 1100bp fragment by a double guide CRISPR/hCAS9 construct that induce early flowering. All expression cassettes are being used on *Prunus* background materials for *Agrobacterium*-mediated transformation using different strains of *A. tumefaciens* (LBA4404, GV3101 and AGL1). Multiple material (both mature explants, from *in vitro* shoot culture, and immature embryonic tissues) from apricot (cvs. Boccuccia spinosa, Bella di Imola and San Castrese) and peach (cvs. Independence, Rich Lady and Royal Glory and GF677) were treated with the diverse constructs and placed onto an appropriate growth medium with the aim to promote regeneration. Infections on seed materials were performed both in the summer 2020 and 2021, and the material is under molecular analysis for the evaluation of possible editing events.