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Activation tagged mutant resource of rice – a gold mine for agronomic applications

Insertional mutagenesis is a technology in which a segment of DNA, about which the investigator has complete information for its tracking, is introduced into the plant genome. These known DNA fragments can be elements such as transposons or jumping genes, which can cause repetitive mutagenesis or the T-DNA that is transferred to the plant genome in the process of genetic manipulation from Agrobacterium. Both the jumping genes and the T-DNAs have been utilized as mutant tools in rice. When transformed, these elements become integrated into the genome randomly, and the target gene becomes disrupted resulting in a *loss-of-function* mutant, if the integration is within the gene. This disruption of the gene might result in phenotypic alterations. However, loss-of-function approaches cannot always uncover gene function as most of the genes occur as families in the genome and loss-of-function in one gene might be compensated by other members of the gene family.

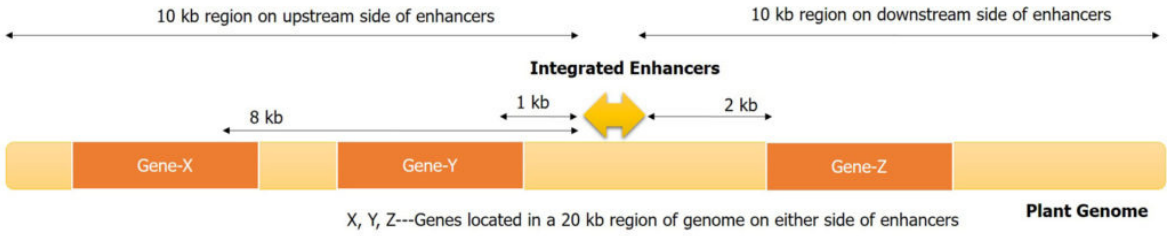

Fig. 1. Representation of a chromosomal map with enhancers integrated in the genome of transgenic plants. Enhancers activate the expression of genes up to 10 kb on either side of their insertion. Genes (X, Y and Z) located on both the sides of enhancers in a 20 kb region of genome would be selected for expression analysis to identify the activated gene(s) responsible for the altered mutant phenotype.

An efficient alternative to the above is *gain-of-function* mutagenesis approach wherein the transcriptional enhancers, usually derived from 35S promoter of Cauliflower Mosaic Virus are randomly introduced into the plant genome. This causes endogenous activation of nearby genes at the sites of their integration. Because the activated genes are associated with a tag, this technique has become popularly known as *activation tagging*. Activation tagging necessitates the development of a large number of transgenic plants each one carrying these introduced enhancer elements at different sites in the recipient genome. This population can be screened for any trait of agronomic importance. Thus, activation tagging not only helps in crop improvement programs, but also identifies novel genes and their significance in a single step.

We have developed a large-scale activation tagged mutant population in a very widely cultivated *indica* rice variety, BPT-5204 (also called Samba Mahsuri). About 3000 such mutants were screened for their ability to sustain under limited water conditions by growing them without normal level of water (not a drought condition). The transgenic plants with better yield-related attributes such as increased tillering, plant height, panicle number, productive panicles and seed yield were selected. Two parameters related to water-use efficiency (WUE), photosynthetic performance using Pulse Amplitude Modulator and carbon isotope composition ($\Delta^{13}C$ measurements) using Isotope Ratio Mass Spectrometer were measured in transgenic plants. Based on their growth and physiology under water deficit conditions, we have identified 200

potential plants with sustained or improved performance, high quantum efficiency and relatively low $\Delta^{13}C$. These plants were analyzed for the DNA sequence on either side of integration of enhancer elements to identify and map the sites of integration on corresponding chromosomes of rice.

We have further selected five mutant plants to study the expression pattern of all the genes situated in a 20 kb region on either side of enhancer integration in the genome (Fig. 1). In two of them, genes coding for proteins involved in ribosomal large subunit assembly, RPL6 and RPL23A became activated by enhancers. Ribosomal proteins (both large and small subunit proteins) are essential in any organism for assembly of ribosomes, which are basic sites of protein synthesis in cells. In addition to their house-keeping function, this investigation identified the novel role of ribosomal proteins in abiotic stress amelioration along with WUE. The other genes that became activated are transcription factors (GRAS, WRKY) and those involved in protein ubiquitination (Cullins) showing that they also function in enhancing WUE in rice.

The principal purpose of this entire study is to generate a sizable activation tagged mutant resource in *indica* rice, which can be utilized for analyzing various traits, followed by identification of genes responsible for the altered phenotypes.

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