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Advances in the Micropropagation and Genetic Transformation of *Abelmoschus Esculentus* (L.) Moench for Insect Resistance

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Abstract

Abelmoschus esculentus (L.) Moench, known as okra, is a common vegetable crop in many diets and serves as a nutrient-rich source. It has a high content of protein, vitamins, minerals and compounds of high medicinal value. India tops in the consumption of pods and ranks first among the worldwide total production. It is now widely cultivated in many countries. Among the factors that hamper okra's marketable fruit yield, insect pests are the major ones. As numerous pests attack vegetables, controlling insect pests is one of the key elements to improve the yield of this crop. A workable approach for improving okra yield is micropropagation. It has been employed for a variety of things, including as large multiplication, inducing somaclonal variation to improve the desirable agronomic traits, maintaining certain genotypes, and genetic modification utilising molecular techniques. In this review, we highlight the most significant research on the micropropagation of okra, which is mediated by a variety of regeneration responses. The media and growth regulators for each of the approaches discussed, we go through how transformation techniques for insect resistance have been made possible via micropropagation. Utilizing this technology might be a workable plan to add genes and enhance particular features. Studying molecular pathways is another option provided by genetic transformation. This offers benefits for developing breeding programmes and optimising field production especially the effective use of CRISPR in genetically diverse lepidopteran insects opened options to study gene functions, insect modification, and pest management.

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Introduction

Agricultural biotechnology has the potential to enhance crop yield.[1] Researchers have developed various techniques to boost crop production and make agriculture more sustainable in the environment.[2] Through genetic engineering, several crop varieties for drought resistance, insect resistance, herbicide tolerance and tolerance to salinity have been developed.[3] Crops that have been genetically modified (GM) have substantially contributed to improving global food security as well as poverty alleviation.[4] Plant transformation technology has grown into a flexible platform in the area of plant biology, with *Bacillus thuringiensis* (*Bt*)-producing transgenic crops being commercialized throughout the world to replace insect pesticides for pest control.[5] Many studies employing *B. thuringiensis* (*Bt*) formulations for the biocontrol of the insect pests of *A. esculentus* were published and have confirmed their effective control along with a significant reduction in the plant population. The major pests that caused damage are *Pectinophora gossypiella*, *Amrasca biguttula*, *Syllepte derogata*, *Earias vittella*, *Acontia intersepta*, *Aphis gossypii*, *Mylabris*, *Odontotermes obesus*, *Nezara viridula*, *Bagrada cruciferarum*, *Helicoverpa armigera*, *Spodoptera litura*, *Tetranychus urticae*, *Tetranychus cinnabarinus* and *Dysdercus koenigii*.[6,7,8,9,10] Based on their structure of protein and genetic variation, *B. thuringiensis* genes were divided into three different classes:CryI, CryII, and CryIII.[11] CryI toxins are found to be deadly against the Lepidoptera insects, whereas CryIII acts against Coleopterainsects.[12] An attempt has been made to transfer the *Bt* Cry1Ac gene into *A. esculentus* plantto confer a high level of resistance against natural pests and improve the crop yield without affecting the environment.[13,14,15]

A. esculentus (L.) (Lady's finger) is an annual or perennial herb that grows in the tropics, sub-tropics and milder climates, belonging to the family Malvaceae. The plant grows to approximately 2 m tall with heart-shaped leaves that are 10–20 cm in length and breadth and palmately lobed with 5–7 lobes. The petals range in colour from white to yellow, and the blooms are big. Seed pods range in length from 3 to 10 inches and taper towards the tip.[16] Immature seed pods of *A. esculentus* are grown and consumed worldwide as a source of vital nutrients and fibre in the human diet. *A. esculentus* is commonly known by a variety of names across the world, such as Lady's Finger, ochro, quiabo and okra. In addition, it is called quimbombó in Spain, gombo in French, bamia or bamya in the eastern Mediterranean, bamies in Arab countries and bhindi in India.[17] This crop was first cultivated in Ethiopia, Sudan and other north-eastern African countries. In terms of pod consumption, India is at the top. At present, it is widely grown in many countries with its distribution spread throughout Africa, Egypt, Asia, America and southern Europe.[18,19] Protein-rich fruits and seeds are low in calories and abundant in vitamins A and C, and iron, glucose, calciumand enzymes.[20,21] *A. esculentus* is a plant of high medicinal importance whose fruits, leaves, roots and seeds are used for several treatments.[22] Soluble fibres in the form of gums and pectin, which help reduce serum cholesterol, lower risks of cardio vascular diseases and are also used as a substitute for plasma for expanding the volume of blood, the pharmaceutical applications include thickeners in oral liquids, disintegrates in tablets, binders, protective colloids in suspensions and gelling agents in gel.[23] The mucilaginous texture offers healing properties for ulcer and may also help reduce acid reflux.[24,25] It also helps in cardiovascular and gastrointestinal health, and has antioxidant and anticancer properties and also the potential benefit against SARS-CoV-2.[26,27,28,29] *A. esculentus* fibre has high cellulose content and may be utilised as a raw cellulosic resourcein the cellulose-based sectors. Yellowing and photochemical deterioration is caused by the low lignin content. It exhibits dyeing fastness, ultimate strength, and other characteristics due to its large-molecular-weight components.[30]

Insecticidal sprays, the most popular approach for controlling insect pests, are hard to implement at the initial stages of plant development due to insufficient area and poor efficiency in the growing leaves.[31] Synthetic insecticides, without a dou*bt*, are effective in controlling insect pests, but, when used on a regular basis, can pollute the environment, cause pests to develop resistance to these over a period of long exposure and also lead to the emergence of secondary pests.[32] Pest management solutions that are environmentally friendly are in high demand across the world. Pest controlusing varietal resistance has been identified as one of the most economical and ecologically safe methods. Insect-resistant cultivars can be developed and utilized

as sole control strategies working in tandem with biological, chemical and cultural control methods to reduce the spreading of pest insects.[33,32,34]

Farmers greatly rely on chemical pesticides to combat the pests of *A. esculentus*. Synthetic pesticides used in vegetable crops are causing enormous problems to the environment. Precisely vegetable crops use around 13–14% of all pesticides used in India.[35] As the young pods of *A. esculentus* are picked on consecutive days, many environmental issues with spraying can result from the reliance on the use of synthetic insecticides. The pesticide residues from vegetable market have been confirmed in *A. esculentus* that contains toxic pesticides such as acetamiprid and thiamethoxam.[36] Therefore, there is an urgent need to develop an alternative strategy of eco-friendly pest control in *A. esculentus.*[7]

Effects Caused by the Chemical Pesticides

Non-target species, such as pests, predators, and parasites, can be harmed by chemical application, and the loss of these useful organisms can disrupt natural biological balances.[37] Pollinating insects such as honeybees and others can be affected by these pesticides. Sprays and vapourdrifts during application along with careless discharge can also cause severe damage and residual problems in crops, livestock, waterways andthe environment, resulting in the extinction of animals and fish.[38] Residues in foods of humans and livestock can be a result of the direct application of a chemical pesticide on the food source, and the presence of pollutants in the environment ortheir transmission and bio-magnification along the food chain.[39] Pesticides that have leached into the ground can also contaminate the water supply. Overuse and improper use of the chemical pesticides might result in the pests' species susceptibility, while its excessive exposure can result in poisoning and other health risks for operators if proper handling protocols are not followed.

Insecticides, herbicides, fungicides, nematicides, rodenticides, and miticides are some of the chemical pesticides used in the chemical approach in okra. The most efficient pesticides for okra shoot borer (*Earias vitella*) control were determined to be fenvalerate (0.01%) and thiodicarb (0.15%).[96] Several pesticides pose risks to people, plants, and the environment.[97] The length of chemical exposure, toxicity, dosage, and route of entry into the body are some variables that affect pesticide toxicity in humans.[98] India has investigated more than 400 genotypes for resistance to the shoot and fruit borer using chemical pesticide, however, none of these lines could be categorized as highly resistant or immune.[99] In okra, traditional and mutation breeding strategies to breed for disease resistance have actually rarely been successful. Because okra germplasm lacks sources of resistance to insect pests and diseases, genetic improvement through traditional plant breeding takes a long time100. However, with the development of genetic engineering techniques, it is now possible to add a variety of genes for disease resistance, insect pest resistance, and nutritional enrichment to this crop. Transgenic plants produced by inserting the genes of insecticidal proteins are showing promising results for effective pest control while causing no harm to beneficial insects.

Biotechnological studies on *A. esculentus*

Tissue Culture Studies

Plant tissue culture is an important field of study for the current transformation procedures for efficient gene transfer. Successful tissue culture protocols are required for *Agrobacterium*-mediated transformation. Tissue culture helps in the selection of explants since each responds differently due to differences in their endogenous hormone level and the efficiency with which transformed plants regenerate. It is a crucial parameter for selecting appropriate plantlets.[40] A critical function is played by the plant growth regulators (PGR) in the development of *in vitro* plants.[41] Their advancement and growth depend upon the type of PGRs, and the concentration varies according to the plant genotype, as one genotype's growing condition may not favour another,[42] so, it is a must to standardise the protocol. The exposure to these PGRs also varies according to the genotype that is used, and media composition determines the regeneration efficiency. The addition of exogenous nutrients such as vitamins, amino acids, inorganic nutrients, casein hydrolysate, proline and glutamine[43,44] exhibit a greater influence on somatic embryogenesis and in shoot regeneration. Temperature and light and dark exposure time also assume a major function in the *in vitro* propagation of tissue culture plants. Standardisation of these parameters paves the way for the development of the different transformation techniques and the development of a successful genetically transformed plant.

A successful transformation of *A. esculentus* is a primary requirement for the establishment of an effective tissue culture procedure.[45,46] But, only a few tissue culture reports on it are available so far. The association of auxin [indole-3-acetic acid (IAA)] and GA_3 on *A. esculentus* for the induction of adventitious root by stem cuttings was studied in a medium supplemented with both IAA and GA.[47] For callus formation and seedling establishment, different explants (hypocotyl, cotyledon, cotyledonary node and leaf segment) were employed. In the presence of naphthalene acetic acid (NAA) or indoleacetic acid, callus and root differentiation were developed. On the cotyledon and cotyledonary node explants grown in a media enriched with benzyl adenine (BAP) and naphthalene adenine (NAA), shoots were generated.[51] In another study on *A. esculentus*, callus was induced from hypocotyl and cotyledonary axil explants on kinetin-supplemented MS medium and rejuvenation of plants by supplementing benzyl adenine was observed.[48] Following this, Haider *et al.*(1993) also regenerated the wholeplant from the hypocotyl callus culture with a combination of BAP and NAA treatment.[49]

Ganesan *et al.* (2007) have also reported a tissue culture study on *A. esculentus*, they devised a method for generating somatic embryos and regenerating plants using hypocotyl explants.[50] They used 2,4-dichlorophenoxy acetic acid and NAA acid for somatic embryogenesis, on MS medium enriched with BAP and gibberellic acid (GA3), plants were regenerated. Kabir *et al.* (2008) reported that the hypocotyl and leaf disc showed 95% callus induction with the combination of BAP and NAA followed by 60.82% highest regenerated shoots from the callus.[51] Another study using hypocotyl and leaf disc was conducted in the same year, and it was discovered that the combination of NAA and thidiazuron (TDZ) produced the most morphogenic callus, with 80% regeneration from callus in the medium containing BAP and IBA (indole-3-butyric acid).[52] TDZ showed better multiple shoot regeneration through direct regeneration method.[53] Direct regeneration of apical shoot showed better response with the combination of IBA and NAA.[54]

A recent study utilizing *A. esculentus* cotyledonary leaf explants revealed a highly efficient procedure for somatic embryogenesis and rejuvenation. When L-glutamine and casein hydrolysate were added to the MS medium, the somatic embryo maturation and plant regeneration frequency were enhanced, along with successful somaticembry ogenesis from *A. esculentus* cotyledonary leaf explants and the genomicintegrity of regenerated plants being affirmed using ISSR markers.[43] A summary of the reports on *A. esculentus* regeneration *in vitro* is provided in Table 1

Table 1: Plant tissue culture and Biotechnological studies reported in *A. esculentus*.

S.No.	Explant	Type of culture	Medium	Phytohormones	References
1.	Hypocotyl and leaf segment	Callus-induced organogenesis	MS basal medium	0.5 mg/l BAP and 2.0 mg/l NAA for callus induction and 2.0 mg/l BAP + 0.1 mg/l IAA and 2.0 mg/l BAP + 0.5 mg/ l NAA gave the most effective for plant regeneration from callus	(51)
2.	Hypocotyl and leaf disc	Callus-induced organogenesis	MS basal medium	2.0 mg/l NAA plus 0.5 mg/l TDZ for callus and 2.0 mg /l BAP plus 0.1 mg/l IBA for shoot regeneration	(52)
3.	Hypocotyl	Somatic embryo genesis through suspension	MS basal medium	2.0 mg dm^{-3} 2,4-D and 1.0 mg dm^{-3} kinetin in suspension culture and 0.2 mg dm^{-3} BAP	(50)

		cultures		and 0.2 mg dm^{-3} gibberellic GA3 on half strength MS medium for regeneration	
4.	Apical shoot regeneration	Direct	MS basal medium	1.0 mg/l IBA and 0.5 mg/l NAA were found to be most effective	(54)
5.	Hypocotyl and cotyledonary axil	Callus culture	MS basal medium	Benzyl adenine (BA) (1.0 mg/l) showed rapid callus induction and presence of 10 and 20 mg/l of silver nitrate to BA (1.0 mg/l) and NAA (1.0 mg/l) significantly increased calli producing multiple shoots	(48)
6.	Stem cutting	Root formation	indole-3-acetic acid, (IAA) and GA_3	At concentrations (10 mg/l IAA + 5 mg/l GA_3) the number of roots formed was greater than the sum of roots formed in the individual treatments	(47)
7.	Hypocotyl, cotyledon, cotyledonary node and leaf segment	Callus formation and root differentiation	MS basal medium	Shoot, root and callus development on cotyledonary node explant cultured on MS medium supplemented with NAA (1.0 mg/l) and BA (1.0 mg/l)	(45)
8.	Hypocotyl	Direct and indirect organogenesis	MS basal medium	1–3 mg/l benzyl adenine (BA) and 0.1–0.3 mg/l NAA induced direct shoot organogenesis and explants indirect shoot organogenesis 0.1–0.3 mg/l BA and 1–3 mg/l NAA supported callus growth followed by addition of 1 mg/l BA developed shoots from callus	(49)
9.	Cotyledonary Node	Direct organogenesis	MS basal medium	0.01 mg/l TDZ found to be best for multiple shoot induction and 0.5 mg/l IBA showed successful root induction in excised micro-shoots	(53)
10.	Cotyledonary leaf	Somatic embryogenesis	MS basal medium	1.5 mg/l 2,4-D and 1 mg/l NAA along with 400 mg/l L-glutamine, 300 mg/l casein hydrolysate and half strength MS medium 1 mg/l BAP and 0.5 mg/l GA3 were used for regeneration and shoot elongation	(43)
11.	Seed embryo	Agrobacterium-mediated transformation	MS basal medium	MS salts, B5 vitamins, zeatin 2 mg/l, agar 0.8%, sucrose 3%, kanamycin 50 mg/l, cefotaxime 500 mg/l and cry1Ac gene used for *Agrobacterium* transformation	(13)
12.	Seeds	*Agrobacterium*-mediated transformation	MS basal medium	MS basal medium containing 250 mg/l cefotaxime and 15 mg/l BASTA. *Agrobacterium tumefaciens* strain EHA105 harbouring	(78)

				pCAMBIA 1301 were used for transformation	
13.	Seed embryo	*Agrobacterium*-Mediated Genetic Transformation	MS basal medium	*Agrobacterium tumefaciens* strain EHA105 was carrying cry1Ac gene against fruit and shoot borer, CaMV 35S as promoter and nptII as plant selectable marker gene	(92)
14.	Pre-cultured seeds	*Agrobacterium*-Mediated Genetic Transformation	MS basal medium	*Agrobacterium*-mediated transformation was performed using LBA4404 strain harbouring the binary vector pBinAR carrying cry3a gene under the control of CaMV35s promoter and npt II gene as a selectable marker	(93)
15.	Seed embryo	*Agrobacterium*-Mediated Genetic Transformation	MS basal medium	cry1Ac gene was borne on the T-DNA of one plasmid while nptII and uidA (GUS) marker genes	(14)

***Bacillus thuringiensis* (*Bt*) Crystal Protein Gene**

Bacillus thuringiensis (*Bt*) is the bacteria that produces *Bt* toxin. Cloning and transformation of the *Bt* toxin genes were done and introduced into host plants, where they were expressed and provided resistance to insects without the need for insecticides.[55] *B. thuringiensis* (*Bt*) crystal proteins have a low toxicity to vertebrates towards non-target organisms, making the man environment friendly alternative to conventional insecticides. They have an essential part in the development of pest resistance being an important tool in modern biotechnology for creating transgenic plants for integrated pest management strategies, where these techniques are utilized with maximum efficiency.[56,57,58] The initial case reported for pesticide resistance was in 1948, and that synthetic insecticide (DDT) was used against insect pests, and six years into its implementation, the population of insecticide-resistant bugs had skyrocketed.[59] Using *Agrobacterium tumefaciens* strain (C58Clrif) bearing the pGV3850: pAK1003 Ti plasmid, Feldmann and Marks(1987) successfully established the first *Agrobacterium*-mediated transformation in *Arabidopsis thaliana.*[60] In order to eliminate specific insect pests as well as reduce the reliance on chemical pesticides, transgenic crops are developed by transforming *B. thuringiensis* (*Bt*) toxins into the host plant.[61] Several transformation works have been carried out by researchers by developing various transgenic plants, including crops of vegetables such as brinjal, tomato, cotton, radish, snake gourd and soybean.[62,63,64,65,66,67,68,69] The role of *Bt* toxins(δ-endotoxins) inside the insect is as follows: solubilization in the midgut once ingested and activation of the gut protease enzymes, cleaving the proteins into a lesser-polypeptides, then, the epithelial cells in the mid gut surface get bound in specific target sites by these toxins and kill the pest.[70,71,72,73,74] The most often utilised *Bt* toxins belong to the Cry1 A family, especially Cry1 Ac in transgenic *Bt* cotton and Cry1Ab in transgenic *Bt* corn.[75]

***Agrobacterium tumefaciens*-Mediated Transformation of A. esculentus**

It is critical to develop *Agrobacterium*-mediated transformation in *A. esculentus* because it may be utilised to introduce *Bt* genes into this crop for pest resistance. *A. esculentus* has a significant set of chromosomes (2n=130) in its genome.[76,77] Both *A. esculentus* and cotton (Gossypium sp.) are members of the Malvaceae family, however, only very few transformation works have been done on *A. esculentus,*[78] whereas many have been carried out on genetic transformation of cotton.[79, 80,81,82,83,84,85,86,87,88] The protocols are genotype-dependent throughout the time periods for the varying transgenic plant's regeneration. Transformation mediated by *A. tumefaciens* can aid in DNA transfer to organisms on awide range alongside several monocot and dicot taxa.[89,90,91]

The first report on *Agrobacterium*-mediated transformation method for *A. esculentus* using transgenic *Bt* plants exhibited tolerance to *Earias vittella*, the target pest that was afruit and shoot borer of *A. esculentus* (Narendran *et al.* 2013) (table 1).

Narendran *et al.* (2013) in their study used CAMBIA 2300, a plasmid containing the Cry1Ac gene that was regulated by an improved CaMV 35S promoter along with a selectable marker for plants in the T-DNA, the gene *nptII*. Plasmid was introduced into the *A. tumefaciens* strain EHA105. Embryos were isolated from *A. esculentus* and punctured for 2–3 times on the plumule area with a syringe needle (23G, 100, 0.6 9 25 mm), followed by inoculation intoa suspension of *Agrobacterium* (EHA105 carrying the Cry1Ac and *nptII* genes), and 20 embryos per petri dish were transferred at a time. Monoclonal antibodies specific to the Cry1Ac protein, coated on an ELISA plate, were used to assess the transgenic plants for Cry1Ac protein expression. The *Bt* Cry1Ac protein was effectively altered, according to Molecular and Genetic Analysis utilising polymerase chain reaction (PCR) and southern hybridization experiments. The insect bioassay study showed mortality of about 83.33 and 100.00 % in the transgenic fruits.[13]

In another study, successful transformation was done on *A. esculentus* genotype Arka Anamika. For this experiment, *A. tumefaciens* EHA 105 was utilised, which carried the binary vector pCAMBIA 1301–bar. Genomic DNA was extracted from 45-day-old transformed *A. esculentus* leaves. Basta resistance was checked on the transformed and non-transformed control *A. esculentus* for herbicide tolerance in transgenic plants.[78] *Agrobacterium*-mediated genetic transformation was used to establish a method for genetic transformation in okra. Explants for transformation were imbibed seed embryos pierced from the meristematic area of the plumule. Cry1Ac gene against fruit and shoot borer, CaMV 35S promoter, and *nptII* plant selectable marker gene were all carried out by *A. tumefaciens* strain EHA105. The potential transgenic plants were validated by polymerase chain reaction to amplify the transgene.[92] A study used LBA4404 strain to undergo *Agrobacterium*-mediated transformations, with the binary vector pB in AR carrying the cry3a gene under the control of the CaMV35s promoter and the *npt* II gene as a selectable marker. The transformation event, which included sonicating the explants for 3 minutes, vacuum infiltration (750 mm Hg) for 2 minutes in *Agrobacterium* (pBinAR-cry3a) and co-cultivation in MS medium with acetosyringone (100M) for 3 days, yielded a 12.5-percent transformation efficiency. Polymerase chain reaction (PCR) was used to confirm the presence and integration of the *npt* II and cry3a transgenes into the *A. esculentus* genome.[93]

Agrobacterium-mediated co-transformation was used to create insect-resistant transgenic okra plants expressing the cry1Ac gene that was free of markers. The cry1Ac gene was found on one plasmid's T-DNA, while the *nptII* and *uidA* (GUS) marker genes were found on the T-DNA of second plasmid. The larvae of the shoot and fruit borer, *Earias vittella*, a significant okra pest, were used to rigorously screen the plants from selected transgenic events in whole plant insect bioassays. Insect bioassays revealed 100 percent larval mortality without infestation in five of the transgenic events, and 5 to 10% infestation in two others, demonstrating the transgenic plants' insect resistance.[14]

These plant transformation methods hold much promise to develop insect-resistant *A. esculentus* for target resistance against insect pests. Commercial insecticides will not be as effective as a well-designed genetic transformation method for *A. esculentus*.

CRISPR/Cas9 Transformation

When it comes to plants, the majority of the early CRISPR/Cas9 findings focused on genome editing. Feng *et al.*, reported the CRISPR/Cas9 investigation with targeted site analysis in different plant taxa.[94] Research using *Bombyx mori* as a model organism for CRISPR/Cas9 technology is driving the evolution of Lepidoptera.[95] These studies provided strong evidence that CRISPR/Cas9 system could be employed in *A. esculentus* plants for insect resistance. The conventional transgenic technique may lack the inheritance of stability due to the introduced genes may get lost or silent within a few generations, CRISPR/Cas9 system will likely be a promising alternative to the conventional transgenic approach and will deliver good results in the field.

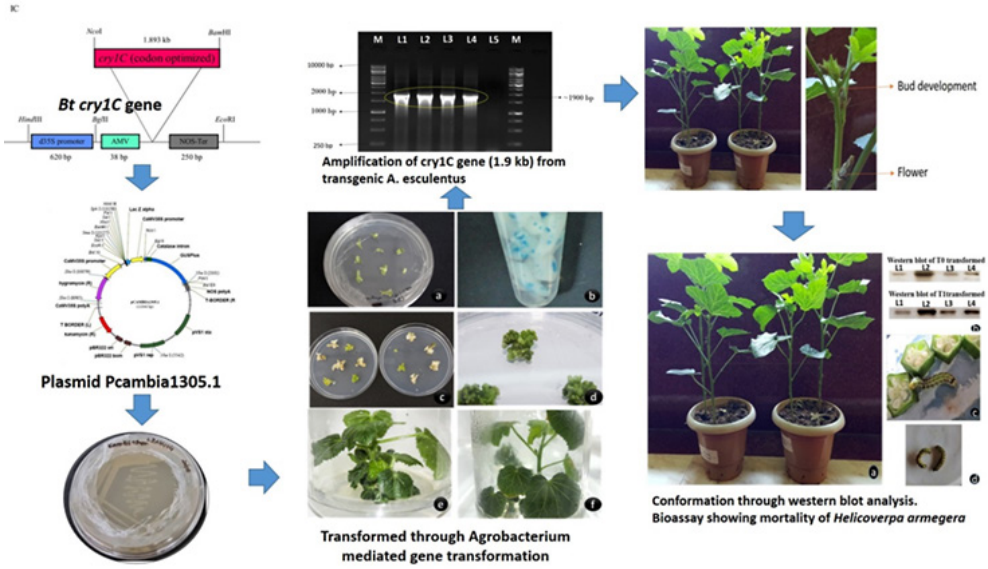

Fig 1: Figure shows the *Agrobacterium*-mediated gene transfer for insect resistance, confirmation and insect bioassay of *A. esculentus* in our laboratory

Conclusion

This review gives a detailed report on the works carried out for insect resistance in okra using different genes and the various explants and their response to different auxins and cytokinins for the rapid growth and development, and rejuvenation and transformation of okra through *Agrobacterium* provide valuable information to the scientific community for further studies in this important vegetable crop. Screening of different genotypes of *A. esculentus* using *in vitro* studies will help isolate elite genotypes for future transformation studies. Further, the *Agrobacterium*-mediated transformation system will be extremely beneficial in introducing useful genes into *A. esculentus* for improving the yield by providing biotic and abiotic stress resistance. The insect-resistant *A. esculentus* plant will also significantly minimize synthetic pesticides usage as well as enhance small-scale farmer's economy who largely depend on this important vegetable crop for their livelihood.

In recent years, and still today, transgenic plants have garnered a lot of media interest. Despite this, the general public still doesn't fully understand what a GM plant is or the benefits of the technology. Many government agencies have strict regulations in place for transgenic crops. The specifications for a thorough risk assessment of transgenic plants and any related food and feed have been outlined by the European Food Safety Authority.[101] For more than 15 years, hundreds of millions of people have consumed foods made from transgenic crops without any negative side effects being noted.

Author's Contribution

All the authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Dr Melvin A Daniel, Dr V. Duraipandiyan and Dr S. Maria Packiam. The first draft of the manuscript was written by Dr Melvin A Daniel, and all the authors commented on previous versions of the manuscript. All the authors read and approved the final manuscript.

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Conflict of Interest

The authors do not have any conflict of interest.

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Abbreviations

Bt: *Bacillus thuringiensis*
IAA: Indole-3-acetic acid
GA: Gibberellic acid
NAA: 1-Naphthaleneacetic acid
BAP: 6-Benzylaminopurine
2,4-D: 2,4-Dichlorophenoxyacetic acid
IBA: Indole 3-Butyric Acid
TDZ: Thidiazuron
MS: Murashige and Skoog's medium
ISSR: Inter-simple sequence repeat
PCR: Polymerase chain reaction