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Original Article

MICROPROPAGATION IN BANANA USING INFLORESCENCE: A REVIEW

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Abstract: ***Bananas and plantains are the major fruit crops belonging to the genus Musa and they provide a well-balanced diet to millions of people. Demand of tissue cultured plants of banana is increasing rapidly for disease free planting material, crop improvement and genetic transformation. The male flower tissue culture technology either through direct organogenesis, indirect organogenesis or somatic embryogenesis creates an environment to provide disease free planting material by mass multiplication and genetic improvement. The purpose of this review is to focus on advances made in male flower tissue culture technology that could be used in the production of disease free planting material and crop improvement.***

Key words: Banana, Inflorescence, Somatic embryogenesis

INTRODUCTION

Bananas and plantains are herbaceous perennial monocots that belong to *Musa* genus of Musaceae family, cultivated in more than 130 countries of the tropics and subtropics. Banana (*Musa* spp.) is the fourth most important food crop after rice, wheat and maize [1], originated in Southeast Asia. Apart from providing a well balanced diet to millions of people the crop also provides livelihood opportunities through crop production, processing and marketing. Unfortunately, banana cultivation is hampered by several diseases and pests, largely due to poor quality clones. In banana, conventional breeding remains a difficult and time consuming process because of high sterility, polyploidy, long generation time and vegetative propagation. Biotechnology involving tissue culture and genetic transformation provides an opportunity to develop new germplasm [2]. The widely used male flower tissue culture technology either through direct organogenesis, indirect organogenesis or somatic embryogenesis, creates an environment to provide disease free planting material by mass multiplication and genetic improvement. Use of male inflorescence as explant for propagation and genetic improvement is a non-destructive method utilizing seasonal structures of the plant thus conserving the mother plant and also rescuing the endangered species.

Vegetative apices from rhizomes of banana have been found to be successful explants in tissue culture. The apical meristem of rhizome as explants had limitations like difficulty in excision and hence floral apex of male bud is an alternative [3]. Banana inflorescence proliferation is used to study the effect of plant growth regulators on organ development [4]. Though disease free micro propagation of banana is

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toilsome, the banana male inflorescence when used as explants in the tissue culture would result in banana bunchy top virus (BBTV), banana streak virus (BSV), banana bract mosaic virus (BBrMV) and cassava mosaic virus (CMV) free plantlets [5]. Micropropagation using male floral meristems showed less or no somaclonal variation as compared to soil grown suckers. There is an opportunity to select male buds with desirable character such as number of hands and fruits per bunch [6]. Hundreds of clones can be obtained successfully from a single flower of inflorescence [7].

The incidence of numerous diseases such as fusarium wilt (*Fusarium oxysporum* var. *cubense*), black sigatoka (*Mycosphaerella fijiensis*), viruses (BBTV, BSV, BBrMV and CMV) nematodes affects the crop production necessitating the need for developing improved varieties. New approaches such as *in vitro* culture and molecular biology techniques can be a complement breeding technique for banana. Genetic transformation is the technique of introducing foreign genes into the plant system, and this requires successful *in vitro* culture system and regeneration of new plants. Somatic embryogenesis or cellular suspension system is the best technique to produce *in vitro* culture system for genetic transformation [8,9,;10]. Different explants were used for somatic embryogenesis in banana such as proliferating meristems [11], young zygotic embryos [8,12,13], basal leaf sheaths and rhizome tissues from *in vitro* plants [9], thin sections from proliferating buds [10]. Among the different explants used, immature male flowers and scalps can turn out to be the most responsive material for initiating embryogenic cell suspension cultures [14].

In this review, we provide an overview on the usefulness of inflorescence in the development of banana plantlets through direct organogenesis, indirect organogenesis and somatic embryogenesis for mass multiplication, somaclonal variation and genetic transformation.

Shoot buds formation/ direct organogenesis: Direct organogenesis using male inflorescence can be mainly used for the micropropagation of disease free plantlets, since rhizome culture fails to come out with disease free micropropgation. Less somaclonal variation, selection of male buds with desirable character such as number of hands and fruit per bunch, and production of hundred clones from a single male bud may be briefed as the other advantage of using male inflorescence for micropropagation.

Size of explants: Innermost male flower buds are used in the flower culture after their bracts have been carefully removed. The size of the explants plays a key role in the successful initiation and establishment of cultures. Doreswamy and Sahijram [3] used male flower apical knob of 3cm for initial culturing in MS media supplemented with 160 mg/l ADS, 5 mg/l BA and 5mg/l IBA. After one month, 0.5mm floral apex was excised and used as explant. These 0.5 mm apices turned brownish in 4-5 days and two months later green globular mass developed, whereas 5 to10 mm long apices didn't turn brownish and developed a green shoot like primordia after 3 to 4 weeks. Darvari et al. [15] reported that the number of cauliflower like bodies clusters increased when the male inflorescence with the size of 20 mm was used for micropropagation. Male flower cluster of 5.5 to 0.8 cm were decapitated and used for inflorescence proliferation and induction of shoot buds [5]. Explants size of 1 to 5 mm were inoculated for shoot proliferation [16]. 4 to 6 cm male buds were surface sterilized and individual male flowers of size 15 to 30 mm were kept for culture [6]. Apical, central and basal male inflorescence had no significant difference in the *in vitro* flower bud cluster development [5].

Contamination free cultures of cv. Kanthali were obtained by treating the floral bud apices with 0.1% $HgCl_2$ for 6 min. Browning due to phenolic exudation was reported in many cultivars of banana, and this phenomenon was observed in cv. Kanthali tissue. This issue can be greatly reduced by pre-soaking of explants in antioxidant solution of 0.125% (w/v) potassium citrate and citrate prior to culture. Browning can also be reduced by incubation of culture in the dark for the first one week, which leads to the downfall of the rate of tissue browning by slowing the enzymatic activity responsible for tissue oxidation. Frequent subculturing to fresh medium cut downs the toxic phenolic compounds, so they do not hinder the activity of plant growth regulators on tissues. The combined treatment will eradicate the phenolic exudation effectively than single treatment [17,18].

Media composition: Media composition greatly influenced the rate of multiplication of male inflorescence and days to which it is produced. Bernando and Purificacion [19] and Perez Hernandez and Rosell-Garcia [20] reported highest multiplication

rate of Cavendish (AAA) male inflorescence in MS basic media with 5 µM TDZ. More number of flower bud clusters were obtained in combination of 2 µM NAA and 5 µM TDZ [5].

Greenish white globular masses were obtained when 'Chandrabale' was cultured in MS supplemented with ADS (160 mg/l), BA (1.13 µM) and IBA (1.02 µM); Rasthali in ADS (2 mg/l), BA (1.13 µM) and IBA (1.02 µM); and Robusta in BA (2.25 µM) and IBA (1.02 µM) [3]. Terminal flower apices of banana cultivar 'Dwarf Cavendish (AAA)' produced multiple shoot clusters in MS media supplemented with 22.2 µM BA and 10% (v/v) coconut water [21]. Resmi and Nair [16] reported that 8.9 µM BA induced multiple shoots on Sannachenkadali (AA); 22.2 µM BA on Red banana (AAA); and 11.4 µM IAA and 17.8 µM BA induced shoot proliferation in triploid cultivars. Lower concentration of BA produced highest number of multiple shoots in diploid cultivar, but same response was observed in triploid cultivars in media with high concentration of BA. Darvari et al. [15] reported that different concentration of cytokinin (TDZ) and the cultivar affects the production of 'cauliflower like bodies' clusters. Banana cv. 'Berangan' showed the highest 'CLBs' cluster induction at 0.4 mg/l of TDZ, Rastali and Nangka at 0.6 mg/l and Abu at 0.8 mg/l. The MS medium supplemented with 8 mg/l of BAP induced the highest 'CLBs' cluster in all the cultivars. Multiple shoot formation and elongation obtained from the male flower buds cultured from *Musa beccarii* (type of ornamental banana under the section of Callimusa) on MS basal medium with 0.014 mg/l BAP, charcoal and coconut water [22]. Punyarani et al. [5] observed direct shoot induction when MS medium was supplemented with TDZ (5 µM) and NAA (20 µM) from the base of male inflorescence without callus phase after two weeks in culture. Hrahsel et al. [6] obtained white bud like structures within 5 to 7 weeks in MS medium supplemented with lower concentration of BA (0.45 µM) and combination with NAA (0.09 µM). Subsequently shoot formation was highest in media containing kinetin (0.43 µM) and NAA (0.09 µM).

Cytokinins have been found to reduce the dominance of apical meristems and induce axillary shoots, as well as formation of adventitious shoot from meristematic explants [23]. The type of cytokinin, concentration of cytokinin and genotype influences shoot proliferation or 'CLB' cluster formation. The multiplication rate of the male flowers was found to be significantly dependent on the type of cytokinins. Cytokinins are pre-requisite of male flower regeneration [15]. BA has the capacity to induce multiple shoots in banana rather than any other cytokinin [24,25]. The cultivars bearing the A genome showed higher rates of multiplication than those with B genome [26].

Indirect organogenesis: Genetic improvement of banana through conventional methods is difficult due to the constraints such as sterility and vegetative propagation. Mutation breeding is one of the breeding methods that can be resort to for the genetic improvement of banana. But the main limitation of this technique is presence of chimeras, which can be overcome by indirect organogenesis through selection of somaclonal variants [27]. *In vitro* regeneration *via* callus culture is not only important for genetic transformation, but also for the selection of useful somaclonal variants.

The male flowers of bracts numbering 24 to 26 gave best response to callus induction in cv. Sabri and maximum (20%) callusing was found in the medium containing 2 mg/l 2, 4-D, 0.5 mg/l NAA and 0.5 mg/l IAA , but 2, 4-D alone did not induce any callusing [27]. Immature male inflorescence of banana cv. Puttabale produced higher callogenic masses in media consisting of MS with 40 g/l sucrose, 8 g/l agar, 160 mg/l adenine sulphate, 100 mg/l tyrosine, 7 mg/l 2,4-D and 0.5 mg/l BA at pH 5.8 incubated at 25$\pm$2 ^{o}C with 12 hrs photoperiod. Callogenesis was proceeded all over the surface of explants and appeared as whitish transparent flashy juicy and compact callus [28]. Effect of 2,4-D was studied for callus formation in media which contained, MS plus 1 mg/l NAA, 1 mg/l biotin and 30 g/l sucrose, and found that the media with 2 or 4 mg/l 2,4-D induced callus. 2,4-D was essential for callus formation in cultivars such as Kluai Namwa, Kluai Hakmuk and Kluai Khai, but vs. like Kluai Hin, Kluai Hom Thong, Tumok and Lep Mue Nang revealed callus formation without 2,4-D in the media [29]. The maximum response on shoot differentiation from callus in cv. Sabri was found in MS supplemented with 1 mg/l BA, 0.5 mg/l IAA and 500 mg/l caesin hydrolysate (Sultan et al., 2011). Shoot differentiated from the callus of cv. Puttabale in media consisted of MS with 160 mg/l adenine sulphate, 100 mg/l tyrosine, 4 mg/l BA and 0.4 mg/l TDZ [28].

Sugiyono et al. [30] reported that time taken for callus formation and percentage of callus formation depended on cultivar. Among the different cultivars studied 'Raja' was found to be most responsive cultivar leading to the highest percentage of callus formation and short callus formation time. On the development of callus formation, 2,4-D and IAA had no significant difference on both callus formation time and percentage of callus formation, but 2,4-D application resulted in higher percentage of embryogenic callus formation and IAA application resulted in least expected senescence callus formation.

Somatic embryogenesis The valuable technology applicable for the development of embryogenic cell suspensions is from immature flowers in seedless cultivars [31-34]. The development of embryogenic cell suspensions can be used for high performance micropropagation and cell regeneration system useful for genetic improvement [31,33,35]. Escalant et al. [36] stated that somatic embryos would be unicellular origin, and would be of great use in genetic transformation since the potential for production of chimeric plants is low. Different explants were used for somatic embryogenesis in banana such as proliferating meristems [11], young zygotic embryos [8,12,13], basal leaf sheaths, rhizome tissues from in vitro plants [9], and thin sections from proliferating buds [10]. Among the different explants used, immature male flowers and scalps could be the most responsive material for initiating embryogenic cell suspension cultures [14]. Embryogenic response was highly dependable on genotype. Ganapathi et al. [33] reported that Rasthali was highly responsive (50%) followed by Shreemanthi (30%), whereas Basrai, Lokhandi and Trikoni were less responsive (8 to 15%).

Immature male buds from positions 0 to 15 (0 being the floral meristems) was successful in somatic embryogenesis of cv. Grand Naine, Yangamba (AAA), French Plantain, Mysore and Silk (AAB) and Pelpita (ABB), of which 74% embryogenic cultures were distributed between positions 7 and 13 [36]. In cases where the banana bears no male bud at all or possesses only one degenerating male bud, the use of immature female flowers is the only option. Plants were cut down and pseudostems were opened lengthwise to extract the buds measuring 10 cm. Embryogenic cultures formed on hands from 1 to 4, and all these hands showed atleast 3 flowers [35]. The flowers in the 8^{th} to 16^{th} position were most responsive explants in embryogensis, those preceding 8th position tends to become necrotic and all those succeeding 16th position produces non embryogenic callus [37]. Flower axis of length 1.5 cm was cut into 2 mm size of 16 to 20 segments and were used for embryogenic initiation [38,39]. Male buds of cv. Nakyetengu of size 1.5 cm width, 2 cm length and 8 to 15th position immature flower hands were used in somatic embryogenesis [40]. Perez- Hernandez and Rosell-Garcia (2008) [20] reported 25 mm to 6 mm width of immature flowers with a total of 24 hands per individual was successful as primary explants.

Media composition: S.S. Ma personally communicated callus induction, embryo initiation and embryo development media for the first time, which helped to induce somatic embryogenesis from immature male inflorescence of banana. The cultures were maintained at 28 ^{o}C under a 12 h photoperiod. Photosynthetic photon flux of 10 µmol/ m^2/ s light was provided by fluorescent lamps for the embryogenic tissue and embryo development phases, and 50 µmol/ m^2/ s for the germination phases [31]. Grapin et al. (2000) [35] maintained all cultures at 30 µmol/ m^2/ s of light intensity.

Callus induction: The callus induction medium that was personally communicated by S.S. Ma consisted of vitamins, macro and micro nutrients according to semi solid MS medium [41], 4.1 µM biotin, 18 µM 2,4-D, 5.71 µM IAA, 5.4 µM NAA, 87 mM sucrose and 7g/l agarose pH was adjusted to 5.7. Later this medium was adopted by most of the researchers for callus induction of banana flowers and was succeeded. The above mentioned media was suitable for banana cultivars such as Grand Naine and Yangambi (AAA); French Plantain, Mysore and Silk (AAB); Pelpita (ABB). The base of the callus showed undifferentiated cells. The middle portion consisted of compact tissue formed to nodular callus and upper portion that was loose, carried the embryos. Proliferation of embryogenic cultures did not occur due to subculturing, but due to permanence of the primary explant or age of the medium or both [36]. After 5 to 6 months, compact yellow calluses and friable white embryogenic tissues were obtained [31]. Ganapathi et al. [33] obtained whitish embryogenic callus in White's medium supplemented with 18.10 mM 2,4-D, 5.37 mM NAA, 5.71 mM IAA, 4.09 mM

d-biotin, 3% sucrose and 0.2% gelrite after 2 to 3 months. MS plus 1 mg/l biotin, 100 mg/l malt extract, 100 mg/l glutamine, 4 mg/l 2,4-D, 1 mg/l IAA, 1 mg/l NAA and 30 g/l sucrose with pH adjusted to 5.8 induced white callus and were transferred to modified medium which composed of above mentioned medium plus 200 mg/l casein hydrolyzate and 2 mg/l proline induced compact calluses and white friable embryogenic tissues with globular structures containing primary somatic embryos [38]. Inclusion of proline and casein hydrolysate increased callus proliferation and production of primary somatic embryos [39]. Embryogenic callus in cv. Nakyetengu was obtained in media consisted of MS salts and vitamins supplemented with 4.09 µM biotin, 4.5 µM 2,4-D, 5.37 µM IAA, 5.4 µM NAA, 20 mg/l ascorbic acid, 30 g/l sucrose and 7.2 mg/l agarose [40]. The Callus induction media personally communicated by S.S. Ma with gelrite (0.2%) was successful for banana cv. Lal Kela belongs to AAA genomic group and it was 83.3% [42]. Elayabalan et al. [43] reported that highest frequency of callus in cv. Virupakshi occurred in media with moderate concentration of 2,4-D, high concentration of proline and glutamine. The media consisted of MS plus 4 mg/l 2,4-D, 1 mg/l IAA, 1 mg/l NAA, 100 ml coconut water, 300 mg/l L-proline, 150 mg/l L-glutamine, 1 mg/l biotin, 30g/l sucrose and 1.5 g/l phtagel at pH 5.3.

Induction of callus required hormones rich in auxins [33]. The higher concentration of 2,4-D (2.0-4.0 mg/l) resulted in a friable callus and remained rather unchanged, that might be suitable for maintenance of embryos. But at the lower concentration (0.5-1.0 mg/l), the callus was relatively compact [44]. Effect of different sugars on cell growth was studied and found that 3% glucose was best for cell growth leads to callus induction. This study in male flowers of banana cultivars viz., Sabri, Jin, Gine and Ranginsagar contained semisolid MS medium supplemented with 2 mg/l 2,4-D +1mg/l NAA +1mg/l IAA +1mg/l Biotin+1mg/l glutamine [45].

The genotype, developmental status of the mother plant, and the type and developmental status (or age) of the plant organ from which the mother explants are obtained had response on somatic embryogenesis [46,47]. Embryogenic callus induction was highest in Lalkela (83.3%) than other cultivars which were in the range (50-40%) of AAA genome [14]. Age of immature male flowers had greatest effect on percentage of embryogenic callus development. Explants derived from two week old male buds of 'Williams' showed highest percentage of embryogenic callus than 1 week old explants, reverse in case of Grand Naine. These inverse responses to the age of the explant sources showed that the effect is dependent on the genotype during initiation of the somatic embryogenesis process. The effect of the ontogenetic age of the male bud of the inflorescence is reflected on the induction of somatic embryogenesis [48]. The embryogenic potential was also affected by the position of immature male flowers. Escalant et al. [36] reported that 74% of embryogenic clusters were formed from immature male flowers positions 7 to 1. Chong et al. [49] reported that the best response occurred within the flowers from the 8th to 15th position. Different types of callus was observed in callus induction medium after four months such as embryogenic callus, yellow nodular callus, white compact callus and translucent callus [48].

Embryo initiation: Embryo initiation medium verbally communicated by S.S.Ma consisted of MS medium plus 4.1 µM biotin, 4.5 µM 2,4-D, 680 µM glutamine, 100 mg/l malt extract and 130 mM sucrose. This medium designated for culturing friable embryogenic tissue to initiate embryogenic suspension culture. The yellow compact embryogenic callus proliferation and embryo induction higher in medium containing MS medium plus 0.22 mM BA, 1.14 mM IAA and 3% mannitol [33]. Initiation of embryogenic cell suspension culture of Virupakshi was obtained in medium containing MS with 2 mg/l 2,4-D, 1 mg/l IAA, 1 mg/l NAA, 45 g/l sucrose and 20 g/l maltose at a pH 5.3, incubated at 18 to 25 $\pm$ 1°C under constant shaking of 100 rpm. Induction of somatic embryos occurred after transfer of 1 ml of suspension culture in media consisted of 8 g/l agarose-solified SH basal medium (pH 5.7) with recommended vitamins, 1 mg/l IAA, 1 mg/l NAA, 0.2 mg/L 2-ip, 40 g/l sucrose, 20 g/l maltose, 10 g/l dextrose [43]. Embryogenic callus and pro embryos were developed in media containing both auxin and cytokinin. Of different auxins tested, 2,4,5-P and 2,4,5-T showed good response towards the initiation of embryos while Dicamba and Picloram showed highest rate of proliferation. Safed Velchi did not show any response to any of the auxins, this due to the genotypic variation [14]. The yellow compact calluses transferred from callus induction medium to embryo initiation liquid

medium became necrotic, meanwhile the friable embryogenic tissues converted to proembryos, isolated cells, aggregates of several groups of cells with uniformly dense cytoplasm and small yellow nodules [31;35]. After one week, proembryos became necrotic and cell aggregrates multiplied and formed aggregrates of size 50 to 1000 µm after three months [31]. Embryogenic calli got converted to proembryos, embryogenic cells and cell aggregates. The proembryos then became necrotic and cell aggregates multiplied to form many lobed structures [38]. Two types of cell clumps were formed in liquid embryogenic suspensions, one type was corresponded to compact masses with a nodular structure and second type was corresponded to cell aggregates with more friable structure [35]. The embryogenic cells were distinct with small size, more spherical in shape and had dense yellow cytoplasm; the non embryogenic cells were largely vacuolated with meagre cytoplasm and irregularly shaped cells [14]. Initial suspension cultures consisted of different kind of cells and cell clusters which were heterogeneous in nature. Upon frequent subculture at 3 to 4 days interval and subsequently at 2 week intervals, the culture became more uniform and consisted of small tightly packed cells with a dense cytoplasm [42].

Embryo development; This medium contains the basic medium of Schenk and Hildebrandt [50], vitamins of MS, 4.1 µM biotin, 680 µM glutamine, 2 mM proline, 100 mg/l malt extract, 1.1 µM NAA, 0.2 µM zeatin, 0.5 µM kinetin, 0.7 µM 2iP, 130 mM sucrose, 29 mM lactose and solidified with 3 g/l gelrite. Average number of embryos formed after 80 days per ml of plated PCV was ca 370 x 103 [31]. Conversion of embryogenic tissues into somatic embryos was successful in MS medium supplemented with 0.1% yeast extract, 1 g/l casein hydrolysate and 1 g/l maltose and 0.2% gelrite [33]. One month after plating on embryo development medium, somatic embryos had a well-defined structure and it had uniform epidermis, a caulinary meristem, a root pole very rich in starch, and provascular strands [35]. Highest percentage of embryos obtained in media containing MS salts, MS vitamins, supplemented with biotin (1 mg/l), malt extract (100 mg/l), glutamine (100 mg/l), NAA (1mg/l), kinetin (0.5 mg/l) zeatin (0.2 mg/l), sucrose (45 g/l), and phytagel (2.6 g/l) under dark condition at 28 ^{o}C. Somatic embryos was observed initially as globular and converted to torpedo within 18 to 21 days [38]. Somatic embryo development of Lal Kela blended well in medium based on Schenk and Hildebrandt [50] which was supplemented with MS vitamins, glutamine (100 mg/l), malt extract (100 mg/l), picloram (1 mg/l), 4.5% sucrose and 0.2 % gelrite [42]. Somatic embryo maturation occurred best in cv. Virupakashi when cultured in medium containing MS plus 1 mg/l NAA, 100 ml/l coconut water, 100 mg/l L-glutamine, 30 g/l sucrose, 30 g/l maltose and 8 g/l agarose at a pH 5.7 [43]. Effect of sugars on somatic embryo formation was studied and the highest rates of embryo formation of Sabri, Gine and Ranginsagar were observed in media containing 3% sucrose plus 1 mg/l BAP and 2 mg/l IAA. The media containing glucose or sorbitol inhibited embryogenesis compared to sucrose [45].

Germination of embryos: Germination of embryos was performed in MS medium salts, vitamins according to Morel and Wetmore [51], 0.2 µM BAP, 1.1 µM IAA, 87 mM sucrose and 2 g/l gelrite [31,35,36]. Two types of germination occurred in germination medium, one with the emergence of chlorophyllious plumule followed by the development of root, another somatic embryo turned into vitrified structure and complete plant emerges. To reduce the vitrification, filter paper was inserted between the semi solid medium [36]. Studies depicts that the medium which contained MS salts, MS vitamin, BA (5 mg/l), sucrose (30 g/l), and phytagel (2.6 g/l) produced a higher number of germinated embryos [38]. Higher percentage of germination was recorded in cv. Nakyetengu using the basic germination media [31,35,36] with slight modification. Instead of 2 g/l gelrite they used 7.2 g/l agarose as solidifying agent [40].

Germination percentage of somatic embryos was highest in media containing 0.22 µM BAP and 1.14 µM IAA compared to media containing 2.22 µM BAP and 11.4 µM IAA; 0.22 µM BAP and free hormones [48]. Plant regeneration of Lal Kela (AAA group) was maximum in medium contained MS supplemented with BAP (2.22 µM), IAA (1.14 µM), sucrose (3%) and 0.2% gelrite [42].

Germination percentage highly depends on the size of the embryos transferred to germination medium. Germination rate of embryos having 100 to 250 µm length was 3%, whereas 800 to 1000 µm was 20% [31]. Escalant et al. [36] reported temporary

Table 1: Response of banana inflorescence with respect to explants, culture media and plant growth regulators

Sl. No.	Explant	Size of explant	Media composition						Response	Reference
			Basic salt	Sucrose	Soldifying agent	Cytokinin 1	Cytokinin 2	Auxin		
1.	Chandrabale Male apical meristem	0.5mm	MS	3%	Gelrite 0.25%	Adenosine sulphate (160 mg/l)	BA (1.13 mM)	IBA (1.02 mM)	Greenish white globular masses	Doreswamy & Sahjiram [3]
2.	Rasthali Male apical meristem	0.5mm	MS	3%	Gelrite 0.25%	Adenosine sulphate (160 mg/l)	BA (1.13 mM)	IBA (1.02 mM)	Mass of green globular shoots	
3.	Robusta Male apical meristem	0.5mm	MS	3%	Gelrite 0.25%	BA (2.25 mM)		IBA (1.02 mM)	Globular green masses	
4.	Sannachenkadali Male inflorescence	2-3 cm	MS	3%	Agar 0.07%	BA (8.9 mM)			Multiple shoots	Resmi & Nair [16]
5.	Red banana	2-3 cm	MS	3%	Agar 0.07%	BA (22.2 mM)				
6.	AAA group	2-3 cm	MS	3%	Agar 0.07%	BA (17.8 mM)		IAA (11.4 mM)		
7.	Berangam (AAA), Rasthali & Nangka (AAB)	20 mm	MS			TDZ (0.09 mM); BA (1.80 mM)			Induction of cauliflower like bodies	Darvari et al. [15]
8.	Berangam (AAA), Rasthali & Nangka (AAB)	20 mm				TDZ (0.09 mM); BA (1.80 mM); TDZ (0.13 mM) TDZ (0.18 mM)			Shooting from flower clusters	
9.	Immature male buds		MS	3%	Clarigar 0.3%	BA (0.45 mM)		NAA (0.09? ? M)	White bus like structures	
10.	Immature male buds		MS	3%	Clarigar 0.3%	Kinetin (0.43 mM)		NAA (0.09 mM)	Shoot induction	Hrashel et al. [6]

immersion system as superior to semisolid medium in somatic embryogenesis, and the multiplication of embryos were found to be three folds the semisolid culture. The phenomenon by which multiplication occurred in temporary immersion system was adventitious embryogenesis. The efficiency of this method can be useful in plant transformation, mass propagation and germplasm conservation. While the plant transformation is achieved through particle bombardment [52], the latter two are employed using the high proliferation of this technique [36] and through synthetic seeds [53]. The translucent embryogenic culture bearing somatic embryos were transferred to temporary immersion medium consisted of MS mineral salts supplemented with Morel's vitamins, 2.2 µM picloram and 87 mM sucrose with the pH adjusted to 5.8.

Temporary immersion system consisted of modified autoclavable filter units which acts as culture vessel. The embryogenic cultures placed in upper compartment were immersed by the medium only when lower compartment was pushed up by air pressure. The explant was kept always in high relative humidity [36]. Somatic embryos were multiplied and maintained in a temporary immersion system and obtained 6000 somatic embryos after six month of subculture. Plant recovery frequency was 60 to 70% [54].

Development of somatic embryos in large scale was by means of liquid culture medium. Cell suspensions were established from embryogenic tissues derived from male flower cultured for six months [36]. These cell suspensions were cultured in liquid medium

described by Bieberach [55], containing MS medium supplemented with 1 mg/l biotin, 100 mg/l glutamine, 100 mg/l malt extract, 1 mg/l 2,4-D and 45 g/l sucrose. At each subculture of the cell suspension, the initial density was adjusted to 3% of the PCV in 15 ml of culture medium. Somatic embryo formation was achieved in basic medium of SH, MS vitamins, 1 mg/l biotin, 100 mg/l L-glutamine, 100 mg/l malt extract, 230 mg/l proline, 0.2 mg/l NAA, 0.05 mg/l zeatin, 0.1 mg/l kinetin, 0.2 mg/l 2iP, 45 g/l sucrose and 10 g/l lactose. Multiplication of globular somatic embryos was made possible due to the MS medium supplemented with 0.3 mg/l BA, 2 mg/l IAA and 30 g/l sucrose. 100 mg initial cell density was the most appropriate for somatic embryo formation. Secondary multiplication of somatic embryos was achieved in liquid media in rotary shaker and in bioreactors. High and constant concentration of dissolved oxygen (80%) produced greater proportion of globular somatic embryos [56].

Secondary somatic embryogenesis is the process of induction of new somatic embryos from pre-existing embryos [57]. Secondary somatic embryogenesis has the potential to produce many plants and may continue to produce embryos over a long period of time. This is suitable for genetic transformation in banana using immature male flowers. Primary somatic embryos were produced in medium containing MS plus 1 mg/l biotin, 100 mg/l malt extract, 100 mg/l glutamine, 4 mg/l 2,4-D, 1 mg/l IAA, 1 mg/l NAA and 30 g/l sucrose with pH adjusted to 5.8 and transferred to modified medium which composed of above mentioned medium plus 200 mg/l casein hydrolyzate and 2 mg/l proline. Proliferating embryogenic callus that developed into secondary somatic embryos were obtained in medium containing MS salts, 30 g/l sucrose, 2.6 g/l phtagel and 10% coconut water. Later secondary embryos were multiplied and maintained for four months in the same medium. Secondary embryos directly entered into globular stage from primary somatic embryos, initially appeared as small, hyaline protuberances. Mature embryos were produced on a medium containing MS supplemented with 5 mg/l BA, above or below this concentration reduced percentage of mature embryos [39].

Abnormal somatic embryos was observed in cv. Rasthali while cultured in media contained Murashige and Skoog medium (MS) supplemented with 18.10µM 2,4-dichlorophenoxy acetic acid (2,4-D), 5.71µM indole 3-acetic acid (IAA) , 5.37µM naphthalene acetic acid (NAA) and 4.09 µM d-Biotin, 3% sucrose and 0.2% gelrite for callus induction and MS supplemented with NAA (1.07 µM), Zeatin (0.23 µM), 2-ip (0.60 µM) and kinetin (0.46 µM) for further maturation. These embryos falls into different categories like, embryos with different size and shapes without proper shoot apical meristem, fusion of embryos, fusion of cotyledons, vascular distortion in fused embryos that lead to multiple shooting and rooting. This delayed culture period in auxin rich media (NAA, IAA, 2,4-D) would have caused abnormal embryos with different shapes and fused cotyledons [58]. Filippi et al. [59] observed two types of somatic embryos: one resembling the zygotic embryo of *Musa acuminate* and the other type showing more similarity to the zygotic embryo of other monocotyledonous species [37]. According to Meenakshi et al. [42], plants regenerated through somatic embryogenesis showed minor variation when assessed by randomly amplified polymorphic DNA (RAPD) and sequence characterized amplified regions (SCAR) markers. Higher concentrations (10 µM to 20 µM) of BA showed complete conversion with shoot and root development. Well-developed somatic embryo derived plantlets ("emblings") grew normally during hardening in the green house. Khalil et al. [39] reported 90% success in developing secondary somatic embryos into complete plantlets when subcultured onto MS medium plus 0.1% activated charcoal, 1 mg/l BA and 1 mg/l IAA. Morphologically normal banana plants developed from all of the regenerated plantlets, the first of which were produced within 6 months of culture initiation.

CONCLUSION

Biotechnology plays an important role in banana production and genetic improvement. Banana production is hampered by abiotic and biotic stress and major focus should be given to genetic transformation since conventional breeding is a cumbersome process. Male flower tissue technology adds more colour to the tissue culture of banana. Direct organogenesis is widely used in banana plantlet production, with the use of male flowers, the production of plantlets can be increased ten times. Production of stable transformants for different characters can be made possible through somatic embryogenesis using male flowers as explant. Somaclonal variation is a major impediment in

production of banana through tissue culture. Technology need to be developed in order to avoid off types. Development of regeneration protocols using flowers for all cultivars of banana will be beneficial for the production of disease free plantlets thereby boosting the global banana production.

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