Agricultural Research & Technology
Open Access Journal
ISSN: 2471-6774

Juniper Publishers
key to the Researchers

Mini Review
Volume 8 Issue 2 - July 2017
DOI: 10.19080/ARTOAJ.2017.08.555734

Agri Res & Tech: Open Access J

Apical Meristem-Targeted In Planta Transformation Strategy: an Overview on its Utility in Crop Improvement

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Submission: June 23, 2017; **Published:** July 17, 2017

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Abstract

Transgenic technology has provided an unprecedented boost to plant biotechnology by the development of various methods to engineer plants with desired traits. Though plant breeding has been successful, trait transfer is limited to different genotypes within the same species, thus highlighting the need of techniques to engineer the plants for greater agronomical value. One such technique has been the non-tissue culture-based In planta transformation strategy to engineer recalcitrant crops that are difficult to regenerate. In this review, we have focussed on the usefulness of one of the In planta transformation protocols that targets the T-DNA to differentiating shoot apical *meristem*. This protocol proves to be advantageous to generate transgenic plants that can alleviate the effects of both biotic and *abiotic* stresses posed by the environment and help in productivity increase.

Keywords: In planta transformation technique; *Chimeric* plants; Tissue culture; Regeneration; Biotic and *abiotic* stresses

Introduction

Biotechnological approaches for crop improvement have gained momentum following the introduction of transgenics. However, one of the bottlenecks in the successful deployment of genes through transgenesis is the amenability of the target crops to regeneration. Not all economically important crop plants are regeneration-friendly. Such plants are labelled as "difficult to regenerate". As an alternative, transformation strategies that totally avoid or minimise the tissue culture steps came into existence [1]. These strategies are commonly called as 'in planta transformation methodologies' as they surpass the regeneration step. In planta transformation protocols have gained much appreciation in the present days due to the viability and ease in the generation of transgenic plants *in vitro* by following simple techniques that are efficient, quick and tissue culture-independent. These techniques also avoid the laborious, time consuming tissue culture practices for crop improvement and have evidenced the potential of genetic engineering in modifying the crops to enhance crop productivity by accelerating disease resistance, pest resistance and stress mitigation by orienting quality changes in the seeds of the plant.

Though physical gene transfer techniques like gene gun, biolistics, micro-injection have the ability to mediate gene transfer to produce plants with desired traits, these sophisticated techniques require expensive equipment and is skill demanding. The conventional plant transformation methods use the biological transfer agent, *Agrobacterium* [2] the only natural genetic engineer that has the ability to engineer the plant genome. The major disadvantage of tissue culture based transformation methods are that they are time consuming, lead to variations that affect both qualitative and quantitative traits of the genetically modified plants and may or may not produce gametes that carry genetic material to the subsequent generations.

Development of a large number of uniform plants *in vitro* in short time, with less labour efforts and minimal reagent requirements are the major advantages of in planta transformation protocols. These methods have been commonly preferred and used for the transformation of genes into recalcitrant plant species. The tissue culture-independent techniques were

first initiated in Arabidopsis thaliana and research with this plant has thrown new insight for the development of high throughput transformation methods referred to as the in planta transformation protocols [3] that avoid/eliminate the plant tissue culture procedures. *Agrobacterium* mediated in planta transformation methods in Arabidopsis [4] such as "clip 'n' squirt" [5] and vacuum infiltration [6] have been successfully used by many researchers. The main advantage of in planta transformation is the ability to produce large number of viable plants that can be screened to achieve transgenic plants which have suffered minimum genetic damage and carry a single insertion event [7,8].

Our group has been working on in planta transformation for the last two decades and we have focussed on the use of differentiating shoot apical meristem for successful transformation [9]. The apical meristem can be considered as the right stage for receiving transgenes as these cells would eventually lead to growth and development of the plant [10] and lead the T-DNA to germ line cells and further into the next generation. *Agrobacterium* infection is directed towards the plumule, cotyledonary node and surrounding regions of aseptically germinated two day old young seedlings (Figure 1). Wounded tobacco leaf extract is added to Winans' AB minimal media to improve the transformation efficiency by increasing the virulence of the vir genes by the phenolic compounds present in the mature tobacco leaves. The seedlings are then transferred to the trays or cups containing autoclaved soilrite and grown in culture chambers for 7-8 days under 16 h photo period. The recovered plants are then transferred to the pots containing soil and grown to obtain mature plants. The seeds of these chimeric plants are then screened in the presence of selective antibiotics to obtain the transformed plants that can be characterised for the presence of the transgene. The methodology helps in the development of a large number of primary transformants. In order to tackle the large number of seeds to be screened in T1 generation, high throughput screening strategies are in place for efficient identification of putative transformants [11- 34].

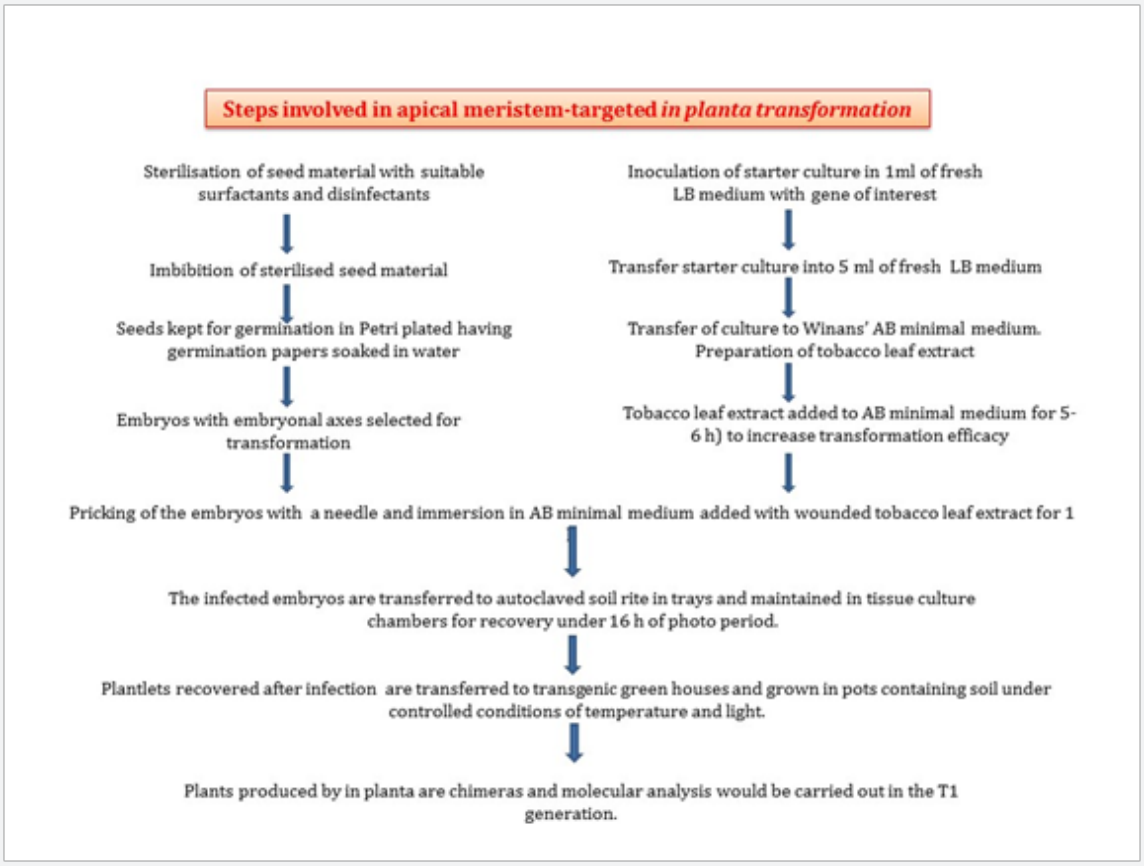

Figure 1: Generalised model of in planta transformation protocol for different recalcitrant crops.

Usefulness of the Apical Meristem-Targeted in Planta Transformation Strategy

Since the conceptualization of the apical *meristem* strategy for transformation, its feasibility has been demonstrated in a wide range of crop species (Table 1). Unambiguous demonstration of the integration and stable inheritance of the T-DNA has been successfully provided using marker genes like *gus* and *gfp*.

Further, the technique has also been used to develop transgenic plants in economically important crops to combat both biotic and *abiotic* stresses (Table 1). The interpretation

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of results from these studies have shown that the transgenic plants do possess superior traits when compared to the non-transformed control plants, thus proving ability of the technique to generate transgenic plants with traits of interest. The methodology can therefore be used to engineer a variety of crop plants of agronomical value by adopting a few modifications and standardisations to the generalised protocol according to the crop species that are to be transformed.

Table 1: List of transgenic crops generated using the apical meristem targeted in planta transformation.

Sl no.	Crop	Gene	Reference
Standardization			
1	Sunflower	uid A	[12]
2	Safflower	uid A	[13]
3	Peanut	uid A	[14]
4	Cotton	uid A	[15]
5	Capsicum	uid A	[16]
6	Capsicum	gfp	[17]
7	Pigeon pea	uid A	[18]
8	Field bean	uid A	[19]
Abiotic stress			
9	Groundnut	epsps	[20]
10	Chick pea	epsps	[21]
11	Rice	AVP1	[22]
12	Rice	AtPCS	[23]
13	Rice	NHX1	[24]
14	Chili	PDH45	[25]
Biotic stress			
15	Groundnut	Cry1X	[26]
16	Chick pea	Cry1AcF	[27]
17	Groundnut	β-1,3-glucanase	[28]
18	Safflower	Chi11	[29]
19	Castor	Cry1AcF	[30]
20	Sunflower	β-1,3-glucanase	[31]
21	Field bean	Cry1AcF	[32]
22	Pigeon pea	Cry1AcF	[33]
23	Sunnhemp	FMDV 1D	[34]

Discussion

The transformation strategy under focus is an addition to the very many non-tissue culture strategies already available. It can be extrapolated to generate stable transgenic plants in both monocots and *dicots*. This technique in spite of its ease has a limitation, the production of chimeras', which can be successfully screened with the adaption of standardised screening protocols. This technique can also be optimised to produce transgenics in crops like wheat that have been reported to have very low transformation efficiency. The production decline due to yield losses caused by insect pests and various environment clues can be mitigated by transferring different genes into the plants using the strategy for management of biotic and *abiotic* stresses. This technology is simple with a good ease of adoption and high rate of success.

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