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MAT vector: a system for production and selection of marker free transgenic plants- A survey and summary

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ABSTRACT: One of the most important transformation methods is the oncogenes (ipt, rol, iaaM/H) of *Agrobacterium* which are used as a selectable markers. In MAT (Multi Auto Transformation) Vector System used the oncogenes of *Agrobacterium* to select transgenic plants. But there is a problem because the oncogenes cause abnormal phenotypes in the transgenic plants. Thus, the MAT vector with the site- specific recombination system R / RS to removed these genes (oncogenes) from transgenic plants after transformation and then produce of normal phenotype in transgenic plants. In this review article, we have evaluated the MAT vector system as a transformation method in plants to generate marker- free transgenic plants.

INTRODUCTION

The main part of the cloning process for transformation is the used of vectors that carry the genes into the host cells and these are responsible for replication independently of the host cells (Minlong *et al.* 2000).transformation vectors are plasmids that have been specifically designed to the generation of transgenic plants. The most commonly used transformation vectors are termed binary vector or co-integrated vector because of their ability to replicate in bacteria (Zelasco *et al.* 2007).

Agrobacterium tumefaciens causes crown galldisease in wide range of plants such as apple, pear and peach. Most of these plants are included Dicotyledonous. *A. tumefaciens* is included Ti Plasmid and part of the Ti plasmid is that called T- DNA. Then, by introduce new genes into the T-DNA we have developed a suitable vector to be used as a tool for transformation (Silvia *et al.* 2007).

To identify transform cells, can be used from selectable markers that inserted into the vectors. A selectable marker is a gene that confers a new trait in transgenic cells. This feature is not available in non-transformed cells (Khan *et al.* 2006).

Today, one of the objections against genetic engineering is the possible harmful effects marker genes in plant transformation vectors for transformation (Li *et al.* 2013). For solving this problem, the MAT vector system is designed for transformation by *Agrobacterium* to use the Oncogene as a selection marker to identify transgenic cells and after the transformation process with the site- specific recombination system R / RS and to remove the cancer phenotype and thus it can help us to generate marker-free transgenic plants (Fig.1) (Ebinuma and Komamine 2001).

MATERIALS AND METHODS

In general, MAT vectors could be divided into two main categories: 1. MAT vector based on ipt gene activity. This vector is used to transformation by *Agrobacterium tumefaciens*. Ipt gene encoding the isopentenyltransferase that it is involved in the catalyzes cytokinin synthesis and leads to proliferationand differentiation of adventitious shoots. Soit could be used as a selection marker for selecting regenerated transgenic cells in plants. 2. MAT vector based on rol gene activity. This vector is used to transformation by *Agrobacterium rhizogenes*. Rolgenes into three groups A, Band C. these genes are involved micropropagation of hairy roots by increasing auxin concentration (Ebinuma *et al.* 2004). After the gene transformation, the transgenic plants exhibit abnormal phenotypes such as reduced apical dominance and folded leaves. MAT vectors should be able to solve this problem. Removal method of selective marker genes includes the following steps: in the MAT vectors with the site- specific recombination system R / RS to removed of oncogenes from transgenic plants cells. The Recombinase gene (R) will be catalysis are combination system. In other words, R gene is separated recombinant DNA fragments and selection markers with repetitive sequences of 34bp that it is called Repeat Sequence (RS).

To apply this system, we need to binary vector for cloning. The first vector containing a selection marker gene that designing with it is transferred to the plant and the second vector contains the recombinase gene. After transformation, expression of recombinase gene leads to the separation of selection marker from plant genome (Fig.2) (Zhao *et al.* 2015).

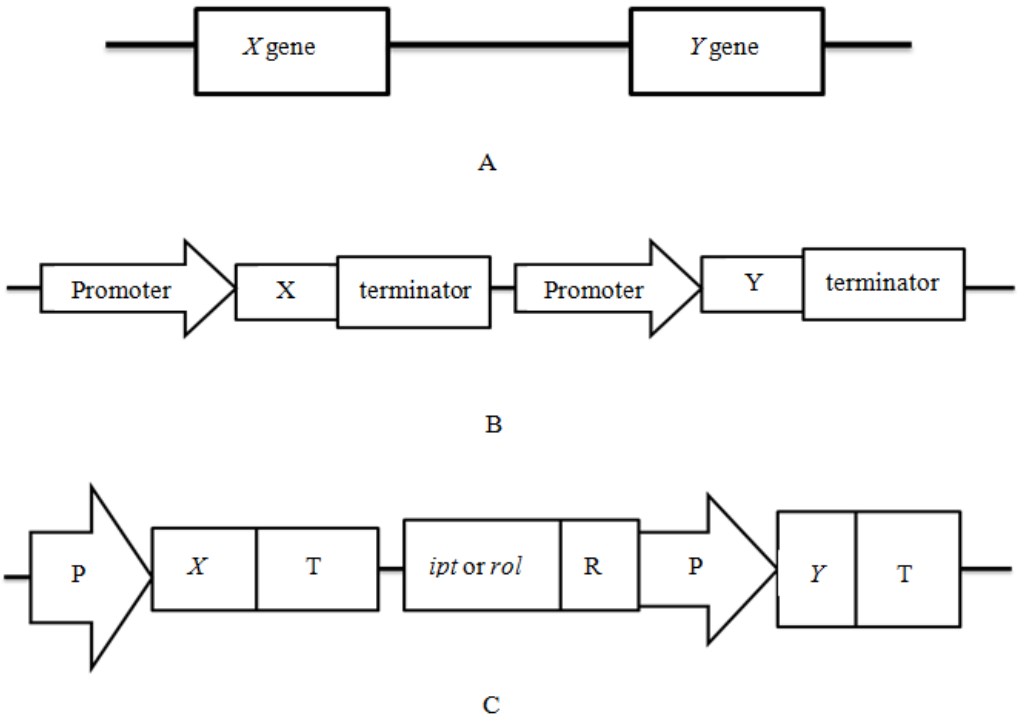

Fig. 1. Marker free gene via MAT vector system: A: integration of transgenes into the wild type genome B: After transformation with the designed vector C: after the recombination that a marker free was obtained.

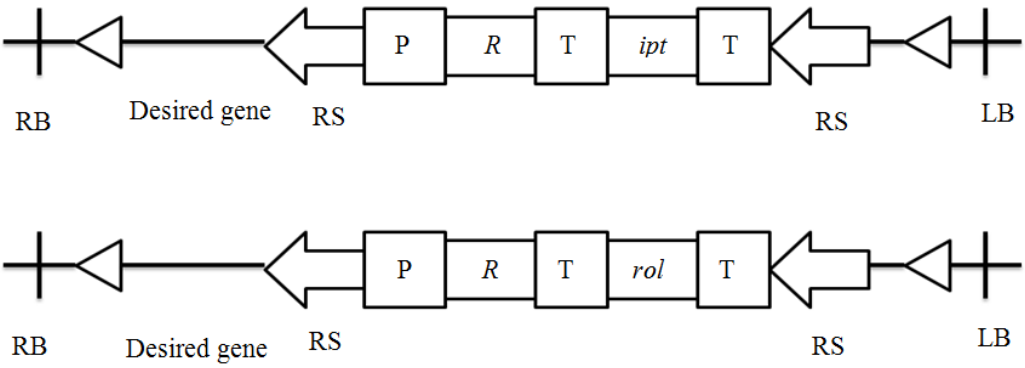

Fig. 2. MAT vector diagram for ipt and rol genes. P: promoter R: Recombination T: Terminator RS: Repeat Sequence RB: Right Border and LB: Left Border.

The application of these vectors in transformation to plants such as tobacco, aspen, rice, white poplar, *Nierembergia caerulea*, lily, eggplant, *Antirrhinum majus* and Snapdragon. PMAT8 is a MAT vector that it is based on ipt gene activities. It is used to generate marker-free transgenic tobacco. PMAT8 containing ipt and R genes with a inducible promoter of the glutathione-S-transferase from corn (*Zea mays*) (JAN and SHINWARI).

Other vectors is pRBI11 that it is based on ipt gene activities.pRBI11 is suitable for the production of transgenic poplar with out sexual crosses. One of the other vectors of this groupisPNP130GFP that it is used for the production of marker-free transgenic rice in a region with high frequency (Khan *et al.* 2014).

Other vector is PNP1702 that it is is based on rol gene activities. In this way, plants were infected with *Agrobacterium rhizogenes*. It is used for the production of marker-free transgenic tobacco and snapdragon (Darwish *et al.* 2014).

CONCLUSION

In conventional transformation systems selected marker genes are essential for the introduce of genes with high economic value to plants and they are very important for the selection of transgenic plants. For example, antibiotics or herbicide genes are selectable markers that they are required to select non-transgenic and transgenic cells. but often these markers leads to negative effects in transgenic or non- transgenic cells. For example, these genes are destroyed non- transgenic cells or may also have negative effects on the development of transgenic cells. Until now, after transformation many selected marker genes remained in transgenic plants. So this selection methods have two major disadvantages: 1. It reduces the ability of plant cells to proliferation and differentiation 2. The uncertainty of the amount of their negative effects on the environment. Recently, number of gene transformation systems are used to produce marker-free transgenic plants. One of these systems is MAT vector which it uses from ipt and rol genes as selectable markers and use of this system has highly successful in the production of marker-free transgenic plants such as tobacco, aspen, rice and white poplar. Ipt gene has a number of characteristics that make it suitable as an alternative to conventional selective markers. These include 1. Cytokinin production during cell division and its differentiation in transgenic plants 2. Regeneration of transgenic plants directly and exhibit phenotypes such as reduced apical dominance and rooting ability. But genes related to current selected markers can be used to select transgenic plants that they are containing genes for antibiotic resistance but they are not suitable for the selection of transgenic plants without genes resistance.

In general, in this paper, we have introduced a transformation method called MAT vector system Because with this system it is possible to produce transgenic plants marker free with high efficiency. Also MAT vector system does not require to selectable markers or Sexual crosses to produce marker free transgenic plants so this method helps to save time.

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