

Transgenic Plant Production using Different T-DNA Technologies as a Tool of Genetic Engineering

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Abstract

The review study was carried out from different research data to find out the innovative latest technology in the transgenic plant production and its application in Agriculture like plant, fruit and vegetables. Transgenic plant production using T-DNA transformation by tissue culture with *Agrobacterium* mediated media in wild potato, potato, tomato, maize, apple, sweet orange, strawberry etc. has been described well. Transgenic plant production applied by dip, swabbing, drip, injection in xylem, phloem tissue, stem, flower and ovary method has been exhibited as innovative from different research data. In addition to that transgenic melon and squash production have been highlighted from different innovative research data. Moreover, Transgenic okra and hard wood plant production by injection using *Agrobacterium* mediated gene. Finally it can be summarized that transgenic ornamental plant, flower, fruit and vegetable can be produced by using different technologies like in vitro cell or tissue culture of leave disc, shoot and root slice having different concentration of *Agrobacterium tumefaciens* (used as *Ti* plasmid and genomic DNA) media by injecting in the stem, xylem and phloem tissue, bud, flower, ovary as well as swabbing, dipping, dripping and micro spraying technologies.

Keywords - Transgenic plant, T-DNA, genetic engineering, *Agrobacterium* mediated gene, injection.

I. INTRODUCTION

The transgenic plant production keeps a superlative role for plant, fruit and vegetable growth and nutritional quality development. It allows research to reach beyond closely-related plants to find useful traits in all of life's vast resources [1]. A far wider set of genetic resources can be directed toward solving problems and creating opportunities. This type of plant is called genetically modified organism (GMO). It is an organism whose genetic material has been altered using techniques in

genetics generally known as recombinant DNA technology [1]. Transgenic plant biotechnology is known as genetic engineering and bioengineering, a certain or known gene (DNA or RNA) for a desired trait is inserted into a plant cell by injection or swabbing, dipping, drip or micro-spray method [2]. One technique of transgenic plant production is cell grown usually in vitro culture to develop explants or plants in a controlled environment or open field [2]. The transgenic (genetically engineered) plant would express the new trait/characteristics, such as a T-DNA from *agro-bacterium* is isolated and inserted to the leguminous plant cell [1]. The soil bacterium *Agrobacterium tumefaciens* causes crown gall disease in plants by transferring the T-DNA region of a tumor-inducing (*Ti*) plasmid into host cells. The T-DNA region of the *Ti* plasmid can be genetically engineered to contain an antibiotic resistance gene (*kan^R*) as well as a foreign gene of interest. Infection of plant cells in culture with bacteria containing this co-integrated *Ti* plasmid allow the foreign DNA to be transferred into the host cell. Integration of the foreign DNA disrupts tumor formation, and only those plant cells with the *kan^R* gene would grow in culture containing antibiotic. Plants are easily regenerated from cultured cells (calluses): the adult transgenic plant expresses the foreign gene [3, 4]. The objective of the study was to review the various techniques and application in plant, fruit and vegetable from different research data.

II. MATERIALS AND METHODS

A. Plant materials

Potato, maize, tomato, apple, strawberry's data have seen reviewed well. Transfer of DNA (T-DNA) Mechanism (In general) has been shown below:

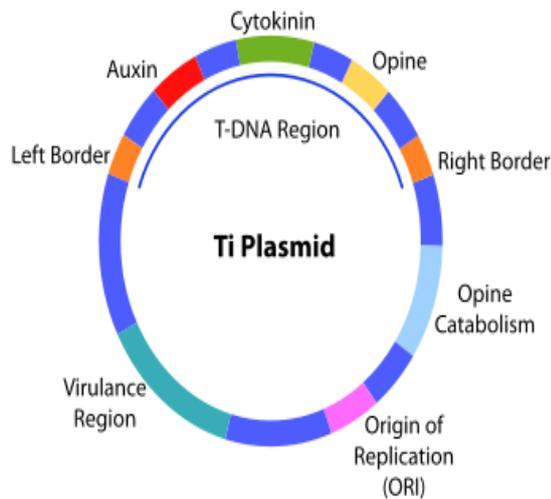


Fig.1. Ti plasmid with T-DNA region [5]

According to the many researchers who explained in other way, the transfer DNA or RNA (T-DNA or T-RNA) is the transferred DNA or RNA of the tumor-inducing (Ti) plasmid of some species of bacteria such as *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes*. It derives its name from the fact that the bacterium transfers this DNA fragment into the host plant's nuclear DNA genome. The T-DNA is bordered by 25-base-pair repeats on each end. Transfer is initiated at the left border and terminated at the right border and requires the *vir* genes of the Ti plasmid. The bacterial T-DNA is about 20,000 base pairs long and contains genes that code for enzymes synthesizing opines and phytohormones. By transferring the T-DNA into the plant genome, the bacterium essentially reprograms the plant cells to grow into a tumor and produce a unique food source for the bacteria. The synthesis of the plant hormones auxin and cytokinin enables the plant cell to grow uncontrollably, thus forming the crown gall tumors typically induced by *Agrobacterium* infection. The opines are amino acid derivatives used by the bacterium as a source of carbon and energy [5, 6].

III. T-DNA transformation by tissue culture

A. T-DNA transformation by tissue culture in Wild potato

Transformation with *Agrobacterium* can be achieved in two ways. Protoplasts or alternatively leaf-discs can be incubated with the *Agrobacterium* and whole plants regenerated using plant tissue culture. *Agrobacterium* is used as a vector to transfer the T-DNA into the plant cells where it integrates into the plant genome. This method can be used to generate transgenic plants carrying a foreign gene.



Fig. 2. Photograph shows *Agrobacterium* transformation. Plant (*S. chacoense*) transformed using *Agrobacterium*. Transformed cells start forming calluses on the side of the leaf pieces [7].

B. Arabidopsis transgenic plant by Dip method

It is a member of the mustard (Brassicaceae) family, which includes cultivated species such as cabbage and radish. A common transformation protocol for *Arabidopsis* is the floral dip method [8, 9]. Inflorescences are dipped in a suspension of *Agrobacterium*, and the bacterium transforms the germline cells that make the female gametes. The seeds can then be screened for antibiotic resistance (or another marker of interest), and plants that have not integrated the plasmid DNA will die when exposed to the correct condition of antibiotic [7].

C. T-DNA mutagenesis in Arabidopsis thaliana (mutation breeding)

The same procedure of T-DNA transfer can be used to disrupt genes via insertional mutagenesis. The inserted T-DNA sequence makes not only a mutation but it also 'tags' the affected gene, thus allowing for its isolation. This method is used widely to study gene function in plants, such as the model plant *Arabidopsis thaliana* (Fig. 3) [10].



Fig. 3. Photograph shows *Arabidopsis thaliana* transgenic plant.

(<http://www.colourbox.com/image/transgenic-plant-of-arabidopsis-laboratory-test-for-modification-image-2005044>) [10].

D. DNA Transformation in Tobacco by tissue culture

It is the process or technique to transfer DNA or RNA or gene from plant to Plant or animal to animal as a whole organism to organism (Fig. 4). It has been stated the following experiment of DNA transformation from tobacco leaves [11].

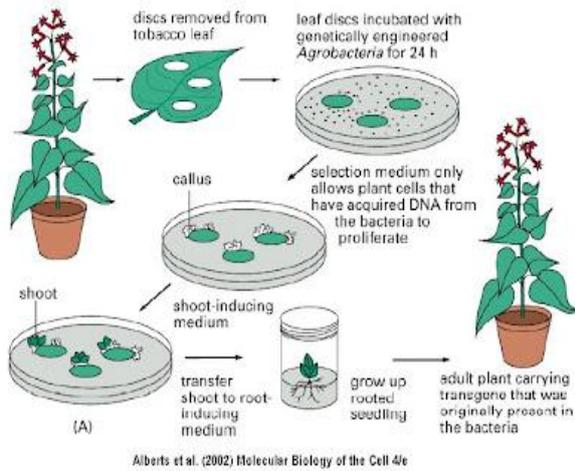


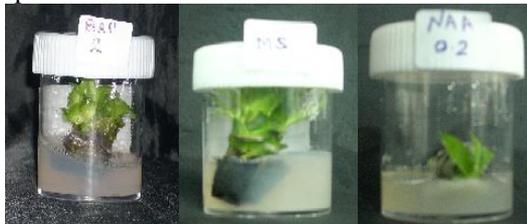
Fig. 4. DNA transformation ([11])

E. Transgenic plant Cell culture/tissue culture

Transgenic Plant Tissue Culture or cell culture is a practice used to propagate plants under sterile conditions, often to produce clones of a plant (Fig. 5) [12]. Different techniques in transgenic plant tissue culture are shown below:



Pineapple crown, Crown slice, Culture in the plastic



Explants after 60 days

Fig 5. Photograph shows the culture from crown of pineapple and subculture from explants at different growth hormones [13].

1. Types or application procedures of Transgenic plant tissue culture

Applications include are given below:

Micro-propagation, Liquid culture in bioreactor, Protoplast fusion, Meristem tip culture, Cell culture, Cell suspension and Somatic embryogenesis [14].

2. Transgenic plants tissue culture Biotechnology

Transgenic plant tissue culture biotechnology can be defined that T-DNA transformation can be done using tissue or cell followed by either short-term testing of genetic

constructs or regeneration of transgenic plants. Transgenic plants biotechnology or Genetically modified crops (GMCs, GM crops, or biotech crops) biotechnology are plants used in agriculture applying technology, the DNA of which has been modified using genetic engineering technology. In most cases the aim is to introduce a new trait to the plant which does not occur naturally in the species. Examples in food crops include resistance to certain pests, diseases, or environmental conditions, reduction of spoilage, or resistance to chemical treatments (e.g. resistance to a herbicide), or improving the nutrient profile of the crop. Examples in non-food crops include production of pharmaceutical agents, biofuels, and other industrially useful goods, as well as for bioremediation [14].

F. Transgenic tomato production

1. Genetically modified tomato

Transgenic tomato, is a tomato that has had its genes modified, using genetic engineering. The first commercially available genetically modified food was a tomato engineered to have a longer shelf life [15]. Currently genetically modified tomatoes are not available commercially, but scientists are developing tomatoes with new traits like increased resistance to pests or environmental stresses. Other projects aim to enrich tomatoes with substances that may offer health benefits or be more nutritious. As well as aiming to produce novel crops, scientists produce genetically modified tomatoes to understand the function of genes naturally present in tomatoes [16].

DNA Plant Technology (DNAP), [17] developed tomatoes that delayed ripening by preventing the production of ethylene,^[18] a hormone that triggers ripening of fruit [18]. All three tomatoes inhibited ethylene production by reducing the amount of 1-aminocyclopropane-1-carboxylic acid (ACC), the precursor to ethylene. DNAP's tomato, called Endless Summer, inserted a truncated version of the ACC synthase gene into the tomato that interfered with the endogenous ACC synthase [18] Monsanto's tomato was engineered with the ACC deaminase gene from the soil bacterium *Pseudomonas chlororaphis* that lowered ethylene levels by breaking down ACC. [17] introduced an S-adenosylmethionine hydrolase (SAMase) encoding gene derived from the *E. coli* bacteriophage T3, which reduced the levels of S-adenosylmethionine, a precursor to ACC (Fig.6).



Fig. 6. Tomatoes that contain the bioengineered ACC synthase gene [19].

2. Transgenic tomato by *bt* gene

Transgenic tomato (cv. *Money maker*) over expressing *Bt* (*Cry 2Ab*) gene was produced using *Agrobacterium*-mediated transformation method. Molecular and biochemical analysis confirmed the expression and integration of the transgene into tomato genome. These results indicate that a significant amount of *Bt* protein was present in all of the transgenic lines and that plants expressing *Cry 2Ab* gene could be used for management of the target lepidopteran insect pests endemic [20].

2.1 Plant transformation

According to the Saker et al [20], seeds of tomato was used. The seeds were surface sterilized by soaking for 1 min in 70% ethanol, washed with sterilized distilled water, and then soaked in 20% commercial Clorox (5.25% sodium hypochlorite) with two drops of Tween 20 for 10 min, and then rinsed with sterilized distilled water for several times (5 min per rinse). Seeds were allowed to germinate on half strength MS medium containing 15 g/l sucrose and 8 g/l agar. pH was adjusted to 5.8 with 1 M KOH prior to autoclaving. The transformation procedure was carried out as described by McCormick et al. Briefly, cotyledon explants of 7–10 day-old seedlings were immersed in a bacterial culture suspension with gentle agitation, blotted to sterilized Whatman paper. The blot-dried explants were placed on co-cultivation medium containing; 4.3 g/l MS medium, 60.0 g/l sucrose, 10.0 g/l glucose, 2.5 mg/l BA, 1.0 mg/l IAA, 3.0 g/l gel right and 100 μ M acetosyringone, and incubated in dark for 2 days at 25 ± 2 °C. After co-cultivation, explants were washed off from bacterial overgrowth using sterilized distilled water containing 500 mg/l cefotaxime. The washing process was repeated three times and then blotted to sterilized Whatman paper. The cotyledonary leaf discs were transferred (placed upside-down) on selection medium fortified with 4.3 g/l MS salts, 2.5 mg/l BA, 1.0 mg/l IAA and 15 mg/l hygromycin B and 300 mg/l cefotaxime. Proliferated shoots (2 cm high) were excised from the mother explants and transferred to rooting medium supplemented with 0.5 mg/l of IAA and 15 mg/l of hygromycin B. Tissue culture-derived tomato plantlets with good developed roots (about 11–14 cm in height) were acclimatized to *ex vitro* conditions [20].

2.2 Production of transgenic tomatoes

The obtained results indicated that about 50% of explants with *Agrobacterium* proliferated shoots on selective medium containing 2.5 mg/l BA, 0.1 mg/l IAA,

300 mg/l cefotaxime and 15 mg/l hygromycin B [20].

G. Transgenic maize

It has been reported that Bt corn is a variant of maize that has been genetically altered to express one or more proteins from the bacterium *Bacillus thuringiensis*. *Bt* hybrids containing the *CryIA(b)* gene under the control of green tissue and pollen-specific promoters showed a significantly higher percentage of damaged ears than *Bt* hybrids carrying the *CryIA(b)* gene under the control of a constitutive promoter (Mon810) [21].

2. Transgenic maize plants by tissue electroporation

Scientists did an experiment on the transformation of regenerable maize tissues by electroporation [22]. They reported that in many maize lines (Fig.7), immature zygotic embryos gave rise to embryogenic callus cultures from which plants was regenerated. Immature zygotic embryos or embryogenic type I calli were wounded either enzymatically or mechanically and subsequently electroporated with a chimeric gene encoding neomycin phosphotransferase (*neo*). Transformed embryogenic calli were selected from electroporated tissues on kanamycin-containing media and fertile transgenic maize plants were regenerated. The neogene was transmitted to the progeny of kanamycin-resistant transformants in a Mendelian fashion. This showed that all transformants were nonchimeric, suggesting that transformation and regeneration are a single-cell event.



Fig. 7. Transgenic maize plants by tissue electroporation [22].

H. Transgenic potato production

Transgenic potato shoots within 4 weeks from the time of initial inoculation of leaf explants by *Agrobacterium tumefaciens* has been established with the *Solanum tuberosum* subspecies *andigena* [23]. To produce callus from leaf explants in 7 days, basal medium was supplemented with optimized concentrations of benzyl-aminopurine and naphthalene acetic acid. Incubation on basal medium supplemented with a combination of zeatin riboside, naphthalene acetic acid, and gibberellic acid induced shoot formation from callus after 28 days of incubation (Fig.8). In this improved protocol, the commonly used pre-

culture of explants with nutrient medium was eliminated and the *Agrobacterium* inoculation medium was not supplemented with any phytohormones. Induction of roots from putative transformed shoots was achieved in hormone-free basal medium supplemented with kanamycin. Normal, healthy root formation was observed within 5 days and 91% of the selected shoots rooted on kanamycin. By using RT-PCR analysis with gene specific primers, all rooted shoots out of 20 selected from five different lines exhibited expression of the full-length *StBEL5* transgene driven by the CaMV 35S promoter [23].

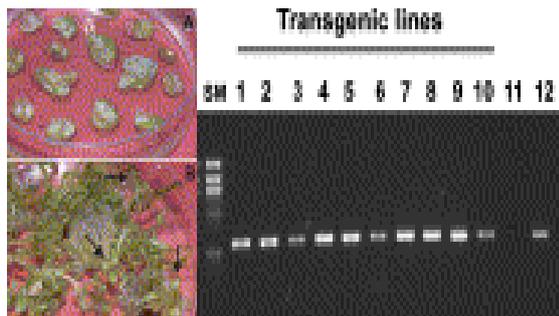


Fig.8. Transgenic potato shoots and transgenic line [23].

1. Transgenic Apple production

According to the Murata et al [24] transgenic apple shoots were prepared from leaf disks by using *Agrobacterium tumefaciens* carrying the kanamycin (KM) resistance gene and antisense polyphenol oxidase (PPO) DNA. Four transgenic apple lines that grew on the medium containing 50 microgram/mL KM were obtained. They contained the KM resistance gene and grew stably on the medium for >3 years. Two transgenic shoot lines containing antisense PPO DNA in which PPO activity was repressed showed a lower browning potential than a control shoot.

2 Transgenic apple by breeding

The genus *Malus* contains up to 122 species [25]. Cultivated apples have been developed mainly from the hybrid species *M. x domestica* Borkh. which has the genetic background of several wild species [25]. Apple breeding has traditionally been carried out by crosspollination and subsequent selection of superior seedlings in which desirable characters have been combined. In the past three decades, two thirds of apple cultivars released in the world have originated from five parents [26]. Because only a small gene pool has been used for apple breeding, many desirable characters (e.g., fungal and pest resistance genes) are not present in the genomes of commercially important apple cultivars.

I. Transgenic straw berry

It has been stated that the genes directly into strawberry had been successfully introduced [27]. The study presented was the first report where transgenic plants of strawberry tolerant to salt stress were produced. Leaf discs of *in vitro* grown plantlets of strawberry (cv. Chandler) were used as explants and *Agrobacterium tumefaciens* strain GV2260 harbouring osmotin gene under the control of CaMV 35S promoter in a binary vector system (pBinAR) was used in co-cultivation experiments. Expression of osmotin gene was confirmed in transgenic lines T_{L3}, T_{L5}, T_{L9} using northern hybridization, while biochemical analyses of these transgenic plants revealed enhanced levels of proline, total soluble protein and chlorophyll content as compared to the wild plants. Leaf disc senescence assay showed that these transgenic lines were tolerant to salt stress [27].

J. Transgenic melon and squash production

It has been reported that transgenic melon and squash containing the coat protein (CP) gene of the aphid transmissible strain WL of cucumber mosaic cucumovirus (CMV) were grown under field conditions to determine the spread of the aphid non-transmissible strain C of CMV [28]. Transgenic melon were susceptible to CMV strain C whereas transgenic squash were resistant although the latter occasionally developed chlorotic blotches on lower leaves. Transgenic squash line ZW-20, one of the parents of commercialized cultivar Freedom II, which expresses the CP genes of the aphid transmissible strains FL of zucchini yellow mosaic (ZYMV) and watermelon mosaic virus 2 (WMV 2) potyviruses was also tested. Line ZW-20 was resistant to ZYMV and WMV 2 but was susceptible to CMV. They also reported that field experiments conducted over two consecutive years showed that aphid-vectored spread of CMV strain C did not occur from any of the CMV strain C-challenge inoculated transgenic plants to any of the uninoculated CMV-susceptible non- transgenic plants[28].

K. Transgenic squash resistance to three viruses

It has been suggested that the risk of escape of a gene construct that confers resistance to three viruses (ZYMV, WMV, CMV) from a transgenic zucchini (*Cucurbita pepo*) to *Cucurbita argyrosperma* ssp. *sororia*, a wild relative at the center of origin of the genus in Mexico [29]. They experimentally generated first and second generation hybrids, as well as backcross progeny (BC), and evaluated their performance. The virus-resistance transgene was successfully inherited into both hybrid generations and also to BC progeny from the crosses of hybrids with the wild relative. The transgene generally followed Mendelian inheritance as a dominant trait. Both hybrid

generations and the BC progeny had lower reproductive output compared to the wild parent. They reported that the hybrid and BC progeny were viable and fertile, the escape and persistence of the transgene is possible via wild populations of *C. argyrosperma* ssp [29].

L. Transgenic inbred squash resistance to mosaic virus

It has been suggested that transgenic inbred squash lines containing various combinations of the cucumber mosaic virus (CMV), watermelon mosaic virus 2 (WMV 2) or zucchini yellow mosaic virus (ZYMV) coat protein (CP) genes were produced using *Agrobacterium*-mediated transformation [30]. They reported that progeny from lines transformed with single or multiple CP gene constructs were tested for virus resistance under field conditions, and exhibited varying levels of resistance to infection by CMV, WMV 2 or ZYMV. Most transgenic lines remained nonsymptomatic throughout the growing seasons and produced marketable fruits, while other lines showed a delay in the onset of symptoms and/or a reduction in symptom severity.

They also mentioned that transgenic lines transformed with a double CP construct containing the CP genes from CMV and WMV 2, designated CW, displayed high level of resistance to CMV and WMV 2. A transgenic line, designated ZW-20, which contained the CP genes from ZYMV and WMV 2 displayed excellent resistance to ZYMV and WMV 2 in that most of the plants showed complete resistance. Transgenic line CZW-3, transformed with the triple CP construct containing the CMV, WMV 2 and ZYMV CP genes, exhibited resistance to all three viruses [30].

M. Transgenic golden rice

It has been reported that Golden Rice is a transgenic variety of rice, with genes for the synthesis of β -carotene taken from the temperate garden favourite *Narcissus pseudonarcissus* (daffodil) and inserted into the genome of a temperate strain of rice, using *Agrobacterium tumefaciens* as the vector, to effect the transfer [31]. The gene construct also contains some genes for enzymes of the biosynthetic pathway of β -carotene, from another bacterium *Erwinia uredovora*. The grains of this Genetically Modified (GM) rice are similar to other varieties, in their crinkly and scabrous husks but the core of the grain is pale yellow, instead of pearly white. The colour is due to β -carotene, which makes this the Golden Rice, more so because β -carotene is very important to our health.

N. Transgenic sweet orange

It has been stated that genetic transformation with genes that code for antimicrobial peptides has been an important strategy used to control bacterial diseases in fruit crops, including apples, pears, and citrus [32]. Asian citrus canker (ACC) caused by *Xanthomonas citri* subsp. They reported that the production of genetically transformed Natal, Pera, and Valencia sweet orange cultivars (*Citrus sinensis* L. Osbeck) with the insect-derived *attacin* A (*attA*) gene and the evaluation of the transgenic plants for resistance to *Xcc. Agrobacterium tumefaciens* Smith and Towns-mediated genetic transformation experiments involving these cultivars led to the regeneration of 23 different lines.

They reported that genetically transformed plants were identified by polymerase chain reaction, and transgene integration was confirmed by Southern blot analyses. Transcription of *attA* gene was detected by Northern blot analysis in all plants, except for one Natal sweet orange transformation event. Transgenic lines were multiplied by grafting onto Rangpur lime rootstock plants (*Citrus limonia* Osbeck) and spray-inoculated with an *Xcc* suspension (10^6 cfu mL⁻¹). Disease severity was determined in all transgenic lines and in the control (non-transgenic) plants 30 days after inoculation. Four transgenic lines of Valencia sweet orange showed a significant reduction in disease severity caused by *Xcc*. These reductions ranged from 58.3% to 77.8%, corresponding to only 0.16–0.30% of leaf diseased area as opposed to 0.72% on control plants [32].

O. Transgenic ladies finger and hard wood plant production by injection and swabbing

Transgenic okra and legume crop can be produced by a certain or known gene (DNA or RNA) like *Agrobacterium tumefaciens* (*ti plasmid*) for a desired trait which is inserted into a okra plant cell by injection (like stem injection, flower injection, ovary injection or xylem injection) and swabbing in hard wood plant (it can be done on the phloem tissue after a bit phloem cut, with a syringe and needle or cotton putting on the top of the syringe [33]. In addition to that injection can be employed on the xylem tissue like in the xylem tissue of wax apple (Fig. 9).

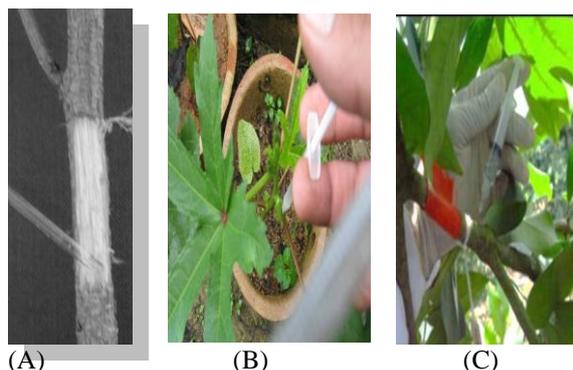


Fig.9. Swabbing method of T-DNA (A) and flower injection of T-DNA (B), injection in the shoot (C)

IV. CONCLUSION

It can be concluded that ornamental plant, fruit and vegetable can be produced by transgenic technology using seed soaking, in vitro culture of leave, shoot and root using different concentration media of *Agrobacterium tumefaciens* (Ti plasmid, genomic DNA) by injecting in the stem, xylem and phloem tissue as well as swabbing, dipping, dripping and micro spraying methods.

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