www.sciencevision.org

Research Forum

Sci Vis Vol 13 Issue No 3
July-September 2013
ISSN (print) 0975-6175
ISSN (online) 2229-6026

Strategies for large-scale production of commercially important banana varieties of Mizoram, India, using plant tissue culture technique

Lalremsiami Hrahsel and Robert Thangjam*

Department of Biotechnology, School of Life Sciences, Mizoram University, Aizawl 796004, India

Received 20 June 2013 | Accepted 29 July 2013

Abstract

The technique of plant tissue culture has been well accepted and applied in the mass propagation of planting materials in various crops and plants. In India numerous micropropagation units are producing millions of plantlets catering the needs for the increasing demand of quality planting materials. The advantages of this technique lie in the production of plantlets that are disease free and genetically identical to the elite mother plants. Application of plant tissue culture technique is the only viable means for the large scale production of banana planting materials which is not possible through conventional propagation. The article discusses the strategies of the mass production of commercially important banana in Mizoram using plant tissue culture techniques.

Key words: Banan; micropropagation; tissue culture; Mizoram.

Introduction

Plant tissue culture is the science of growing plant cells, tissues or organs isolated from the mother plant, on artificial media under aseptic conditions. The plant tissue culture technology owes its origin to the concept of totipotency of cell, introduced by Haberlandt, 1902 which led to the successful culture of tomato roots.[1] The number of successful cases is continuously increasing. Numerous publications and reports have come out with regard to the basic procedures and method involved in plant tissue culture in various plants.[2-4] The simpler techniques that are found to be applicable directly in propagation and genetic improvement of plants are (i) micropropagation, (ii) meristem culture, (iii) somatic embryogenesis, (iv) somaclonal variation, (v) embryo culture, (vi) *in vitro* selection, (vii) anther culture, and (viii) protoplast culture.[5] For the large-scale sustainable production of plants, a number of superior quality planting materials is required, which is difficult to obtain by conventional methods of propagation. In contrast, thousands of plants could be derived from a single cell or tissue in a relatively short amount of time, thus having a great potential for

Corresponding author: Thangjam
Phone:
E-mail: robertthangjam@gmail.com

mass propagation of commercially important crops such as banana. Initial plant material could be obtained from an inflorescence, proliferating meristem, zygotic embryos and rhizome and leaf sheaths; depending on the type of species at hand. This technology could thus be commercially used for mass propagation of quality planting materials by micropropagation (i.e. *in vitro* clonal propagation), mass production of quality and useful secondary metabolites, development of new varieties (via mutagenesis, etc.) as well as the production and enhancement of natural pharmaceutical, nutraceutical and cosmaceutical compounds. Plant cell culture is a requirement as well for genetic engineering in the production of designer plants, whereby these could be designed for desirable attributes such as disease resistance, tolerance towards environmental strains and mass production of antibodies and bioplastics. It can become a versatile tool for mass multiplication of elite clones, elimination of disease in planting material, creation of super genotypes of agricultural crops, which hither to it was not possible through conventional plant breeding methods. Tissue culture propagation can thus heighten our ability to produce consistently uniform superior planting material for export and domestic market. Micropropagation through tissue culture techniques thus offers rapid and reliable means of producing large number of genetically uniform clonal planting material within a short time.

Advantages of Plant Tissue Culture Technique

- ⊕ Micropropagation results in rapid propagation of a superior plant while maintaining the genetic make-up and also helps in storage of germplasm.
- ⊕ Established aseptic cultures such as flower buds, shoot buds, somatic embryos may be packaged as artificial seeds which are encapsulated for distribution and protected with a complex of agar and other gel-forming compounds such as sodium-alginate beads, and stored in a protective, hydrated gel with nutrients for a long period under ultra low temperature.[6] Slow growth techniques can also be applied for the maintenance of the culture for a longer time in limited culture media.
- ⊕ The plantlets that are derived from these techniques are free from fungal and bacterial diseases since the contaminated plants fail to respond and gradually die out. Viral diseases could be eliminated from plant propagative material through quarantine and virus indexing. These checks are recommended for verification of the disease-free planting materials.[7]
- ⊕ Somaclonal variations derived from callus and cell suspension cultures can be utilized for the induction of desirable, heritable changes in regenerated plants by subjecting a population of cells to a selection pressure.
- ⊕ Regeneration from callus, cell suspention and pollen cultures helps to produce homozygous, pure-breeding lines of plants for hybrid production and genetic studies and also to improve the efficiency of *in vitro* selection. The use of colchicine may be needed to double the chromosome number of haploid plants.[8-9]
- ⊕ Protoplast culture helps to incorporate potentially useful genes from one plant species to another by fusion of protoplast and regeneration from the hybrid cell line. It also helps to transfer specific genes into protoplasts and regenerate transgenic plants.[10]
- ⊕ Plant cell and tissue culture can also be used for large scale harvesting of medicinally important secondary metabolites, which otherwise, will need a large number of plants from the natural population. Similarly, there are number of cultured cells producing metabolites not synthesized by the plant itself e.g. *Lithospermum erythrorhizon* cultures have been observed to synthesize rosmarinic acid - a characteristic of lemon plants, through metabolic engineering.[11]

Application of Plant Tissue Culture Technique for Production of Quality Planting Material in Banana

In spite of the availability of many reports on *in vitro* propagation in banana, in which the protocols are complicated, the standardization of specific protocols for a specific cultivar is essential. Development of new banana varieties through conventional breeding programs remains difficult because of sterility and polyploidy of most edible cultivars. Banana being one of the most widely distributed fruit crops in the world, it is cultivated in more than 120 countries covering about 10 million hectares, with an annual production of 130 million tons.[12] It is the fourth most important food crop after rice, wheat and maize.[13] The crop is strongly believed to have originated from Southeast Asia, and many of the species and clones have India as their homeland.[14] Natural hybridization, mutation and polyploidy have contributed a lot for wide diversity among Indian bananas which have perpetuated through vegetative propagation over.

As a result of various shortcomings including lack of uniformity, high disease and pest infection rates, as well as the bulkiness of conventional propagation via suckers, the application of various biotechnological approaches has become an integral part of the banana industry.[15] In particular, the use of plant tissue culture via clonal propagation of superior cultivars has been an immense benefit to commercial banana farmers globally.[16] *In vitro* propagation provides excellent advantages over traditional propagation, including a high multiplication rate, physiological uniformity, the availability of disease-free material all the year round, rapid dissemination of new plant materials throughout the world, uniformity of shoots, short harvest interval in comparison with conventional plants, and faster growth in the early growing stages compared to conventional materials.[17-19]

Banana is a long duration crop of one and a half years. The production of suckers varies in different genotypes ranging from 5-10 per plant per year. Crop productivity and maturity is dependent on the size and age of suckers and uneven maturity extends the duration by 3-4 months. Suckers also carry soil nematodes, disease causing organisms such as bunchy top virus, leaf spot etc. thereby affecting the crop production considerably. In this regard, biotechnological approaches such as cell and tissue culture, protoplast fusion and gene transfer offer as useful tools.[20] *In vitro* propagation of banana through shoot tip cultures is useful in the rapid multiplication of desirable disease free plantlets. In addition, careful selection and updating of mother plants results in improved crop yield.

Strategies for Production of Quality Planting Material of Banana in Mizoram

North-east India is considered as the reservoir for the large gene pool of banana genetic resources, and is the meeting point of *Musa balbisiana* of the Indian subcontinent and *Musa acuminata* of Southeast Asia.[21] With the loss of crop genetic resources at an alarming rate, the future of global food crops depend on the sustainability of the genetic pool at their centre of diversity. The northeastern states of India, namely Assam, Arunachal Pradesh, Meghalaya, Tripura, Mizoram and Manipur have been richest sources of natural diversity. Altogether 39 different accessions of banana have been collected and characterised.[14] From the state of Mizoram, 14 different accessions have been collected and characterized.[22] The important commercial banana varieties of the state are vaibalhla (*M. acuminata* AAA group), lawng balhla (*Musa* AAB group) and banria (*Musa* ABB group). The strategies for mass propagation of these commercially important banana varieties are as follows:

1. Characterization of the different cultivars of edible banana grown in different phytogeographical regions of Mizoram and identification of the superior genotypes:

The prospective mother plants should be thoroughly evaluated and selected for its superior agronomic characteristics and freedom from diseases. The selected plants should be maintained in a protective area where soil and plantation hygiene are in place. For the proper validation of superior genotypes, molecular markers such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), simple sequence repeat (SSR) can be used. Continuous evaluation for disease signs and symptoms should be made in the plantation areas and the plants itself. Viral diseases like banana bunchy top virus (BBTV) should be screened using enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) techniques. The plantation area should be free from any disease plants. Only the plants that are healthy looking, having superior agronomic traits and free from viral symptoms should be selected as mother plants for the mass propagation.

2. Standardization of in vitro regeneration system of superior genotypes for rapid multiplication of genetically stable planting materials:

a) Initiation of aseptic culture: Various explants (male flower buds, shoot tips, immature zygotic embryos) taken from the selected superior mother plants materials can be tested for their response in various culture media such as MS,[23] B5,[24] White media,[25] etc. under standard culture conditions. The effect of various growth regulators such as cytokinins, auxins, etc. on the *in vitro* regeneration potential of selected banana genotypes should be evaluated.

b) *In vitro* regeneration: Some of the explants from the above culture may result in the direct or indirect regeneration of shoots or roots. The obtained *in vitro* organs can be transferred to different media and hormonal combinations for further multiplication and rooting.

c) Hardening and acclimatization: The rooted regenerated plantlets should be transferred into pots containing sand and soil mixture for primary hardening in a growth chamber then proceed for secondary hardening and acclimatization in the polyhouse under standard conditions. The successfully hardened and acclimatized plantlets should be transferred to the field.

3. Standardization of in vitro regeneration system from encapsulated aseptic cultures:

The established aseptic cultures such as flower buds, shoot buds, somatic embryos can be encapsulated with the help of sodium alginate and regenerated into plantlets.

4. Genetic fidelity testing of the regenerated plantlets:

For testing of genetic fidelity of the regenerated plantlets, leaf samples of the hardened plantlets should be used for isolation of genomic DNA and compared with mother plants using RAPD or SSR markers.

Conclusion

Mizoram is located in the centre of diversity of Musa germplasm which indicates that it is imperative to take necessary steps at all levels for the conservation and sustainable production of banana genetic resources. Application of biotechnological tools such as tissue culture and DNA profiling techniques could serve as the best option for these programs.

Acknowledgment

The authors gratefully acknowledge the Bioinformatics Facility (BIF) Centre in the Department of Biotechnology, Mizoram University sponsored by Department of Biotechnology, Govt. of India, for extending and providing the facilities for the preparation of the manuscript.

References

1. White PR (1943). A Handbook of Plant Tissue Culture. Jacques Cattell Press, Lancaster, PA, pp. 277.
2. Bhojwani SS & Razdan MK (1983). *Plant Tissue Culture* -

Theory and Practice. Elsevier, Amsterdam.

3. George EF & Sherrington PD (1984). *Plant propagation by Tissue culture*. Exegetics Ltd, Basingstoke, UK.
4. Pierik RLM (1987). *In vitro Culture of Higher Plants*. Martinus Nijhoff Publishers, Dordrecht, Netherlands.
5. Smith MK & Drew RA (1990). Current applications of tissue culture in plant propagation and improvement. *Aust J Plant Physiol*, **17**, 267-289.
6. Ganapathi TR, Suprasanna P, Bapat VA & Rao PS (1992). Propagation of banana through encapsulated shoot tips. *Plant Cell Rep*, **11**, 571-575.
7. Quak F (1977). *Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture*, (Reinert J, Bajaj YPS eds.), Springer-Verlag, Berlin, pp. 598-615.
8. Collins GB & Genovesi AD (1982). *Application of Plant Cell and Tissue Culture to Agriculture and Industry* (Tomes DT, Ellis BE, Harney PM, *et al.*, eds.). The University of Guelph, Guelph, pp. 1-24.
9. Hu H & Zeng JZ (1984). *Handbook of Plant Cell Culture of Crop Species* (Ammirato PV, Evans DA, Sharp WR, *et al.* eds). Macmillan Publishing Company, New York, 3, 65-90.
10. Binding H (1986). *Cell Culture and Somatic Cell Genetics, Vol. 3.* (Vasil IK, ed.), Academic Press, Orlando, pp. 259-274.
11. Fukui H, Yamazaki K & Tabatam M (1984). Two phenolic acids from *Lithospermum erythrorhizon* cell suspension cultures. *Phytochem*, **23**, 2398-2399
12. FAOSTAT (2009). FAOSTAT Agriculture Data. http://faostat.fao.org
13. Uma S & Sathiamoorthy S (2002). Names and synonyms of bananas and plaintains of India, National Research Centre for Banana (ICAR), Tiruchirapalli, India, pp. 16-43.
14. Uma S, Shyam B, Selvarajan R & Sathiamoorthy S (2001). Collection and characterisation of banana and plantains of northeastern India. In: *Advancing Banana and Plantain R & D in Asia and the Pacific. Vol. 10* (AB Molina, VN Rao & MAG Maghuyop, eds). Proceedings of the 10th INIBAP–ASPNET Regional Advisory Committee meeting held at Bangkok, pp. 103-109.
15. Vuylsteke DR, Crouch JH, Pellegrineschi A & Thottappilly G (1998). The biotechnology case history for *Musa*. *Acta Hortic*, **461**,75-86.
16. Jain SM (2004). Introduction: cellular biology and biotechnology including mutation techniques for creation of new useful banana genotypes. In: *Banana Improvement: Cellular, Molecular Biology, and Induced Mutations* (Jain SM, Swennen R, eds) Science Publishers, Inc., Enfield (NH), pp. xv-xvi.
17. Vuylesteke DR (1989). Shoot tip culture for the propagation, conservation and exchange of *Musa* germplasm. In: *Practical Manual for Handling Crop Germplasm in vitro 2*, IBPGR, Rome, Italy.
18. Daniells J & M Smith (1991). Post-flask management of tissuecultured bananas. The Australian Centre for International Agricultural Research (ACIAR) technical reports, Canberra, pp. 8.
19. Arias O (1992). Commercial micropropagation of banana. In: *Biotechnology Applications for Banana and Plantain Improvement*. INIBAP, San Jose, Costa Rica, pp. 139-142
20. Novak FJ, Brunner H, Afza R, Morpurgo R, Upadhyay RK, Van Duren M, Sacchi M, Hawz JS, Khatri A, Kahl G, Kaemmer D, Ramser J & Weising K (1993). Improvement of *Musa* through biotechnology and mutation breeding. In: Biotechnology applications for banana and plantain improvement. *Proc. of the Workshop. INIBAP*, pp. 143 - 158.
21. Molina AB & Kudagamage C (2002). The international network for the improvement of banana and plantain (INIBAP): PGR activities in south Asia. In: South Asia Network on Plant Genetic Resources (SANPGR) meeting held on December 9-11 at Plant Genetic Resources Center (PGRC), Peradeniya, Sri Lanka, pp. 1-7.
22. Lalrinfela PC & Thangjam R (2012). Genome characterization of banana genetic resources of Mizoram. *Sci Vis*, **12**, 32-36.
23. Murashige T & Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant,* **15**, 473-497.
24. Gamborg OL, Miller RA & Ojima K (1968). Nutrient requirements of suspension cultures of soybean root cells. *Exp Cell Res*, **50**, 151-158.
25. White PR (1934). Potentially unlimited growth of excised tomato root tips in a liquid medium. *Plant Physiol*, **9**, 585-600.