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Review Article

AN INSIGHT INTO *IN VITRO* MICROPROPAGATION STUDIES FOR BANANA- REVIEW

CHAUDHARY DEEPIKA[1], BRAR BASANTI*[1], DUHAN JOGINDER SINGH[1], KAJLA SUBHASH[2] AND POONIA ANIL[2]

[1]Department of Biotechnology, Chaudhary Devi Lal University, Sirsa, 125055, Haryana
[2]Centre for Plant Biotechnology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana 125004
*Corresponding Author: Email-basantibrar@gmail.com

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Abstract- Bananas are the most essential economically tropical fruit crops that come second in area as well as production of fruit crops. It constitute fourth very important food crop following wheat, rice and maize. It grown in more than 100 countries is harvested in an approximately 4.84 mha area, with 95.6 million annual productions. Bananas are given that a cheap and easily providing source of energy and very essential for food security of country. It is rich source of vitamins A, C, B6 along with β-carotene. In addition, bananas contain serotonin, melatonin and tryptophan that act as mood elevators. In various developing countries states that the crop is act as an important source of revenue, at a time providing the chief source for income in rural areas. Hence plays an essential role in case of poverty mitigation. In vitro multiplication of banana has gained more attention for proving genetically uniform, disease and pest free true to type plants. *In vitro* micropropagation of banana can be done using the shoot tip culture for the production of disease free plants. Banana plants *in vitro* micropropagation has an immense profitable prospective due the speed of propagation, lower space requirements and capability to grow elite clones having better growth with increased stress tolerance capability. The present review provides the upto date knowledge of different steps involved for *in-vitro* multiplication of banana.

Keywords- Banana, food security, micropropagation, shoots proliferation, rooting.

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Introduction

Banana (*Musa* spp.) belonging to family Musaceae is one of the oldest fruits of the world [1]. For millions of people in developing countries in the tropics and subtropics, banana provides a basic food source of great importance [2]. The largest banana-producing regions in the world are Africa and Latin America, which represent 74.2 and 22.5 % of the world production, respectively followed by Asia with 3.3 % [3].

Bananas are thought to have originated from Malayan Peninsula or tropical parts of Asia. It is a tropical plant and grows well in warm and humid conditions. It is propagated by suckers. Ripe fruits are rich in vitamin A, C & B complex and phosphate. It contains 18 – 20 % sugar. It is produced in islands of Honduras and Jamaica, Brazil, Costa Rica, Cuba, Central America, Canary Islands, West Indies, India, Taiwan and Indonesia, China, Philippines, Australia, Colombia, Ecuador. All above countries except India export banana. India ranks number one in production of banana followed by Brazil, Indonesia, Philippines, China and Australia. In India, the banana producing areas are Maharashtra, Gujrat, Karnataka, Kerala, Tamil Nadu, Andhra Pradesh, Orisa, Bihar, Madhya Pradesh, Assam, Tripura and Manipur and West Bengal. India takes a lead in world banana production but exports are not substantial due to internal consumption, post harvest problems and disease affecting the plants.

Recent Production Trends

World production of bananas is around 88 million tonnes, with plantation of 30 million tonnes. Nearly half the world production of banana comes from Asia. Worldwide, 10 million ha area is devoted to banana cultivation. Approximately 98 per cent of world production is grown in developing countries. More than 73 per cent of total banana production is contributed by ten major banana-producing countries. India, Ecuador, Brazil and China alone produce half of total banana output. India is the biggest producer of bananas with 17 million tonnes or 17 per cent of global production, followed by Uganda with 14 million tonnes. But, the cost of production in India is three times that of big global exporters. Banana farming is very commercial enticing amount of Rs. 40,000 to Rs. 60,000 per acre. The top most banana cultivating states are Orissa, Andhra Pradesh, Kerala, Maharashtra, Tamil Nadu, Madhya Pradesh, West Bengal, Karnatka. When we consider the area under cultivation of banana Tamil Nadu ranks first and in production Maharashtra is on the top [4].

Security for Food and Income

Micropropagation is easy and cost effective technique providing high economic input [5]. Banana has 2.8% of agriculture GDP in India. It is not only a good source food and income throughout the year but also an important crop for farmers. Banana is basically a hot-climate plant. Edible bananas are of two types—cooking bananas, known as plantations which can be considered starchy vegetables, and the fruit bananas. That is why banana is blend closely as national heritage and being used as a food from many years. It is the ancient fruit known to people.

Being a rich source of potassium, banana is recommended for patients suffering from high blood pressure. It is being used to prepare squashes and juices. Despite 75 percent water content, banana has not been used for long to prepare juice because when compressed, it simply turns to pulp. The technique of extracting juice from banana pulp was developed in 2004 by scientists of BARC (Bhabha Atomic Research Centre) and also been patented. Banana is a good source of vitamins C and B6. It has a high content of carbohydrates/fibre, but is fat-free and

low in proteins. Fruits are good for the treatment of gastric ulcer, diarrhea, cancer and heart diseases.

Nutritional Requirements

Nitrogen is an important element in banana nutrition. Banana plant cannot store nitrogen. But oversupply of nitrogen produces large plants with dark green leaves. Sometimes the peduncle breaks off just inside the pseudo stem causing bunch loss. Phosphorus requirement is less than Nitrogen and Potassium requirement. Banana plants accumulate the Phosphorus they require over a long period; they lose relatively little through the fruit they distribute the balance readily to the sucker. Banana plants absorb Phosphorus mostly during the period between three to nine months after planting. Potassium is the most important element among nutrients required for the banana. The Potassium requirement of the developing bunch is high and if soil cannot supply enough quantity after its flowering stage, the leaf system could collapse due to Potassium being withdrawn from leaves for supply to the bunch. Banana also requires Calcium, Magnesium, Sulphur, Zinc, Manganese, Boron, Iron and Copper in traces as part of its nutritional requirements [6].

Challenges and Measures

Banana cultivation is facing problems like non-availability of standardized, good-quality planting material and quality certification of plant material. Though banana is a highly perishable crop, currently no insurance facility is available for farmers. Air tariffs for banana are also very high. There is an urgent need for standardization, certification and rapid multiplication for yielding good-quality material through tissue culture. Through tissue culture, it would be possible to multiply a single clone into a thousand, which effectively means possibility of further multiplication.

Tissue-culture banana cultivation is gaining popularity in states like Tamil Nadu. With an initial investment of Rs 80,000-85,000 per acre, the farmers could get a yield of 38-40 tonnes per acre, which amounts to Rs 150,000 to Rs 160,000 per acre as gross income, and a net income of Rs 70,000 to Rs 75,000 per acre annually. Farmers are advisedto go in for two ratoons which give them a net income of about Rs 1 lakh each per acre. Therefore farmers can earn amount approximately of Rs 2.25 to Rs. 2.40 lakh per acre in around 27 – 30 months by vigorous cultivation.

There are centers for research in banana in Andhra Pradesh, Assam, Bihar, Gujarat, Karnataka, Kerala, Maharashtra, Tamil Nadu and West Bengal. The National Research Centres for Banana (NRCB) at Thayanur near Tiruchi has a dozen scientists working on banana in a 38-ha farm. Work is in progress on transgenic varieties and tissue culture that have been yielding positive results. The NRCB is also collecting germ plasm of Indian varieties. There are around 1200 accretions.

Research and Development Programmes In India

Earliest banana improvement programme in India started in the year 1949 at Central Banana Research Station in Aduthurai in Tamil Nadu. The programme continued in Tamil Nadu Agricultural University after its formation in 1971. Improvement was also initiated in Kerala Agricultural University in Thrissur in 1982. National Research Centre on Banana (NRCB), Trichy, established by the Indian Council of Agricultural Research (ICAR) in 1993, has also initiated and pursuing banana improvement programmes, coordinating at national level. Viewing the polyclonal situation in India and the research needs, the objectives of banana improvement programme at national level are includes collection, conservation and evaluation of Musa germplasm; credentials of successions tolerant to main constraints like *fusarium* wilt (*Fusarium oxysporum*sp.*cubense*), leaf spot diseases and nematodes (*Radopholussimilis*, *Meloidogyne incognita* and *Pratylenchuscoffeae*) as well as hybridization and selection to evolve potential synthetic diploids and primary tetraploids to further generate triploid hybrids having good horticultural traits coupled with resistance or tolerance to pests/diseases.

Tissue Culture

Generally, four to five suckers are obtained per plant which are insufficient to replace the infected or affected farms with healthy and disease free planting materials. The area under cultivation, as well as, per hectare production could be increased if appropriate technologies supplement the conventional practices. Tissue culture has been proven as a potential technology to produce millions of identical plantlets, which are disease free and true to parental type. Micropropagation has several advantages over conventional vegetative propagation, i.e. elimination of systemic pathogens, preservation of breeding stocks as juvenile plants and international exchange of disease free germplasm [7]. Through this technology, (*Musa sapientum*cv. Sagar) from a single shoot tip or an axillary bud, a large quantity of uniform and disease free plants with good genetic potential can be produced within a short time [8,9]. The protocol for micropropagation of bananas has been achieved. While some researcher used shoot tips [10-12] and others chose floral apices for large scale multiplication [13-17]. There are also reports of somatic embryogenesis and regeneration in liquid medium [18,19]. Due to its soft and delicate root system, the rate of acclimation of *in vitro* regenerated banana plants is not satisfactory.

Hence culture conditions for high frequency shoot multiplication; rooting and transplantation in banana require optimization. Now days 95.6 million tons of banana is produced worldwide on an area of 4.84 million ha [20]. Tissue culture is the best way of producing disease free plants in large scale [6,21]. The cultivar Nanjanagud Rasabale was once a leading cultivar of Mysore distinct but now a day under threat of extinction because of panama disease [22]. This cultivar Nanjanagud Rasable was earlier grown in 600 acres of land in the district of Mysore, Karnata, but now confined to only 30 acres of land [23]. This cultivar is famous for its unique taste and has a huge demand all across the country [23].

Studies were also performed to establish best growth hormone concentrations and concentration of phytohormones [24]. *In vitro* micropropagation of banana species were done by using different concentrations of BAP (Benzyl Amino Purine*)*, NAA (Naphtalic Acetic Acid) and the effect of the BAP & IBA (Indole-3-butyric acid) was studied [25]. Ms media containing 2.0 mg/l N6-benzylaminopurine reported most excellent for shoot number, length, multiplication as well as number of leaves, while MS media with1.50 mg/l of IBA showed very good numbers of roots and root length after 20, 40 and 60 days following inoculation of Basrai banana variety micropropagation [26]. With increased concentration of cytokinins (4.0 mg/l BAP) the formation of multiple shoots were increased and buds is also promoted [23]. So, the *in vitro* culture techniques have been standardized for commercial banana plantations, because of the advantage of producing disease-free planting materials [13,21,27,28].

Shoot establishment

Meristem is used as an explant on medium (agar or liquid) for producing multiple shoots of banana [13]. Twenty different combinations with various concentrations of BAP, KIN (Kinetin) and NAA alone were used for bud initiation on shoot tip explants. Among all the combinations used either 4.0 mg/lBAP alone was found to be most effective for establishment in both the banana cv. Robusta and G9 [29,30]. Previous researchers showed that 5 mg/l (22.2 µM) BAP was the optimum concentration for most banana cultivars [8,31]. Shoot tip explants were cultured on MS liquid medium supplemented with different antioxidants like ascorbic acid 50 mg/l helps in controlling browning. Shoot proliferation and shoot growth were enhanced by using the concentration of BA 4.0 mg/l + NAA 0.50 mg/l in MS medium, respectively [31-33].

Shoot multiplication

Thirty different combinations with various concentrations of BAP with NAA and KIN with NAA was used to analyze the shoot multiplication capacity of the MS medium. They found that two mg/l of BAP and 0.5 mg/l of NAA and 3.0 mg/l of BAP and 0.5 mg/l of NAA of medium showed good results respectively for banana cv. 'Robusta' and 'G-9' for shoot multiplication [29,30]. Many of the previous reports on banana micropropagation used more than one type of media for shoot initiation, multiplication and rooting [27,34-37]. BAP and NAA were used successfully in multiplication of banana cultivars in many studies [16,38,39].

However, the effective concentration of these growth regulators in the media

varied with different banana cultivars. A combination of 0.2 mg/l IAA (Indole acetic acid) and 2 mg/l BAP and 1mg/l NAA and 5 mg/l BA in MS were found to be effective in the concentration and development of banana plantlets [40]. Likewise, the combinations of BAP with IAA or IBA were effective for *in vitro* multiplication of bananas and plantains [41]. Higher shoot multiplication but a reduction in the length of shoots in media with a combination of BAP and IAA in triploid cultivar by using inflorescence explants [42]. Reduction in the number as well as length of shoot has been observed with exposure to high levels of BAP alone (44.44 µM) in banana cv. Nanjanagud rasabale (AAB) [31]. Various treatments of BAP and NAA, BAP 2.0 mg/l + 2 weeks dark incubation +1 week light incubation and NAA 1.00 mg/l were found most effective for tissue culture propagation of Banana cv. Ney Poovan [43].

Newer chemicals

Different concentrations of newer chemicals i.e. TDZ, putericine, spermidine and thiourea and additives i.e. adenine sulphate were also used to study their effect on *in vitro* multiplication of shoot tip of banana cultivar i.e. Robusta and G-9. The maximum numbers of shoots were observed on 0.4 mg/l concentration of TDZ for both banana cultivars *viz.* Robusta and G-9 which is much lower than other growth hormones used [30,44]. Diphenyl urea derivatives were used in various cell-culture systems including both callus cultures and micropropagation of many woody-plant species [32,45]. BAP supplemented with different concentrations of adenine sulphate results in increase in number of shoots as comparison to the BAP alone [29]. The maximum number of average shoots was observed at 30 mg/l of adenine sulphate in combination with 2.0 mg/l BAP + 0.5 mg/l NAA. In addition to these it is reported that with the increasing BAP concentration upto 5.0 mg/ml increase the numbers of shoots but above this concentration shoot numbers decrease [46]. Some other growth regulators like phloroglucinol also react along with auxins and cytokinins. For the development and production of Musa spp. Cultivar *Grand Naine* phloroglucinol is used *in vitro* Phloroglucinol. By adding 200µM of phloroglucinol in MS medium *in vitro* the roots and shoots elongation was increased, while its higher concentrations (400-1000µM) leads to decrease in its development and growth [47].

Rooting

The best result in rooting with (2.0 mg/l NAA) for Robusta with number of days taken (10.97±0.16 and 12.23±0.30) while in case of G9 best results were obtained in 3.0 mg/l NAA [31]. Root development with IAA and IBA in combination was found to be superior to using alone and maximum roots in banana of an average of 8 per plantlets in ~78% cultures were obtained on MS supplemented with 1.0 mg/l IBA with 0.5 mg/l IAA [48]. Similarly, root numbers varied with different concentrations of IBA and IAA was reported [49]. The highest number of roots was produced by 0.5 mg/l IAA + 0.5 mg/l IBA [49].

The different concentrations of BAP growth hormones on rooting in different species of banana such as Ardhapuri, Basrai, Shrimanti has been studied and the best rooting response was observed in BAP 1mg/l+ IBA 3mg/l [50]. In the case of Grand naine variety, the concentration of 1.5mg/l IBA alone treatment had positive effects on multiple root formation and MS + 0.5 mg/l IBA for highest length of roots [39,51].

Acclimatization & hardening

Plantlets produced *in vitro* must be acclimatized gradually to withstand the harsh natural environment. Misting, spraying or covering the pots with polythene bags may serve to fulfill the above objectives. Different types of substrates have been used during the acclimatization period such as soil-vermiculite mixture [52], sterilized sand [53,54] and soil [55]. To increase the survival of transplanted *in vitro* cultured banana plantlets, the best method involved transplanting the plantlets into plastic bags. Before transplanting, plantlets were soaked in a 600 times dilution of carbendazin for 1 minute for sterilization. When the first new leaf appeared after planting in bags, 0.1% urea solution was added. When transplanted plantlets grew to a height of 20-30 cm after 45-60 days, the plant material was suitable for planting in the field [23,56].

Rooted plantlets of two cultivars ('Robusta' and 'G-9') were transferred the pots containing different potting mixture having potting mixture- PM_1= Sand, PM_2= Sand + FYM (Farm Yield Mannure) (1:1), PM_3 = Sand + Soil + FYM (1:1:1), PM_4= Sand + Soil + Vermi compost (1:1:1). Highest survival of 100.0 percent plantlets was recorded in both cv. 'Robusta' and 'G-9' in PM_2, PM_3 and PM_4 [30]. The potting mixture that contains only sand led to 80.0 percent survival of *in vitro* raised plantlets of both cv. 'Robusta' and 'G-9'. Both the varieties respond equally on different potting mixtures [30].

Conclusion

The present paper reviewed the *in vitro* micropropagation studies on banana. Microprapagation of plants gives us appropriate substitute to traditional methods. Micropropagation is cost effective method and useful for the production of disease free plants. In micropropagation method small explant is used during starting experiment. It has been used to multiply novel as well as endangered plant species. It is very helpful to provide an enough number of seedlings to plant from the same plant that is not able to produce seeds as well as not well respond to vegetative reproduction.

Application of research: *In vitro* micropropagation is technique of vegetative propagation of plants under sterile/aseptic conditions. Explants in very small size are used for this technique. It can be capable of producing disease free true to type plant production. The main benefit of this technique is enormously high multiplication rates. Hence, this method is greatly suited for fast multiplication of rare/endangered genotypes.

Research Category: Micropropagation Studies for Banana

Abbreviations:

FYM: Farm Yield Mannure
BAP: Benzyl Amino Purine
NAA: Naphtalic Acetic Acid
NRCB: National Research Centre on Banana
ICAR: Indian Council of Agricultural Research

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***Research Guide or Chairperson of research: Dr Joginder Singh Duhan**
University: Chaudhary Devi Lal University, Sirsa, 125055, Haryana
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