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Genetically modified organism (GMO) production using hormones, T-DNA, tissue culture and its regulated gene expression

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Abstract
Genetically modified (GM) technology keeps a significant act to produce GM organism (GMO) and GMO derived food. The review study was conducted different innovative research data in the GMO production like plant, fruit and vegetables and its related gene expression. GMO production using hormones (GA_3, IAA & ABA) and gene transformation (T-DNA) in plan has been noted well. GMO using hormone application by injection, bacteria like Bacillus thuringiensis, tissue culture with Agrobacterium mediated media technology has been exhibited as innovative from different research data. Moreover, different innovative research data have been highlighted and regulated gene expression was documented well.

Keywords: modified, organism, hormones, DNA, regulated

Introduction
Genetically modified (GM) technology keeps a significant act to produce GM organism (GMO) and GMO derived food. It is generated by genetic engineering technology. GM technology 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 [1,2,3].
Artificial 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 [4, 5]. Genetically modified bacteria were used to produce the protein insulin to treat diabetes. Similar bacteria have been used to process bio-conversion [6], plant and human growth hormone to treat various forms of dwarfism. In addition, various genetically engineered micro-organisms are used as sources of enzymes for the manufacture of a variety of processed foods. The objectives of the review study were to find out innovative technology for genetically modified plant (GMO) using hormone application and T-DNA techniques in plants and to observe the regulated gene expression.

2. Application of Genetically modified technology
2.1. Using plant hormones
The experiment was conducted using pumpkin plant [5]. Local cultivar was grown at the experimental Field, University of Malaya, Malaysia. 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. It was stated [2] 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 [5] (Fig. 1a).

Injecting hormone **seedless ladies finger**

Fig 1: Injecting hormone solution into the flower and seedless ladies finger production [5].

It was evaluated [1, 2] 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. It was conducted [7] an experiment to know the genetically dwarf of peach tree by growth inhibition hormone. They also reported that 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 [7] that this study had dwarfing effect on vigorous peach trees grafted on vigorous rootstocks. They suggested that there might be genetically dwarf peach trees produced and shoot growth reduced 95% at 2000ppm ABA. Almost same proportion of root growth was reduced in the case of both concentrations of ABA [7].

2.2. Using T-DNA transformation (Transgenic plant) in tissue culture

Transformation with *Agrobacterium* can be done 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 [8,9,10]. This method can be used to generate transgenic plants carrying a foreign gene Figure 1b shows the gene transfer procedure [11].

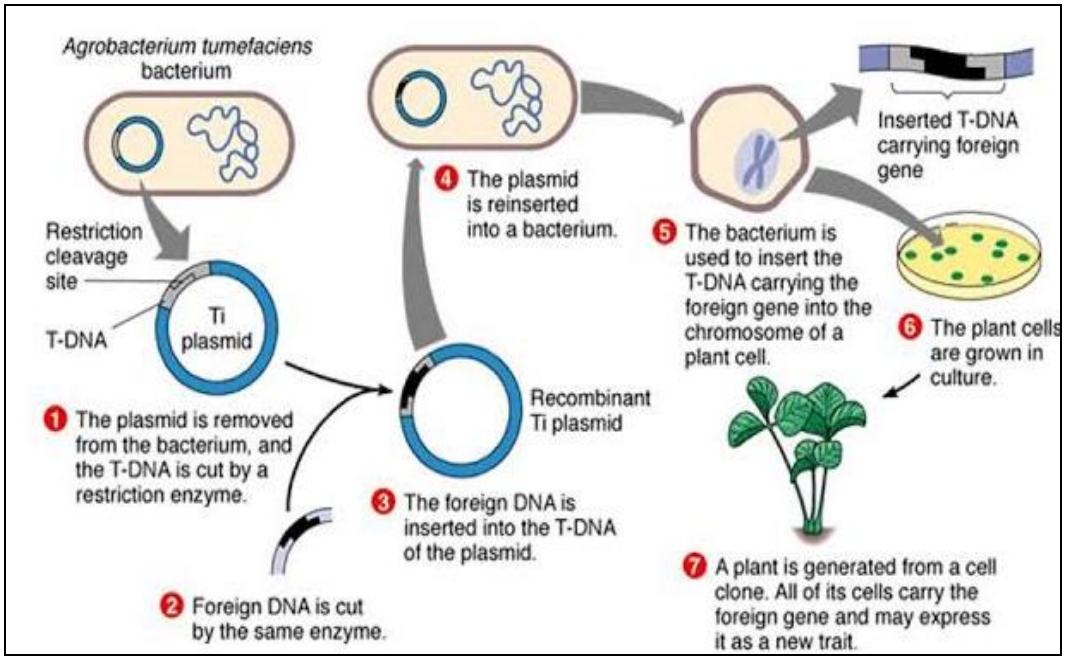

Fig 2: Photograph shows gene transfer procedure using Agrobacterium. https://slideplayer.com/user/7062904/ [11].

It was conducte [12] an experiment to produce transgenic soybean and reported that advantitious shoot induced with Agrobacterium. Tumefaciens and healthy explants exhibited compared to control.

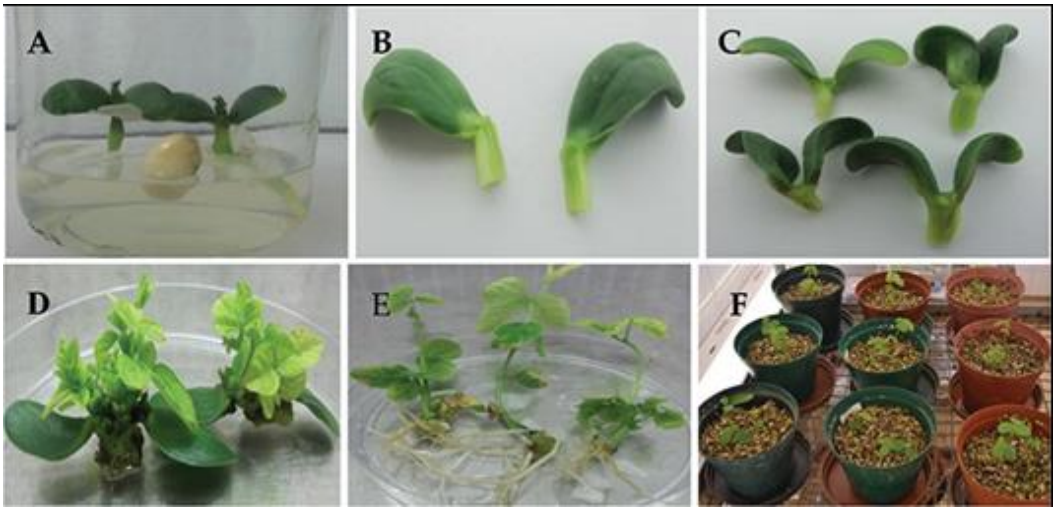

Fig 2: Photographs show the production of transgenic soybean. A. Seedlings to serve as explant source, B. Single coty-node explants, C. Double coty-node explants, D. Adventitious shoot induction on double coty-node explants infected with A. tumefaciens, E. Rooted shoots obtained from PGR-free MS medium with some callus at the base and F. ex vitro acclimatised[12]. https://www.intechopen.com/books/soybean-the-basis-of-ield-biomass-and-productivity/challenges-of-in-vitro-and-in-vivo-agrobacterium-mediated-genetic-transformation-in-soybean) [12].

It was reported that a blue rose flower was produced in 2004 [13]. 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 [13]. 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 [14]. The roses are commercially sold in Japan, the United States, and Canada [14].Florigene has also created and sold lavender-colored carnations that are genetically engineered in a similar way [13].

3. Regulation of Gene expression

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

It was suggested [15] that auxin (4-chloroindole-3-acetic acid [4-Cl-IAA]) and gibberellins (GAs) regulated GA biosynthesis in pea (Pisum sativum) fruit. 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.

3.2 ABA regulated gene expression

It was stated [16] 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. Some of these genes are also expressed during the normal embryogenic program when seeds desiccate and embryos become dormant [17]. Although different sets of ABA-responsive genes exhibited at different patterns of developmental and tissue-specific expression, some of them appear to be part of a general reaction to osmotic stress. This system was a normal part of the embryogenic program but was inducible in vegetative tissues at other times in the plant life cycle. Severa1 ABA-responsive genes had been isolated [18]. It was observed [19]. that previously observed in Arabidopsis suspension cells that (Diacylglycerol Pyrophosphate) DGPP content was increased consecutively to ABA treatment and that the application of dioleoy lDGPP was able to trigger the expression of *RAB1 8*[20]. Application of dioleoylDGPP (300 μm, 3 h) also induced expression of these genes. Fig. 3. RT-PCR analysis of ABA response gene expression in hda19-1 mutants, two-week-old Ws wild-type and hda19-1 plants were treated with 100 μm of ABA for 3 h. Total RNA for RT-PCR analysis was isolated from leaf tissues. Ubiquitin (UBQ) was shown as an internal control [21].

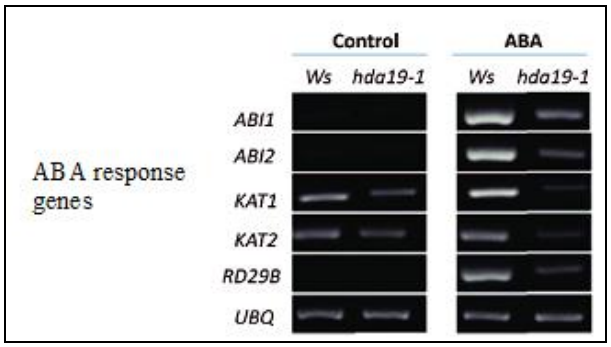

Fig 3: RT-PCR analysis of ABA responsive gene expression in hda19-1 mutants. two-week-old Ws wild-type and hda19-1 plants were treated with ABA [21] (https://www.researchgate.net/publication/47370643_Role_of_histone_deacetylases_HDA6_and_HDA19_in_ABA_and_abiotic_stress_response/figures?lo=1).

3.2. Agrobacterium (T-DNA) regulated gene expression

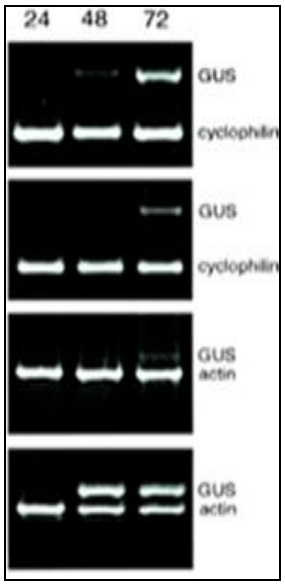

Fig 4: Gus (Colorimetric solution) expression analysis. *A.* thaliana root (A) and leaf tissue (B), tobacco BY-2 cells (C), and A. conyzoides cells (D) at 24, 48, and 72 h after inoculation with Agrobacterium containing a GUS–intron construct. Primers for constitutive control genes were used in the RT-PCR analysis: cyclophilin primers for Arabidopsis and actin primers for BY-2 and Ageratum. https://www.pnas.org/content/98/19/10954 [22].

It was conducted [22] an experiment on plant response to infection and transformation by *Agrobacterium tumefaciens* and they compared compared the cDNA-amplified fragment length polymorphism (AFLP) pattern of *Agrobacterium*- and mock-inoculated *Ageratum conyzoides* plant cell cultures (Fig.

4). From CDNA fragments analyzed, 251.6 were differentially regulated 48 h after cocultivation with *Agrobacterium*. From 75 strongly regulated fragments, 56 were already regulated 24 h after cocultivation. Sequence similarities were obtained for 20 of these fragments and reverse transcription PCR analysis was carried out with seven to confirm their cDNA-AFLP differential pattern. Reverse transcription PCR analysis indicated that four genes involved in defense response are regulated in a similar manner by nonpathogenic bacteria, whereas one gene putatively involved in signal transduction appeared to respond more strongly to *Agrobacterium* [22].

4. Conclusion

From the review study, innovative research data on the GMO production in plant were described well. GMO production using hormones like GA_3, IAA & ABA and gene transformation using *Agrobacterium* (T-DNA) in plant has been documented. Moreover, ABA response genes, *RAB, ABI 1, ABI 2, KAT 1, KAT 2,* Agrobacterium (T-DNA) regulated gene, *RD29B* and GA3 regulated gene, PsGA3ox1 were noted.

5. References

1. Hossain ABMS. Development of Seedless Star Fruit and its Antioxidant, Biochemical Content and Nutritional Quality by Gibberellic Acid Hormone as Genetically Modified Component. Int. J. Plant Breed. Genet. 2015; 10(1):23-30.
2. Hossain ABMS. Seedless Pumpkin Vegetable Production Using Gibberellic Acid (GA3) As Plant Hormone and Genetically Modified Technique. Global j. Biology, Agri. and Health Sciences. 2015; 4(3):6-8.
3. Hossain ABMS and Musamma Muddin, Genetically Modified Organism Using ABA GA_3 and IAA Hormone: Regulated Gene Expression. Asian Journal of Biological Sciences. 2018; 11:197-202.
4. Hossain ABMS. Plant Physiology and Biotechnology: Recent innovation LAP Lambart Academic publishing Co. Paperback, Germany, 2012, 603.
5. Hossain ABMS. Plant Biotechnology and Genetic Engineering. LAP Lambart Academic publishing Co. Paperback, Germany. 2014; 433.
6. Chronicle C. Biologists invent gun for shooting cells with DNA Wayback Machine, 1987, 3.
7. Hossain ABMS, Mizutani F, Onguso JM, Ali R, El-Shereif. Effect of interstock and spiral bark ringing on the growth and yield of peach trees. Bulgarian Journal of Agriculture. 2005; 11(3):316-320.
8. Steven CJ, Andrew BF. Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana". The Plant Journal. 1998; 16(6):735-743.
9. York M. Plant tissue culture, GE plant and Applications. Presentation on theme: Lec, 2019. https://slideplayer.Com /user/7062904.
10. Mangena P, Phatlane WM, Roumiana VN. Book Chapter, Intechopen, Challenges of *In vitro* and *In vivo* Agrobacterium-Mediated Genetic Transformation in Soybean https://www.intechopen.com, 2017. DOI: 10.5772/66708.
11. Dan N. Suntory Creates Mythical Blue (Or, Um, Lavender-ish) Rose. Popular Science, Retrieved, 2012.
12. Kyodo (11 September 2011) Suntory to sell blue roses overseas. The Japan Times, Retrieved, 2012.
13. Ozga JA, Yu J, Reinecke DM. Pollination-, development-, and auxin-specific regulation of gibberellin 3beta-hydroxylase gene expression in pea fruit and seeds. Plant Physiol. 2003; 131(3):1137-46.
14. Marco Y. Biological control of bacterial wilt in Arabidopsis thaliana involves abscissic acid signaling. New Phytologist. 2012; 194:1035-1045. doi: 10.1111 /j.1469-8137.2012.04113.x
15. Dure L, Greenway SC, Galau GA. Developmental biochemistry of cottonseed embryogenesis and germination. XIV. Changing mRNA populations as shown by *in vitro* and *in vivo* protein synthesis. Biochemistry. 1981; 20(41):62-41 68.
19. Baker J, Steele C, Dure L. Sequence and characterization of 6 Lea proteins and their genes from cotton. Plant MOI. Biol. 1988; 11:277-291.
23. Christene ZS, Paradis R, Maldine Y, Habricot E, Miginiac J, Rona E, *et al.* Induction of Abscisic Acid-Regulated Gene Expression by Diacylglycerol Pyrophosphate Involves Ca^{2+} and Anion Currents in Arabidopsis Suspension Cells. Plant Physiol. 2006; 141(4):1555-1562.
24. Zalejski C, Zhang Z, Quettier AL, Maldiney R, Bonnet M, Brault M, *et al.* Diacylglycerol pyrophosphate is a second messenger of abscisic acid signaling in Arabidopsis thaliana suspension cells. Plant J. 2005; 42:145-152.
25. Wu K, Chen L-T. Role of histone deacetylases HDA6 and HDA19 in ABA and abiotic stress response, 2010.
26. Renata F, Eugene D, Nester W, Comai L. Plant gene expression response to *Agrobacterium tumefaciens* PNAS. 2001; 98(19):0954-10959.