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Research Article
Genetically Modified Organism Using ABA GA_3 and IAA Hormone: Regulated Gene Expression

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Abstract

Background and Objectives: Genetically Modified (GM) technology retains an important role to produce GM organism (GMO) and GMO derived food. The study was carried out to investigate GM seedless pumpkin, seedless ladies finger and dwarfed peach plant employing different concentration of GA3 (100 and 150 ppm) and ABA (2000 ppm) hormones and its related gene expression. **Materials and Methods:** The treatments were used in expt. 1 as water control and abscisic acid (ABA) 2000 ppm applied to the partially peach barked tissue. Injection method was used in expt. 2 to make seedless pumpkin. Star fruit flower buds were dipped for 2 weeks with GA3 150 ppm and water control in expt. 3. The concentration of IAA 200 ppm were applied to the ladies finger's flower by injecting in Expt. 4. **Results:** From the results it was observed that bark tissue, shoot and root were inhibited by ABA hormone. Whereas in control plant, there was no inhibition occurred. In expt. 2, it had been shown that fruit weight was higher in the GA3 treated fruit than in control. However, seed number and per seed weight were higher in the control than the GA3 treated pumpkin. Glucose, fructose potassium and calcium content were found higher in the GA3 treated pumpkin than the control. In expt. 3, fruit weight was higher in the GA3 treated fruit than in control. However, seed number was higher in the control than the GA3 treated fruit. In expt. 4, the maximum pod length, TSS and vitamin C were obtained in IAA 200 mg L^{-1} compared to the control. The highest seedless pod was found in IAA 200 mg L^{-1}. **Conclusion:** From the results it has been seen that maximum seedless pod was 100%. Positively gene was expressed by ABA 2000 ppm and GA3 150 ppm.

Key words: GMO, plant hormone, ABA, GA3, gene expression

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Genetically Modified Organism (GMO) technology keeps a significant role to produce GM organism (GMO) and GMO derived food. The GMO is generated by genetic engineering technology. The first GMOs were bacteria in 1973. The GM mice were generated in 1974. Insulin-producing bacteria were commercialized in 1982 and genetically modified food has been sold[1] since 1994. The GMO is the source of plant, animal, medicines and genetically modified foods, also widely used in scientific research and to produce other goods[2]. The 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 and 10% of the world's croplands were planted with GM crops[2,4] in 2010. 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 and e: Grafting and dwarfism[6,7].

Many 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[3,8,9]. Genetically modified bacteria were used to produce the protein insulin to treat diabetes. Similar bacteria had been used to produce bio-fuel[4], human growth hormone to treat various forms of dwarfism. In addition, various genetically engineered micro-organisms were routinely used as sources of enzymes for the manufacture of a variety of processed foods. The objectives of the study were to investigate an innovative technology of genetically modified plant (GMO) using ABA, IAA and GA3 hormone application in plants, fruits and vegetables as well as to evaluate the regulated gene expression.

MATERIALS AND METHODS

Experiment 1: Dwarf plant production by abscisic acid as GMO: A 2 year peach plant was used in this experiment. A total of 12 trees (4 replicates) used in the experiment. The treatments were water control (no inhibitor) and abscisic acid (ABA) 2000 ppm applied to the partially barked tissue at two weeks interval (Fig. 1) and continued for 3 month.

Experiment 2: Seedless vegetable (pumpkin, ladies finger) production by hormone treatment seedless pumpkin by GA_3: Pumpkin plants (local cultivar) were grown at the University experimental Field. Local cultivar was used. The concentration of 150 ppm GA3 was used for 5 replicates and water used for the control. Injection method by using micro-syringe was used to make seedless pumpkin or reduced seed by flower injection before blossoming (opening the flower).

Experiment 3: Seedless star fruit production by hormone treatment: Five branches were used for GA3 150 ppm treatment in three tree of star fruit (Fig. 3) and also for control (total of 6 trees, 10 years old) by which flower buds were dipped twice per week for 2 weeks with GA3 (150 ppm) during the growing season of the flower bud formation.

Experiment 4: Seedless ladies finger by IAA: One and a half mL of the concentration of IAA (100 mg L^{-1}) were applied on the ladies finger's flower by injecting once with needle before anthesis while control was distilled water (Fig. 4). Green pod length, green pod diameter, healthy seeds/pod, seedless or aborted seeds/pod were measured.

RESULTS AND DISCUSSION

Experiment 1: From the results, the effects of the growth inhibitor of abscisic acid (ABA) at the concentration 2000 ppm on the shoot and bark tissue growth inhibition (%) had been presented (Table 1). It had been shown that the bark phloem tissue was inhibited 100% at ABA 2000 ppm (Table 1). Figure 1 had represented the photograph of the growth inhibition of shoot and root.

From the results it had been exhibited that ABA 2000 ppm had showed the highest inhibition of the peach shoot and bark phloem tissue growth. This is might be due to the effectiveness of the given concentration. About 100% inhibition was found in these concentration. Cell division and

Fig. 1(a-b): Inhibition of peach plant growth leaf, root, shoot and phloem tissue inhibition as dwarf trees using genetically modified technique by ABA hormone, (A) Water control ABA (Shoot) and (b) Water control ABA (Root)

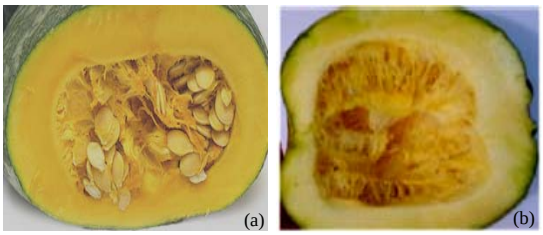

Fig. 2(a-b): (a) Seedless and (b) Seeded pumpkin by genetic engineering techniques

Fig. 3(a-f): Fruit growth, maturity and color at different treatments, (a-c) Water control and (d-f) Seedless fruit by GA3 150 ppm

Table 1: Growth inhibition (%) as affected by ABA

Hormone treatment	Shoot growth inhibition (%)	Bark tissue growth inhibition (%)	Root growth inhibition (%)
Water control	0	0	0
ABA 2000 ppm	64.2[a]	100[a]	73.4[a]

LSD test at 5% significant difference. Means followed by the common letters are not significantly different at the 5% level by least significant different test (LSDT)

Table 2: Pumpkin weight and seed number measurement

Treatments	Fruit wt (g)	Seed wt (mg)	Seed No.	Seedless (%)
Control	700.5±0.5	13.3±0.2	98.0±0.5	0
GA3	750.4±0.6	6.6±0.1	3.0±0.1	96.9±0.1

Mean±SE (N = 5)

Table 3: Biochemical and mineral content like glucose, fructose, potassium and calcium determination

Treatments	Glucose*	Fructose*	P (ppm)	Ca (ppm)
Control	5.0±0.3	7.0±0.5	310.2±0.4	36.1±0.2
GA3	5.9±0.2	7.6±0.5	340.5±0.3	50.2±0.1

*Percentage

differentiation might not be occurred and genetically modified by the concentration of hormone. That is why organs growth was inhibited 100% by ABA 2000 ppm concentration. Hossain and Mizutani[9] reported that growth of the different organs of peach plant was inhibited by using ABA 1000 ppm. They also reported that shoot and root growth were inhibited 46% at 1000 pm ABA. Hossain[8] observed that ABA 1000 and 2000 ppm reduced the trunk and flower growth.

Buttner-Mainik[5] stated that ABA had shown the various developmental and physiological processes that inhibited the growth performance of crop plants.

Experiment 2: Figure 2 showed the difference between the seeded and seedless pumpkin. In the Table 2 it had been shown that fruit weight was higher in the GA3 treated fruit than in control. However, seed number and per seed weight were higher in the control than the GA3 treated pumpkin. Besides, 96.9% seedless pumpkin was found by the treatment of GA3 compared to the control (Table 2). Glucose, fructose potassium and calcium content were found higher in the GA3 treated pumpkin than the control (Table 3).

It had been reported that gibberellic acid was widely used in the grape-growing industry as a hormone to induce the production of larger bundles and bigger grapes, especially Thompson seedless grapes were produced[10] by GA3. It has been stated that seedless melon was produced nowadays by GA3 and was also used as a growth replicator in the cherry industry[11]. They also stated that regulation of transcription was happened by gibberellin (GA) and abscisic acid (ABA). In addition, GA stimulated and ABA prevented the transcription of genes for α-amylases and other secreted hydrolytic enzymes.

Fig. 4(a-b): Injecting IAA hormone solution into the (a) Flower and (b) Seedless ladies finger production

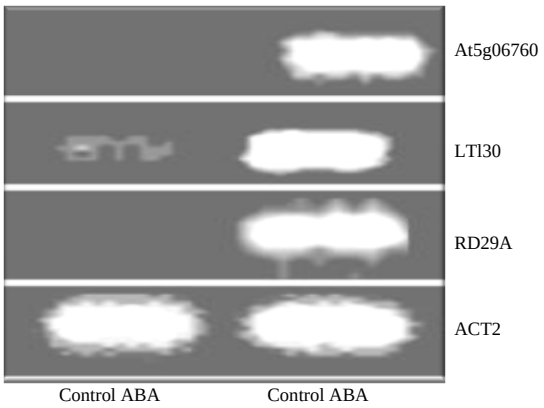

Fig. 5: Expression of ABA regulated genes. Northern blot analysis of At5g06760, LTI30 and RD29A expression in peach have been shown

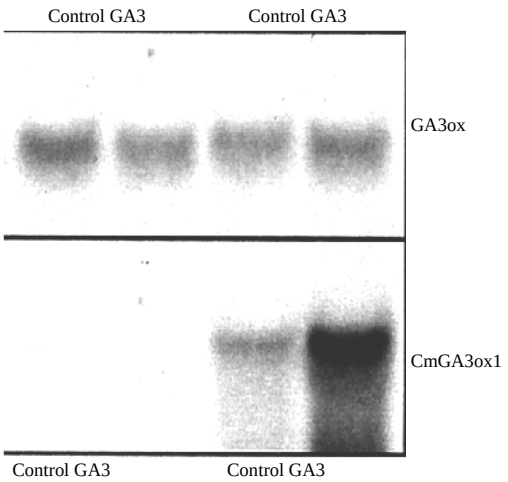

Fig. 6: Expression of GA3 regulated genes in cucurbita maxima

Experiment 3: It had been shown that fruit weight was higher in the GA3 treated fruit than in control. However, seed number was higher in the control than the GA3 treated fruit. Besides, 75% seedless (aborted seed) star fruit was found by the treatment of GA3 compared to the control (Table 4). Moreover, fructose content was found higher in the GA3 treated fruit than the water control fruit. It was reported that gibberellins might promote cell division and elongation[12,13]. The increase of fruit weight was attributed to the effect of GA3, acting synergistically increasing fruit diameter and size. Localized application of GA3 to bilimbi was known to increase the sink strength enabling them to attract photoassimilates from the foliage and to develop fully formed fruits[13-15]. The photosynthesis rate occurred rapidly which led to the higher levels of carbohydrate in the fruits. That is why higher the fructose, the better its quality[16-22].

Table 4: Star fruit weight and seed measurement

Treatments	Fruit wt (g)	Seed No.	Seedless fruit (%)	Fructose (%)
Water control	49.1±0.4	4.0±0.5	0	8.5±0.3
GA3 150 ppm	52.2±0.3	1±0	75±0.1	10.7±0.2

Table 5: Ladies finger pod length and biochemical content measurement

Treatments	Pod length (cm)	TSS (%)	Vitamin C (mg/100 g)	Seeded pod (%)	Seedless pod (%)
Water control	4.28[b]	2.2[a]	11.7[a]	84.3	0
IAA 200 ppm	7.39[a]	2.3[a]	11.8[a]	0	100

Experiment 4: The maximum pod length, TSS and vitamin C were obtained in IAA 200 mg L^{-1} compared to the control (Table 5). The significant highest seedless pod was 100% in IAA 200 mg L^{-1} followed by zero in control 200 mg L^{-1}. However, seeded pod was 84.3% in the control followed by zero in the IAA treated pod. From the results it had been seen that maximum seedless pod was 100%. It might be due to the genetically modified by IAA hormone. It had been reported that pericarp PsGA3ox1 expression was hormonally regulated and the conversion of GA (20) to GA (1) occurred in the pericarp and was regulated by the presence of seeds and 4-Cl-IAA for fruit growth[23].

ABA regulated gene expression: The ABA regulated genes were expressed by northern blot[24] and found new genes like At5g06760, LTI30, RD29A (Fig. 5). Marco[25] reported that high protection rates associated with a significant decreased in the multiplication of *R. solanacearum* in plants pre-inoculated with a DhrpB mutant strain. Neither salicylic acid, nor jasmonic acid/ethylene played a role in the establishment of this resistance. It also showed that 26% of the up-regulated genes in protected plants are involved in the biosynthesis and signaling of abscisic 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*. They suggested that inoculation with the DhrpB mutant strain. The ABA levels increase in tissues subjected to osmotic stress by desiccation, salt or cold[26]. Under these conditions, specific genes are

expressed that can also be induced in unstressed tissues by the application of exogenous ABA[27]. Some of these genes are also expressed during the normal embryogenic program when seeds desiccate and embryos become dormant[28]. 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. Several ABA-responsive genes have now been isolated[29]. Christine *et al.*[30] stated that *Diacylglycerol Pyrophosphate* (DGPP) content was increased consecutively to ABA treatment and the application of dioleoyl DGPP was able to trigger the expression[31] of RAB18. Application of dioleoyl DGPP (300 μm, 3 h) also induced expression of genes.

Auxin (GA3 and IAA) regulated gene expression

GA3 regulated gene expression: The A3 regulated genes were expressed in Cucurbita maxima by northern blot[24] and found new gene like CmGA3ox1 (Fig. 6). It had been studied that auxin (4-chloroindole-3-acetic acid [4-Cl-IAA]) and gibberellins (GAs) regulated GA biosynthesis in pea (Pisum sativum) fruit[32]. They observed that expression of the gene PsGA3ox1 that coded for the enzyme that converted GA (20) to biologically active GA (1). They also described 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.

Auxin (IAA) regulated gene expression: In current experiment, IAA regulated gene was not investigated. However, some research literature data had been shown. 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[33]. They discovered that Auxin/IAA- ARF2A was regulated. ARF2A expression was ripening regulated and reduced in ripening mutants.

CONCLUSION

From the above results it can be concluded that GMO technology can be applied using hormonal injection technology, swabbing in xylem and phloem technology exhibited as innovative in pumpkin, ladies finger, peach and star fruit. Therefore, GMO plant, fruit and vegetable can be produced by using different concentrations of GA_3, IAA, ABA. It can be concluded that 96.9% seedless pumpkin was found at GA3 150 ppm. Besides, GA3 150 ppm was the best treatment compared to others showing the 75% seedless as aborted seed, biggest size and highest TSS and biochemical content of star fruit. In addition, the nutrient like Na, Ca and K was higher in GA3 150 ppm. Moreover, ABA regulated gene was expressed by ABA 2000 ppm concentration.

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