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## A NEW RICE PLANT IDEOTYPE THROUGH GENOME EDITING: THE SUSRICE PROJECT

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Rice (*Oryza sativa*) is the second most cultivated cereal in the world, after corn. Italy is the first producer in Europe, and rice consumption is constantly increasing, in Italy and within the European community. Rice is also one of the first crops in which genome editing (GE) has been applied. Within the BIOTECH project funded by Masaf to CREA, the editing of three rice genes, in the CREA japonica varieties Vialone Nano and Roma, was the focus of the Susrice subproject, aimed at rice breeding with the ultimate goal of creating a new plant ideotype with improved resilience and sustainability. The target genes were *DRO1*, influencing the root growth angle; *NRT1.1B*, which increases in its indica allele the efficiency of nitrate absorption in comparison to the japonica varieties, and *IPA1*, which regulates the architecture of the plant, through less unproductive tillers, stronger culms and more grains per panicle. The target of *DRO1* editing, carried out by the Cas9-VQR variant, was the auxine Response Element 1 (*RE1*) in its promoter region, in order to avoid gene expression inhibition, thus allowing roots deepening. *NRT1.1B* editing aimed at a nucleotide substitution, by prime editing, in its coding sequence, in order to obtain the Thr327Met mutation of the indica allele. *IPA1* is negatively regulated

by miRNA156: target of its editing was the mutation of the miRNA156 binding site, thus obtaining gain of function mutant plants, accumulating *IPA1* transcripts. Edited plants of Vialone Nano and Roma have been produced, despite a certain recalcitrance of these varieties to genetic transformation. Seedlings of the T2 generation have been genotyped and are currently being evaluated for the characters subject to improvement. In particular, for seedlings edited for the nitrate transporter, *NRT1.1B*, the first assessments indicate that when germinated in a liquid medium containing nitrate as a source of nitrogen, they display greater vigour, in terms of length of both culm and roots, in comparison to the wild type. As regards *DRO1* gene, the root growth angle of seminal, nodal and lateral roots is being evaluated and measured in edited plants compared to control plants using 2-D rhizoboxes in which plants grow vertically between two wet filter paper sheets and in soil-filled rhizotrons placed vertically at an angle of 30°. The first results of root phenotypic evaluation will be presented.