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T-DNA Tagging: A Promising Tool for Functional Genomics in Medicinal Plants

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Nowadays, herbal medicines have been widely used in health care and disease treatment and the share of herbal medicinal products in pharmaceutical market is increasing rapidly in many countries. One of the main resources of active pharmaceutical ingredients of herbal medicines is secondary metabolites, many of which are structurally complex and difficult to synthesize chemically. Thus, increasing the yield of some secondary metabolites through bioengineering approaches is highly desired. However, it is difficult to achieve at present because the genetic background of most medicinal plants is not clear and the genes involved in the biosynthesis of secondary metabolites are largely unknown. Intensive studies on functional genomics in medicinal plants seem to be a key for solving this problem.

The ever-growing research tools for plant functional genomics include large-scale sequencing of expression sequence tags (ESTs) or genomic DNA sequences, DNA chips, two-dimensional polyacrylamide *gel* electrophoresis (PAGE), mutagenesis-based methods, and so on [1]. EST sequencing is a rapid and relatively economic tool for transcriptome analysis. Through EST sequencing and subsequent computational analysis, some key enzyme genes involved in the biosynthesis of secondary metabolites have been identified and characterized in various medicinal plants, such as *Glycyrrhiza uralensis* [2], American ginseng [3], *Ginkgo biloba* [4], *Digitalis purpurea* [5], *Panax notoginseng* [6], *Euphorbia fischeriana* [7], *Bupleurum chinense* [8], *Camptotheca acuminate* [9], *P. ginseng* [10], *Salvia miltiorrhiza* [11,12], and *Taxus cuspidate* [13]. Whole-genome sequencing is the other effective tool for functional genomics. Whole-genome sequence analysis, combined with gene expression pattern analysis and systemic evolution analysis, has been successfully used to reveal forty genes involved in terpenoid biosynthesis in *S. miltiorrhiza* at a genome-wide level [14]. cDNA microarray, a hybridization-based technique, is an alternative way for analysis of plant genes. This method has been used for the identification of tanshinone biosynthesis-related genes in *S. miltiorrhiza* [15].

Genome-wide mutagenesis is a direct route to determine the function of a gene product *in situ* [16]. It is usually induced by radiation, chemicals, T-DNA or transposons, of which T-DNA and transposons are more attractive and have been used more often than radiation and chemicals in recent studies. This is because T-DNA and transposons can generate mutants tagged with known fragments. It makes the insertion sites in the genome be trackable, which is very convenient for gene identification. Transposons have been successfully used to generate mutant populations for various plant species, such as *Arabidopsis* and rice, and have been proved to be an effective tool for gene function verification [17]. However, the 'jumping' frequency of transposons is significantly varied among plant species, which results in the inability to control transposon activity in some plant species [18]. Additional disadvantages of transposon tagging include that the operative vectors of transposon tagging are limited to some plant species and the insertion of transposons in plant genome is not very stable. Therefore, T-DNA-mediated mutagenesis appears to be more suitable for generation of large-scale mutant populations of medicinal plants.

In the past years, the genome-wide T-DNA tagging strategy has been successfully employed in constructing mutant populations for various plant species, such as *Arabidopsis*, rice, maize, sorghum, soybean, tomato, et cetera, and has been proved to be powerful in elucidating gene functions through the generation of knockout mutants, activation-tagged transgenics, and promoter or enhancer trap lines [19,20]. Various web-based databases for T-DNA-tagged mutants have been constructed for two model plants, *Arabidopsis* and rice. The phenotypes of mutants and T-DNA insertion sites in the genome are available on the web. It has greatly accelerated systematic analysis of gene functions in *Arabidopsis* and rice. Thus, T-DNA tagging must be a very useful tool for the analysis of gene functions in medicinal plants. However, there is only a few reports on T-DNA-tagged mutants of medicinal plants and the mutant populations generated are small [21]. It could be due to the lack of whole-genome information and the unavailability of T-DNA-mediated transformation systems for most medicinal plant species. Other reasons probably include the costliness, time-consumingness and laboriousness in the generation of saturated mutant population and subsequent mutant screening and gene identification.

Although it is a herculean task, application of the T-DNA tagging technique in the studies on medicinal plant functional genomics is very attractive. This is particularly true with more and more genomes of medicinal plants decoded using the next-generation sequencing techniques and highly efficient transformation systems established for medicinal plants [21,22]. In addition, the development of some new methods, such as the rolling circle amplification-mediated hairpin RNA (RMHR) library construction technique, will greatly improve the efficiency of T-DNA tagging [23]. Thus, application of the T-DNA tagging technique in medicinal plant functional genomics is also practicable. Since one can expect tremendous outcomes once large-scale mutant populations are generated for medicinal plants and valuable information of gene functions are obtained, it is reasonable to believe that T-DNA tagging is a promising tool for functional genomics in medicinal plants.

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