

IMPROVING ITALIAN ELITE RICE VARIETIES FOR DIFFERENT AGRONOMIC TRAITS

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breeding, Marker-Assisted Selection, rice blast, CRISPR/Cas9, TEA

Rice cultivation is hampered by several abiotic and biotic stresses that contribute to the reduction of rice productivity. Crop breeding is, therefore, constantly required to ensure the release of new varieties that can guarantee satisfactory productivity and adaptability to novel and arduous growing conditions. Molecular approaches, such as Marker-Assisted Selection (MAS) can already be efficiently used to speed up the selection process and in addition the recent development of the CRISPR/Cas9 technology has enlarged the number of tools available for plant breeding.

We applied MAS to introduce in an elite Italian rice variety (recurrent parent) favourable alleles related to agronomic traits as grain yield (*OsCKX2*), nitrate use efficiency (*NRT1.1B*) and resistance against rice blast (*Pib*). A Chinese high-yielding cultivar was used as a donor variety for the breeding program. We designed different kinds of markers such as High-Resolution Melting PCR (*OsCKX2*) and tetra-ARMS-PCR (*NRT1.1B*) while we exploited available markers for *Pib*.

We also utilized a targeted mutagenesis approach, in a proof-of-concept project, as a tool for plant breeding since it allows the editing of more genes (traits) simultaneously reducing the time and labour required for crop improvement. More specifically, we exploited a multiplexing CRISPR/Cas9 system to target two genes. The first, *Pi21*, is a recessive blast resistance gene encoding a proline-rich protein with a putative heavy metal binding domain and a protein-protein interaction motif. It is

considered as one of the few R genes conferring broad-spectrum resistance and its recessive resistant allele (non-functional allele) is thought to prevent susceptibility of the plant to *Magnaporthe oryzae* infection. The second one *OsCKX2* (*Gn1a*), which encodes for a cytokinin oxidase/dehydrogenase, is correlated to the number of grain per panicle and important polymorphism, such as an 11 bp deletion in the fourth exon, were identified in high-yielding variety like 5150. Expression data and RNAi interference experiment suggest the hypothesis that either reduction or loss of function of *OsCKX2* is responsible for the increase of plant productivity. Different independent lines characterised by frameshift mutations, in both the target genes, were created through in vitro culture and *Agrobacterium*-mediated transformation. Plants were then propagated for the selection of non-transgenic mutant lines that will undergo phenotypic screening for the traits under investigation. An Italian elite rice variety, characterized by susceptibility to rice blast infection and a functional *OsCKX2* allele was selected for the mutagenesis.