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Gene Tagging Strategies in Plants

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INTRODUCTION

Gene tagging refers to a broad range of approaches that involve the identification of an existing DNA sequence or the introduction of a new DNA that can act as a label/tag for the gene of interest. More specifically, it refers to the identification and marking of a particular gene which is responsible for any beneficial output by inserting a foreign DNA that can act as a tag or label for the gene of interest (Tagu and Moussard 2006). In general, gene tagging relies on the use of a foreign DNA sequence capable of inserting itself into different places in the genome, in a given gene or close to it, can act as a tag by way of affecting the function of the gene in which it is inserted and causing mutation in it. Several tagging strategies are also used to assess protein expression, localization and function. Here, we summarize some important gene tagging approaches that are either being used or could possibly be utilized in plants.

T-DNA tagging: Genome wide mutagenesis is an approach to determine the function of a gene *in situ* and is generally induced by chemicals, radiation, T-DNA, transposons, etc. T-DNA tagging strategy, which utilizes the T-DNA of Agrobacterium as an insertional mutagen, has been successfully employed in several plant species such as Arabidopsis, rice, maize, sorghum, soybean, tomato, etc., for elucidating functions of genes through generation of knockout mutants, activation-tagged transgenics, and promoter or enhancer trapped lines. Different types of T-DNA tags are available that can be used to tag promoters, enhancers, exons or introns (Walden 2002).

Transposon tagging: In case of transposon based gene tagging, a transposon sequence is used to identify DNA sequence adjacent to the transposable element. It is an efficient tool to isolate recessive loss of function and dominant gain of function mutations and isolate plant genes by identifying a mutation tagged by a DNA insertion.

Transposable elements move from chromosome to chromosome or plasmid to chromosome, or chromosome to plasmid. Transposons can cause mutation through insertional inactivation by incorporating into the coding regions or regulatory sequences. However, if a transposable element consists of an enhancer sequence, integration of that element in an intergenic location can lead to overexpression of the flanking genes. Generally two types of transposable elements are found in plants, autonomous and non-autonomous elements. An autonomous transposon element (Activator or Ac) can encode its own transposase and is self-mobile while a defective or deleted version of it is non-autonomous (Dissociator or Ds) which requires the presence of Ac elements for transposition. The maize *Ac*/*Ds* transposon system has been successfully applied in many species for transposon based tagging (Overduin 1994).

Activation tagging: Activation tagging, an approach that complements conventional insertional mutagenesis, uses a T-DNA or transposon to generate gain of function mutations. Gain-of-function phenotypes can either be caused by mutations in the coding region that lead to constitutive activation of the resulting protein, or by mutations that alter levels or patterns of gene expression. Activation tagging results in upregulation of gene expression by placing strong promoters or transcriptional enhancers (viz. multimeric CaMV 35S enhancers or promoters) near genes, facing outwards (Jeong et al. 2002). A complete CaMV 35S promoter pointing outward from a transposable Ds element has been used to identify dominant mutations at the *Arabidopsis* loci tiny, late elongated hypocotyl (LHY) and short internodes (SHI) (Wilson et al. 1996). The Ac/Ds transposon based insertional construct for activation tagging carries an Ac element along with a marker gene for its detection in the genome, a Ds element which consist of a 35S multimeric enhancer or a strong promorter and a marker gene for detection of Ds element in the genome. Integration of the Ds element allows identification and characterization of the flanking gene(s) because of the upregulation of the expression of the gene(s) due to the presence of the enhancer, which acts as a 'tag' (Johnson et al. 2007).

Epitope tagging: Epitope tagging is a method for rapid and effective characterization, purification and *in vivo* localization of protein products of cloned genes. The process involves inserting a polynucleotide encoding a short continuous epitope into a gene of interest and expressing the gene in an appropriate host (Fritze et al. 2000; Jarvik et al. 1998). Tagging a protein with an existing epitope is a simple procedure that reduces the time and expense required for isolating and characterizing antibodies against multiple proteins. Genes fused to epitope tags can be easily purified using antibody tags and the method can be efficiently used for detecting low abundance and poorly immunogenic proteins for which antibodies are not available. Such tags are used for immunochemical detections, biochemical manipulations and live imaging.

Molecular marker based gene tagging: Molecular marker based gene tagging is used for identification of desired gene(s) or quantitative trait loci (QTLs) closely linked to a known molecular marker. A molecular marker is a short sequence of DNA which can tag a gene and is mostly used in Marker Assisted Selection (MAS) by tagging for an important trait in breeding programs (Miah et al. 2013). Gene tagging followed by MAS of desired genes is the prerequisite for molecular breeding strategies. Several molecular tags including restriction fragment length polymorphism (RFLP) tags, random amplified polymorphic DNA (RAPD) tags, microsatellites, minisatellites etc. have been identified and utilized for molecular breeding in various plant species.

CRISPR assisted gene tagging: The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) based genome editing technology utilizes the CRISPR-associated 9 (Cas9) nuclease to disrupt genes for loss-of-function analysis, or to introduce or reverse genomic polymorphisms. It is a simple,

efficient and powerful tool to modify any sequence of interest and thus offers a precise route for site specific tagging of endogenous genes. This system is highly useful for tagging genes with a fluorescence marker or tag peptides. Fusing a fluorescent protein sequence to an endogenous gene helps in tracking subcellular locations and real-time monitoring of expression and dynamics of the protein which cannot be accomplished by conventional techniques (Xiang et al. 2019). Additionally, CRISPR-assisted insertion tagging (CRISPaint) allows precise and efficient site-specific insertion of large heterologous DNA cassettes into eukaryotic genomes at user-defined genomic locations (Schmid-Burgk et al. 2016).

CONCLUSION

Gene tagging comprises a range of techniques widely used for identification, isolation and characterization of gene(s). Among the various approaches, insertional mutagenesis through T-DNA tagging and transposon tagging are being used extensively in several plants. Activation tagging systems have further emerged as an important strategy through which important genes implicated in plant development; metabolism, stress tolerance and disease resistance have been identified. In addition, functional tags have proved to be extremely valuable tools for detection of expression and functional manipulation of genes.

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