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Identifying gene function

The rice genome contains about 30,000 genes, half of which have an as yet undetermined role. The function of these genes can be found by analysing of a large population of plants in which a mutagen has been randomly inserted into each gene. Mutagens are short DNA sequences of different types that are used as tools to map and isolate genes ("molecular labels" so to speak).

In the framework of the national plant genomics initiative Génoplante (see page 16), a team of researchers from Cirad-INRA-IRD-CNRS / University of Perpignan based at Montpellier has created a collection of rice insertion mutants large enough (100,000) so that each gene has a good probability of being interrupted at least once by an insertional mutagen, here the T-DNA from Agrobacterium tumefaciens. The collection of mutants will be progressively screened under various constraints in a controlled environment (confinement greenhouse, phytotrons) so as to identify plants of altered morphology, physiology or tolerance of environmental constraints, and subsequently to isolate the affected genes that govern the characteristics in question (forward genetics). The genome sequences adjacent to the T-DNA insertion sites in each mutant will be entered in a database. They will then be used to study the function of any gene with a known sequence, by searching for and then evaluating the relevant

mutant carrying the altered sequence (reverse genetics). This will enable isolation of the rice genes involved in plant morphogenesis (architecture, flowering, embryogenesis...) and in the tolerance of biotic stress (diseases and pests) and abiotic stress (drought, salinity, mineral deficiency



A reporter (blue) gene reveals gene expression in vessels of a rice flower

or toxicity). Molecular mapping of these genes will shed light on the genetic control of quantitative traits and will greatly facilitate varietal improvement in rice and other cereals (wheat, barley, maize, sorghum...).

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Characterizing gene function

• Functional genomics aims to characterize the expression of the genome and its integration in the major metabolic functions.

- insertional mutagenesis (see above "Identifying gene function using rice mutants") creates mutants by randomly inserting in the genome a DNA fragment that can be mapped. When this fragment is integrated in a gene, thus generating a mutant, it alters the gene's function, thereby modifying the trait of interest. The mutated gene is located thanks to the inserted fragment, and its function is identified using the affected trait. This technique has been developed for different model plants (Arabidopsis, Medicago truncatula, for example). The Montpellier research teams are studying rice as a representative of the grass family (Poaceae);

- partial sequencing of expressed

genes (Expressed Sequence Tags or ESTs) allows the characterization of genome expression patterns. The analysis of RNA extracted from certain organs of cultivated plants, under given conditions and at a given moment, yields an image of all the genes expressed under these conditions. Among these genes are some of agronomic value that it would useful to clone. Depending on the characteristics of the sequences or of the expression patterns, some of these ESTs can be attributed a hypothetical function, thus making them candidate genes. This is now under way using model plants and will be extended to the major crops. The precise functions of the candidate genes, identified through genomic approaches, are characterized in two stages:

- *genetic transformation* to test the effect of a gene or of a candidate sequence on the phenotype of a plant, through its insertion in the genome and/or the modification of

its expression in the plant; - the fine evaluation of transformed material to elucidate all facets of the function of a gene, by means of cellular and molecular biology, and physiology.

These technologies, applied to some model plants and to a range of more complex cultivated plants, can build bridges between genomes. Genetic information can be transferred between several species because of the conservation throughout evolution of certain elements of the general genome organization, like the similarity in gene distribution between chromosomes (conservation of synteny, see next page "Synteny and parallel chromosome walking in the Poaceae") or the colinearity between homologous chromosomes. Genomics gives an impetus which unites research teams formerly specialized in different cultivated plants.