Genetically Modified Plant (GMO) Using Hormone and T-DNA Technology: Regulated Gene Expression

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Abstract

Genetically modified (GM) technology retains a superlative role to produce GM organism (GMO) and GMO derived food. The review study was carried out employing different innovative research data to attain the latest modern technology in the GMO production like plant, fruit and vegetables and its related gene expression. GMO production using hormones (GA_3, IAA, NAA, ABA) and T-DNA/gene transformation in ornamental plant, fruit and vegetables has been described well. GMO using hormonal injection technology, bacteria like Bacillus thuringiensis, tissue culture with Agrobacterium mediated media, cross breeding, inter-stock breeding, swabbing in xylem and phloem technology has been exhibited as innovative from different research data in pumpkin, ladies finger, peach plant, rose, carrot, star fruit, tobacco, tomatoes and S. chacoense plant has been highlighted from different innovative research data and regulated gene expression was documented well. Finally it can be summarized that GMO ornamental plant, flower, fruit and vegetable can be produced by using in vitro tissue culture of leave, shoot and root or in vivo having different concentration of GA_3, IAA, NAA, ABA and Agrobacterium tumefaciens (Ti plasmid, genomic DNA/T-DNA) *media as well as* by injecting in the stem, xylem and phloem, flower, ovary tissue applying swabbing, dipping, dripping and micro spraying methods.

Keywords: *GMO, T-DNA, plant hormone, Agrobacterium, gene expression*

I. INTRODUCTION

Genetically modified (GM) technology keeps a significant act to produce GM organism (GMO) and GMO derived food. GMO is generated by genetic engineering technology. The first GMOs were bacteria in 1973. GM mice were generated in 1974. Insulin-producing bacteria were commercialized in 1982 and genetically modified food has been sold since 1994 [1]. Genetically modified organism (GMO) is the process of producing any organism whose genetic material has been altered using genetic engineering techniques (i.e. genetically engineered organism). GMO is the source of plant, animal, medicines and genetically modified foods, also widely used in scientific research and to produce other goods [2]. GMO is also considered as transgenic Organism. This is an organism whose genetic makeup has been altered by the addition of genetic material from other related or unrelated organism [3]. At first farmers had widely adopted GM technology. Between 1996 and 2013, the total surface area of land cultivated with GM crops increased by a factor of 100, from 17,000 square kilometers (4,200,000 acres) to 1,750,000 km2 (432 million acres) [*Büttner-Mainik,2011*] 10% of the world's croplands were planted with GM crops in 2010 [2, 4]. In the US, by 2014, 94% of the planted area of soybeans, 96% of cotton and 93% of corn were genetically modified varieties [2]. In recent years GM crops expanded rapidly in developing countries. In 2013 approximately 18 million farmers grew 54% of worldwide GM crops in developing countries [5].

Genetic modification involves the mutation, insertion or deletion of genes. Inserted genes usually come from a different species in a form of horizontal gene-transfer. In nature this can occur when exogenous DNA penetrates the cell membrane for any reason. This can be accomplished artificially by a. attachment of the genes to a virus, b. the DNA can be inserted into the nucleus of the intended host (transgenic organism), c. using electroporation, d. Firing small particles from a gene gun, e. hormonal treatment as mutation breeding, f. cell and tissue culture, g. cross breeding, e. grafting and dwarfism [6, 7].

Other methods have been highlighted that natural forms of gene transfer such as the ability of *Agrobacterium* to transfer genetic material to plants or the ability of lentiviruses to transfer genes to animal cells [3, 8]. Genetically modified bacteria are used to produce the protein insulin to treat diabetes. Similar bacteria have been used to produce bio-fuel, [4] human growth hormone to treat various forms of dwarfism. In addition, various genetically engineered micro-organisms are routinely used as

sources of enzymes for the manufacture of a variety of processed foods. The objectives of the review study were to describe innovative technology of genetically modified plant (GMO) using hormone application and T-DNA techniques in plants, fruits and vegetables as well as to evaluate the regulated gene expression.

II. APPLICATION OF GM TECHNOLOGY IN PLANT, FRUIT AND VEGETABLE

A. Seedless vegetable (pumpkin, ladies finger) production by hormone treatment

1. Seedless pumpkin by GA_3

Pumpkin plants (local cultivar) were grown at the experimental Field, University of Malaya [7]. Local cultivar was used. Five plants were used for the concentration of 150ppm GA3 and five plants were used for the control. Injection method by using syringe was used to make seedless pumpkin or reduced seed by flower injection before blossoming (opening the flower). He stated that 96.9% seedless pumpkin (GMO) was found by the treatment of GA3 compared to the control.

Fig. 1 Photo shows the seedless pumpkin by genetic engineering techniques [7].

2. Seedless ladies finger by IAA

It was stated that seedless ladies finger had been produced by the applying of Plant growth hormone like Indole Acetic acid (IAA) at 100 PPM concentration. He reported that the techniques applied were the stem injection and flower injection before flower opening [3].

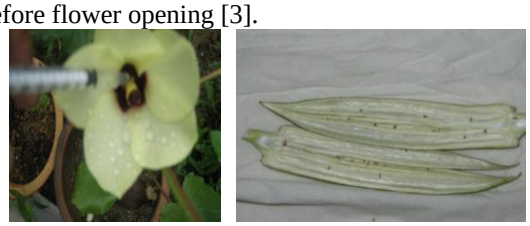


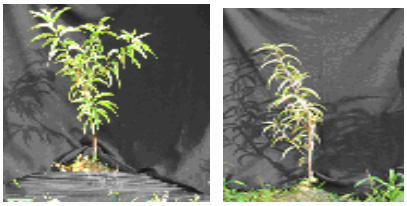
Fig. 2. Injecting hormone solutions into the flower and seedless ladies finger production [3].

3. Seedless star fruit production by hormone treatment

Hossain [6, 7] stated that GA3 150 ppm was the best treatment compared to others showing the 75% seedless as aborted seed which was genetically modified, biggest size (length and diameter) and highest TSS and biochemical content of star fruit. Five branches were used for GA3 150 ppm treatment in one tree by which flower bud were dipped at 3-4 times (twice per week until 2 weeks) with GA3 (150 ppm) during the growing season of the flower bud formation.

B. Dwarf plant production by Abscissic acid as GMO

An experiment had been conducted to know the genetically dwarf of peach tree by growth inhibition hormone [9]. It was reported that by this study, it was possible to produce peach tree greatly dwarfed (small tree size) by using ABA 2000ppm applied to the bark strip of partially ringed trees. In the research technique, bark ringing and growth inhibitor (ABA) were used by swabbing method with cotton to the bark band (strip) (Two and one year trees) surface only. It has been found that this study had dwarfing effect on vigorous peach trees grafted on vigorous rootstocks. From the figure, externally also we can explain that there might be genetically dwarf peach trees produced and we found all shoot growth reduced 95% at 2000ppm ABA. Almost same proportion of root growth was reduced in the case of both concentrations of ABA.



Water control ABA 2000pm

Fig. 3. Inhibition of Peach plant growth leaf, root, shoot and phloem tissue inhibition) as dwarf trees using genetically modified technique by ABA hormone [9].

C. Different GM lower plants produce Biomedicine (biopharmaceuticals)

Simple plants and plant cells have been genetically engineered for production of biopharmaceuticals in bioreactors as opposed to cultivating plants in open fields. Work has been done with duckweed Lemna minor,[10] the algae Chlamydomonas reinhardtii [11] and the moss Physcomitrella patents [*5, 12*] An Israeli company, Protalix, has developed a method to produce therapeutics in cultured transgenic carrot and tobacco cells [13] Protalix and its partner, Pfizer, received FDA approval to market its drug Elelyso, a treatment for Gaucher's disease, in 2012 [13].

D. Production of new color in Suntory rose

After thirteen years of collaborative research, an Australian company, Florigene, and a Japanese company, Suntory, created a blue rose (actually lavender or mauve) in 2004. The genetic engineering involved three alterations adding two

genes, and interfering with another. One of the added genes was for the blue plant pigment delphinidin cloned from the pansy [14]. The researchers then used RNA interference (RNAi) technology to depress all color production by endogenous genes by blocking a crucial protein in color production, called dihydroflavonol 4-reductase) (DFR) and adding a variant of that protein that was not blocked by the RNAi but that allowed the delphinidin to work [15] The roses are commercially sold in Japan, the United States, and Canada [15]. Florigene has also created and sold lavender-colored carnations that are genetically engineered in a similar way [14]. Fig. 1 shows the Suntory blue rose.

Fig. 4. Suntory blue rose (www.en.wikipedia.org/file/blue _rose), [14, 15].

E. Production of transgenic plants

1. Transgenic plant by the use of retrovirus

Recombinant DNA from retrovirus consists of antisense gene (AG). Antisense gene was incerted into the tomato cell (Ti plasmid). Then developed a transgenic tomato which could not produce ethylene itself to ripe. Fig. 2 shows the transgenic tomato by the use of retrovirus.

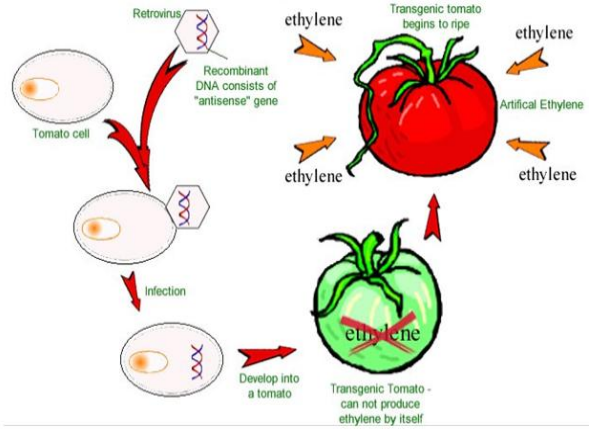


Fig 5. Production of transgenic tomato [16] (https://www.google.com.sa/, Fwww.ied.edu.hk%2Fbiotech%2Feng)

2. Transgenic plant by T-DNA transformation in tissue culture

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 (Ti plasmid) into the plant genome. This method can be used to generate transgenic plants carrying a foreign gene [17, 18, 19].

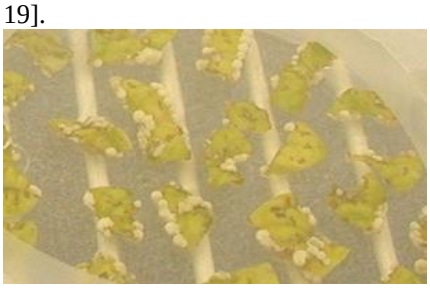


Fig. 6. Photograph shows *Agrobacterium* transformation. Plant *(S. chacoense)* transformed using *Agrobacterium*. Transformed cells start forming calluses on the side of the leaf pieces. (http://en.wikipedia.org/wiki/Agrobacterium) [17].

F. GMO by grafting breeding

People generally like for genetically engineered fruits because they are seedless, taste better and have a longer shelf life, so there is less fear of fruits getting rotten quickly. Cultivars of grapes and avocardo are grown as grafted composites as two genotypes [3]. Rootstock (lower portion) and scion (upper) Lev-Yadun and Sederoff [20] stated that it was possible to graft a nontransgenic scion with rootstock before reproductive age. In some species vegetative propagation was induced by the rootstock or other adventitious rootstock resulting transgenic flower production. They suggested that double grafting starting with transgenic shoot grafted (interstock) to a wild type rootstock then grafted again nontrasgenic scion resulting a transgenic scion between nontrasgenic portions. It has been investigated an experiment on interstock grafting of two varieties of peach fruit plant and got genetically dwarfed peach trees and different fruit color [9].

G. GMO BT CORN

It has been described that corn (Zea mays L.) grown in many areas of the United States suffers from a variety of insect species that attack virtually all parts of the growing plant [21]. Many conventional pest management programs have been developed to combat these insects with varying degrees of success. The initial target of Bt corn, which contains insecticidal protein encoding genes from Bacillus thuringiensis (Bt), were stalk boring insects, such as the European and southwestern corn borers. Within a few years of the introduction of Bt hybrids for stalk boring insects, Bt hybrids targeting western and northern corn rootworms were introduced. Since their introduction, however, Bt corn hybrids have come under considerable scrutiny.

H. GMO by cross breeding

It has been stated that corn plants that had traditionally been allowed to cross-pollinate

freely were artificially self-pollinated for generations and crossed to other self-pollinated lines in an effort to achieve a favorable combination of alleles [22]. The hybrid corn is the result of the strategy of self-pollination followed by cross-pollination to produce vigorous hybrid plants that is called GMO (corn).

III. REGULAION OF GENE EXPRESSION

A. ABA regulated gene expression

Marco [23] reported that high protection rates associated with a significant decrease in the multiplication of R. solanacearum inplants pre-inoculated with a DhrpB mutant strain. Neithern salicylic acid, nor jasmonic acid ⁄ ethylene played a role in the establishment of this resistance. Microarray analysis showed that 26% of the up-regulated genes in protected plants are involved in the biosynthesis and signal ling of abscissic acid (ABA). In addition 21% of these genes are constitutively expressed in the irregular xylem cellulose synthase mutants (irx), which present a high level of resistance to R. solanacearum. We proposed that inoculation with the DhrpB mutant strain.

ABA levels increase in tissues subjected to osmotic stress by desiccation, salt, or cold [24]. Under these conditions, specific genes are expressed that can also be induced in unstressed tissues by the application of exogenous ABA [25]. Some of these genes are also expressed during the normal embryogenic program when seeds desiccate and embryos become dormant [26]. Although different sets of ABA-responsive genes exhibit different patterns of developmental and tissue-specific expression, some of them appear to be part of a general reaction to osmotic stress. This system is a normal part of the embryogenic program but is inducible in vegetative tissues at other times in the plant life cycle. Severa1 ABA-responsive genes have now been isolated [27].

Christine et al [28] stated that previously observed in Arabidopsis suspension cells that (Diacylglycerol Pyrophosphate) DGPP content was increased consecutively to ABA treatment and that the application of dioleoylDGPP was able to trigger the expression of *RAB18* [29]. To evaluate the importance of DGPP for the expression of genes induced by ABA, three supplementary genes were chosen. *At5g06760*, *LTI30* (also named *DHNXERO2*, *At3g50970*), and *RD29A* (also named *LTI78* or *COR78*, *At5g52310*) were selected because they were characterized as ABA up-regulated genes (Leonhardt et al., 2004). Reverse transcription (RT)-PCR analysis of *At5g06760*, *LTI30*, and *RD29A* shows that their expression was stimulated by 10 μm ABA in the suspension cells within 3 h (Fig. 7). Application of dioleoylDGPP (300 μm, 3 h) also induced expression of these genes (7). Expression of *At5g06760* and *LTI30* was neither induced by dioctanoylDGPP nor by dioctanoylPA and dioleoylPA. Weak expression of *RD29A* was recorded with the short fatty acid chains dioctanoylDGPP and dioctanoylPA, whereas dioleoylPA was less efficient than dioleoylDGPP in triggering *RD29A* expression (Fig. 7).

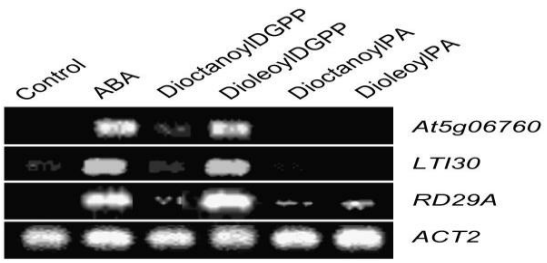


Fig. 7. DioleoylDGPP induces expression of ABA up-regulated genes. RT-PCR analysis of *At5g06760*, *LTI30*, and *RD29A* expression in Arabidopsis suspension cells is shown [28].

B. Auxin (IAA and GA) regulated gene expression in pea fruit (PsGA3ox1)

It has been studied that auxin (4-chloroindole-3-acetic acid [4-Cl-IAA]) and gibberellins (GAs) regulated GA biosynthesis in pea (Pisum sativum) fruit [30]. They observed that expression of the gene PsGA3ox1 that codes for the enzyme that converted GA (20) to biologically active GA (1) using real-time reverse transcription-polymerase chain reaction analysis. PsGA3ox1 mRNA levels were minimally detectable in prepollinated pericarps and ovules (-2 d after anthesis [DAA]), increased dramatically after pollination (0 DAA), then decreased by 1 DAA. Seed PsGA3ox1 mRNA levels increased at 4 DAA and again 8 to 12 DAA, when seed development was rapid. These data showed that PsGA3ox1 was expressed and developmentally regulated in pea pericarps and seeds. These data also showed that pericarp PsGA3ox1 expression was hormonally regulated and suggested that the conversion of GA (20) to GA (1) occurs in the pericarp and was regulated by the presence of seeds and 4-Cl-IAA for fruit growth.

C. Auxin regulated gene expression in tomato (ARF2)

It was stated that the involvement of ethylene in fruit ripening was well documented, though knowledge regarding the crosstalk between ethylene and other hormones in ripening was lacking [31]. They discovered that AUXIN RESPONSE FACTOR 2A (ARF2A), a recognized auxin signaling component, functions in the control of ripening. *ARF2A* expression was ripening regulated and reduced in the *rin*, *nor* and *nr* ripening mutants. It was also responsive to exogenous application of ethylene, auxin and abscisic acid (ABA). Over-expressing *ARF2A* in tomato resulted in blotchy ripening in which certain fruit regions turn red and

possess accelerated ripening. *ARF2A* over-expressing fruit displayed early ethylene emission and ethylene signaling inhibition delayed their ripening phenotype, suggesting ethylene dependency. Both green and red fruit regions showed the induction of ethylene signaling components and master regulators of ripening. Comprehensive hormone profiling revealed that altered *ARF2A* expression in fruit significantly modified abscisates, cytokinins and salicylic acid while gibberellic acid and auxin metabolites were unaffected.

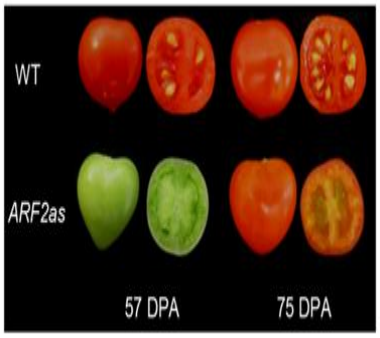


Fig. 8. Photo shows auxin regulated gene expression in tomato (ARF2) [31].

D. Auxin, NAA regulated gene expression in strawberry fruit

It was evaluated that strawberry fruit contain the allergenic Fra a proteins, members of the pathogenesis-related 10 protein family that caused oral allergic syndrome symptoms [32]. Fra a proteins were involved in the flavonoid biosynthesis pathway, which might be important for color development in fruits. In this study, they treated strawberry fruits with exogenous auxin or auxin inhibitors at pre- and postharvest stages and analyzed. Fraa transcriptional and translational expression levels during fruit development by real-time PCR and immune blotting. Pre-harvest treatment with 1-naphthaleneacetic acid (NAA) alone did not affect Fra a expression, but applied in conjunction with achene removal NAA promoted fruit pigmentation and Fra a protein accumulation. Theresponsewasdevelopmental stage-specific: Fra a 1 was highly expressed in immature fruit, whereas Fra a 2 was expressed in young to ripe fruit. In post-harvest treatments, auxin did not contribute to Fra a induction. Auxin inhibitors delayed fruit ripening; as a result, they seemed to influence Fra a 1 expression. Thus, Fra a expression was not directly regulated by auxin, but might be associated with the ripening process and/or external factors in a paralog-specific manner. Keywords: allergen; auxin; auxin inhibitor; Fragaria × ananassa; oral allergic syndrome; pathogenesis-related; phytohormone; post-harvest; pre-harvest; ripening regulation.

E. Auxin, GA and BR regulated gene expression in wild type plant tissue

It was identified a recessive, brassinolide-insensitive mutant caused by a deletion allele (*bri1-201*) of the brassinosteroid (BR) receptor *BRI1[33]*. The *bri1-201* mutant displayed altered expression levels of genes differentially regulated by gibberellin (GA). RNA-blot analysis revealed that BR and GA antagonistically regulated the accumulation of mRNAs of the GA-responsive *GASA1* gene and the GA-repressible *GA5* gene. Expression studied with cycloheximide indicated that the antagonistic effects of GA and BR on *GA5* required the novo protein synthesis. Reporter transgene analysed and RNA-blot analysis showed that BR and GA modulated *GA5* expression, at least in part, at the transcriptional level, and that the signals were independent and subtractive. It was reported that cross-talk might occur between BR- and GA-signaling pathways, like mRNA of the GA-responsive γ-*TIP* gene accumulated ectopically in BR-deficient and BR-signaling mutants, suggested that BR and GA antagonistically regulated γ-*TIP* expression [34]. γ-*TIP* encoded a tonoplast-intrinsic aquaporin or water channel and its antagonistic regulation by BR and GA might reflect differences in the mechanisms by which the two hormones modulated cell growth and size by regulating turgor pressure or solute flow. In contrast, mRNA levels of the *MERI-5* gene [35] were regulated positively by either BR or GA treatment [34]. *MERI-5* probably encoded a xyloglucan-endohydrolase involved in cell wall loosening, thereby modulated cell expansion and growth.

IV.CONCLUSION

From the above data and results it can be concluded that GMO technology can be applied using hormonal injection technology, bacteria like *Bacillus thuringiensis*, tissue culture with *Agrobacterium* mediated media, cross breeding, inter-stock breeding, swabbing in xylem and phloem technology exhibited as innovative in pumpkin, ladies finger, peach plant, rose, carrot, star fruit, tobacco, tomatoes and *S. chacoense* plant. Therefore, GMO ornamental plant, flower, fruit and vegetable can be produced by using in vitro tissue culture of leave, shoot and root or in vivo using different concentrations of GA_3, IAA, NAA, ABA and *Agrobacterium tumefaciens* (Ti plasmid, genomic DNA/T-DNA) media. In addition, swabbing, dipping, dripping and micro spraying technologies can be applied by injecting in the stem, xylem and phloem tissue, flower, ovary tissue as an innovative technology.

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