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Genetic engineering in Banana and Plantain

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Abstract

Bananas are one among the world's leading food crops, after rice, wheat and maize. Almost ninety percent of production is consumed in the production areas, especially in the poorest countries in Africa, Latin America and Asia. In certain regions, pureed banana is the first solid food given to infants. Bananas contribute to reducing food insecurity in producer country populations. Their composition, which includes high carbohydrates and minerals, makes them a staple calorie resource for over 500 million inhabitants of tropical countries. Considering the nutrition aspect, it is the world's leading fruit crop, and in terms of economical value it is ranked as fifth economically important agricultural crop in world trade. In the global production of banana India contributes 29.19% as leading country.

Bananas face numerous environmental challenges, particularly with fungal, bacterial as well as the major threatening disease like banana bunchy top virus. The problem is further aggravated by the limited diversity of banana cultivars around the world. Conventional breeding methods have limited success due to low female fertility, sterility, ploidy levels and poor seed set, besides the process is time consuming. These problems point to the necessity of developing alternate strategies for banana improvement through advancement of biotechnology tools like tissue culture and transgenic technology to improve the bananas. In this regard I will be discussing the current status of Banana improvement using biotechnology and future prospects.

Keywords: Bananas; Plantain; Biotechnology; *Agrobacterium*

Introduction

Banana belongs to the family *Musaceae*. Members of the genus Musa were considered to be derived from the wild diploid species of *Musa acuminata* (*AA*) and *Musa bulbisiana* (*BB*). Banana serves as the staple food for approximately 500 million people in the world (INIBAP, 2000). It is a popular commercial fruit crop grown all over the world in 132 countries with average productivity of 15,730 kg/ha. India is the largest producer of bananas in the world with a production of approximately 29.19 million tonnes from an area of 0.57 million hectare contributing 19.71 % to the global production.

In Tamil Nadu banana is cultivated over 81,498 ha with a production of 34.62 lakh tonnes Singh [1]. Banana cultivation is affected by various diseases. Among them, bunchy top disease caused by the banana bunchy top virus (BBTV) and *Fusarium* wilt caused by the fungus, *Fusarium oxysporum f. sp*. Cubense are the most serious diseases. Conventional breeding is not successful in imparting disease resistance due to long generation time, various levels of ploidy, lack of genetic variability and sterility of most edible cultivars.

Materials and Methods

Banana gene delivery targeted explant: Embryogenic cell line system

Banana is known to be a recalcitrant crop for tissue culture and regeneration. Several explants have been tried for callus induction viz., Immature male flower bud, scalp, leaf sheath, rhizome etc. Among them immature male flower bud is known to respond well and produce higher proportion of embryogenic calli with high regeneration potential [2-5]. Banana suspension is commonly employed for the multiplication of regenerable embryogenic cells of embryogenic calli, that will be later used for the induction of somatic embryos followed by regeneration into whole plant [3,4,6-10] (Figure 1).

Gene delivery methods in Banana

There are several reports now available on the genetic transformation of banana [5,11-13] even then still it is far from routine in most of the labs due to long incubation time in the callus induction, difficulty in developing regenerable embryogenic cell suspension, prolonged incubation in the selection medium with very low potential to regenerate in the presence of a selection agent. Agrobacterium-mediated transformation is a method of choice for banana transformation. Few reports are also available on the Biolistic gene gun-mediated transformation of banana [14,15] (Figure 2).

Results of Genetic Engineering in Banana and Plantain

Agrobacterium-mediated transformation of Banana

Sagi [16] suggested a method that combined both Agrobacterium and micro projectile bombardment methods. Apical meristems and underlying corm tissues were bombarded with naked gold particles and then infected with *Agrobacterium*. Tissues were allowed to heal for three days on a non-selective regeneration medium. Tissues after recovery were co-cultivated for 30 min with 16 h culture of *Agrobacterium* strain, *LBA4404* harboring PNI 141 vector. Tissues were then transferred to non-selective medium containing acetosyringone and incubated for three days and transferred to regeneration medium with selection agent to recover transgenic plants.

Hernandez et al. [17] studied chemotaxis of *Agrobacterium* towards banana tissue. Excised and *in vitro* proliferating tissues from different land races were able to elicit a positive chemotactic reaction. Presence of bacteria individually bound or massively attached to single banana cell and tissues was demonstrated. Dugdale et al. [15] assessed

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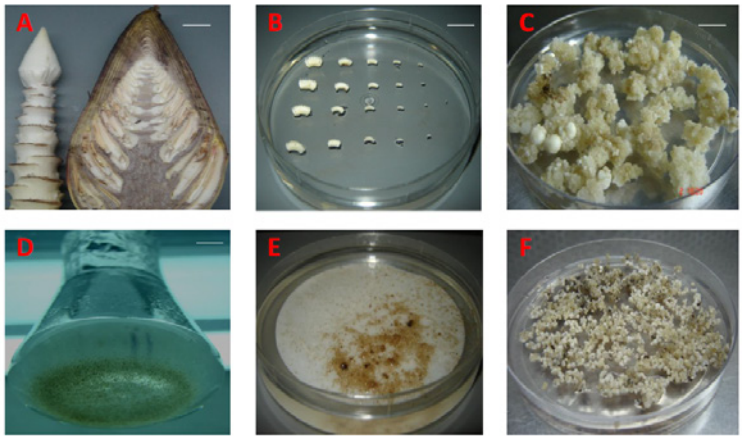

Figure 1: Banana embryogenic cell line developmental stage, banana male flower to embryogenic cell line; 11-12 months for initiation of embryogenic cell lines.

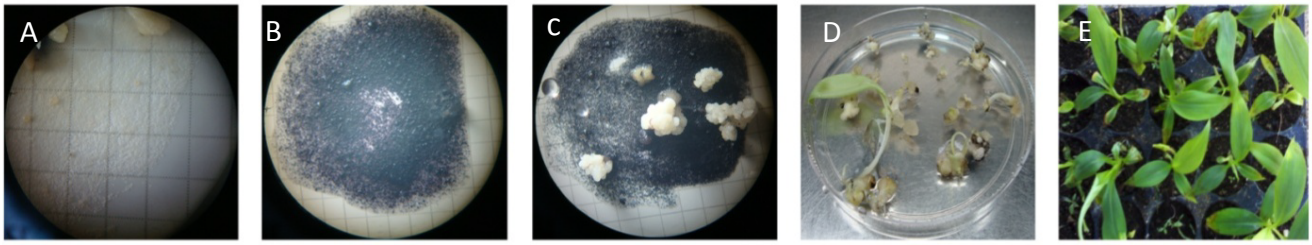

Figure 2: Gene delivery methods in Banana: Agrobacterium mediated gene transformation system in banana. A: Co-culturing of ECS; B: Selection and death of non-transformed ECS; C: Transformed ECS to induction of somatic embryos; D: Germination of transformed Somatic embryo; E: Hardening of transformed plants, molecular analysis of the plants and bioassay.

promoter activity in transgenic banana (*Musa sp. Bluggoe*) containing the intergenic regions of banana bunchy top virus DNA 1 to DNA 5 fused to Green Fluorescent Protein (GFP) and uid A reporter gene.

Ganapathi et al. [5] achieved Agrobacterium-mediated transformation of embryogenic cell suspensions of banana cv. Rasthali (AAB) using strain EHA containing the binary vector *pVGSUN* with acetolactase synthase (*ALS*) gene as a selectable marker and gusA reporter gene. Pineda et al. [18] reported that ECS of Dominico hartón (AAB) was infected with the *Agrobacterium* strain *AT650/pLIGh*, which has the binary plasmid *pLIGH* harboring two chimeric reporter genes *gusA* and *hph*. Further they demonstrated transient GUS expression and regenerated putatively transformed lines on a selective medium.

Chakrabarti et al. [19] reported the Agrobacterium-mediated transformation of Rasthali (AAB) using plant expression binary vectors *pMSI164* and *pMSI168*, imparting fungal disease resistance. Transgenic banana plants were obtained for both *pMSI164* and *pMSI168* transformations and showed resistance to *F. oxysporum f.sp.* cubense and *Mycosphaerella musicola*. The transgenic nature of the transformants and expression of the peptide was confirmed through Polymerase Chain Reaction (PCR) and Reverse Transcription (RT)-PCR.

Khanna et al. [12] carried out Centrifugation Assisted Agrobacterium-Mediated Transformation (CAAT) of ECS of banana (Musa cv. Cavendish AAA and Lady Finger AAB) using strain AGL1 and LBA 4401 binary vectors carrying *hpt, gusA* and modified GFP (Green Fluorescent Protein). Escoffie et al. [20] evaluated two Agrobacterium-mediated plant transformation protocols for the generation of transgenic banana tissues (*Musa acuminata* var. Grand Naine).

Co-cultivation versus vacuum infiltration of meristematic banana tissues was compared. The binary vector *pCAMBIA* 2301 was used for the initial transformation and the histochemical detection of GUS. PCR assays demonstrated that the transformation protocol was successful. Infiltrated samples transformed with *pCAMBIA* 2301 showed a wider GUS response than the co-cultivated tissues. The specific β-glucuronidase activity was also higher in the infiltrated tissues than in co-cultivated ones.

Kumar et al. [21] achieved expression of Hepatitis B surface antigen in banana fruits through Agrobacterium-mediated gene transfer using embryogenic cell suspension of Rasthali (AAB). Tripathi et al. [22] reported Agrobacterium-mediated transformation of plantain *Musa sp. Agbagba* (AAB) using shoot tips as the explant. *Agrobacterium* strain *EHA105* with the binary vector *pCAMBIA* 1201, having the hygromycin resistance gene as a selection marker and *GUS-INT* as a reporter gene was used in this study. Transient expression of the β glucuronidase (uid A) gene was achieved in transformed apical shoot tips. The hygromycin resistant shoots were regenerated four to five weeks after co-cultivation of explants with Agrobacterium.

Human lysozyme (HL) inhibits *Fusarium oxysporum* (FocR4) growth *in vitro* Wang et al. [23]. To obtain transgenic bananas (*Musa spp.*) that are resistant to Panama wilt (*F. oxysporum*), Pei et al. [24] introduced an HL gene that is driven by a constitutive CaMV 35S

promoter into the banana via Agrobacterium-mediated transformation with the assistance of particle bombardment. In this study 51 individual transgenic plants harboring the HL gene were obtained and 24 displayed resistance to FocR4 in the early vegetative growth stage. Hence, Agrobacterium-mediated transformation with the assistance of particle bombardment is a way in which a larger number of transgenic banana plants may be obtained.

A high efficient Agrobacterium-mediated transformation protocol of *Musa acuminate cv.Mas* (AA) was developed by Huang et al. [25] (2007). They co-cultivated Male flower derived ECS in liquid medium with Agrobacterium strain EHA105 harboring a binary vector *pCAMBIA2301* carrying nptII and gusA gene in the T-DNA. Depending upon the conditions and duration of co-cultivation in liquid and semisolid medium, the liquid medium was significantly superior to semi solid medium in quantity of initial explant used, duration of transgenic plant recovery and transformation efficiency. Transgenic plants were not obtained in parallel experiments carried on semi-solid media. Histochemical GUS assay and molecular analysis in several tissues of the transgenic plants demonstrated that foreign genes were stably integrated into the banana genome.

Khanna et al. [26] reported a 100% transformation efficient protocol by introducing a novel animal derived anti apoptotic genes in Banana ECS. They presented evidence for *Agrobacterium* induced cell death in banana cell suspensions and importantly, they inhibited *Agrobacterium* induced cell death by expressing the animal antiapoptosis genes *Bcl-xL, Bcl-2 Bcl-3* untranslated region, and CED-9. Inhibition of cell death resulted in upto 90% of cell clumps transformed with *Bcl-xL*, a 100-fold enhancement over vector controls, approaching the transformation and regeneration of every transformable cell.

Using immature male flowers of Cavendish banana cultivar Robusta (AAA) Ghosh et al. [9] established ECS. ECS obtained was co-cultivated under different co-cultivation conditions with Agrobacterium strain EHA105 harboring *pCAMBIA* 1301 plant expression vector. Up to 30 transgenic plants/50 mg of settled cell volume (SCV) was obtained with co-cultivation in semisolid medium whereas no transgenic could be obtained with parallel experiments carried out in liquid medium.

Elayabalan et al. [10] reported for RNAi technology to impart BBTV resistance in banana. One of the most severe viral diseases of hill banana is caused by banana bunchy top virus (BBTV), a nanovirus transmitted by the aphid Pentalonia nigronervosa. In this study, we reported the Agrobacterium-mediated transformation on a highly valued hill banana cultivar Virupakshi (AAB) for resistance to BBTV disease. The target of the RNA interference (RNAi) is the rep gene, encoded by the BBTV-DNA1. In order to develop RNAi construct targeting the BBTV rep gene, the full-length rep gene of 870 bp was polymerase chain reaction amplified from BBTV infected hill banana sample DNA, cloned and confirmed by DNA sequencing.

The partial rep gene fragment was cloned in sense and anti sense orientation in the RNAi intermediate vector, *pSTARLING-A*. After cloning in *pSTARLING-A*, the cloned RNAi gene cassette was released by NotI enzyme digestion and cloned into the NotI site of binary vector, *pART27*. Two different explants, embryogenic cells and embryogenic cell suspension derived microcalli were used for co-cultivation. Selection was done in presence of 100 mg/L kanamycin. In total, 143 putative transgenic hill banana lines were generated and established in green house condition. The presence of the transgenes was confirmed in the selected putative transgenic hill banana lines by PCR and reverse transcription PCR analyses. Transgenic hill banana plants expressing RNAi-BBTV rep were obtained and shown to resist infection by BBTV. The transformed plants are symptomless, and the replication of challenge BBTV almost completely suppressed. Hence, the RNAi mediating resistances were shown to be effective management of BBTV in hill banana.

Particle bombardment/Biolistic-mediated transformation of Banana

Ma et al. [24] transformed somatic embryos of banana cv Grand Naine, through particle bombardment using of a chimeric *β-glucuronidase* (GUS). Somatic embryos of multiplication stage were bombarded with microprojectile coated with PBPFA5 and pDH 1001 plasmid. Dugdale et al. [15] transformed cv Grand Naine using micro projectile bombardment. When assayed for gus expression, all plants transformed with pUGR73 showed high expression.

Sagi et al. [16] (2000) were able to transform Cavendish banana (*Musa sp*. AAA group cv Grand Naine) via micro projectile bombardment based on three lines of evidences, the antibiotic-resistant phenotype, stable expression of the *gusA* reporter gene in various plant tissues, and the presence and stable integration of the introduced genes in transformed plants.

Matsumatto et al. [27] developed a simple and routine particle bombardment system to generate transgenic bananas using a herbicide resistance gene as selectable marker. Embryogenic cells were bombarded with a plasmid vector compiling the AHAS gene, under the control of the AHAS promoter from *Arabidopsis thaliana*. The bombarded cells were cultured with the herbicide containing medium for 30 d, and putative transformed plants were regenerated.

A plant regeneration method with cell suspension cultures of banana, and the effect of biobalistic on regeneration potential was described by Kido et al. [28]. Cell transformation using particle bombardment with three different plasmids containing the uid-A gene, resulted in a strong GUS expression five days after bombardment; however, plant regeneration from bombarded cells was much lower than non bombarded ones.

Conclusion

Banana crop faces numerous environmental challenges, particularly with fungal and bacterial pathogens as well as pests and abiotic stresses. The problem is aggravated by the limited diversity of cultivars. Conventional breeding methods have limited success in banana crop improvement due to low female fertility, sterility, ploidy levels and poor seed set; besides the process is time consuming.

For many banana diseases like Fusarium wilt, BBTV, CMV and moko disease and no chemical treatment exists, and destruction of the affected plants is the only method of control. For the others, the cost of chemical treatment is increasing as more virulent strains appear, or even prohibitive for small farmers in developing countries or else damaging to the environment. These problems point to the necessity of developing alternate strategies for banana improvement. Biotechnological approaches such as tissue culture and genetic transformation has the potential to overcome this important disease.

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