Plant Tissue Culture Techniques of Tropical Tuber Crops Utilized For Developing Pest and Disease Free Plantlets

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Tissue culture is a technique which is used to produce plants in sterile nutrient rich controlled environment. This technique works based on the property of the plant cells called totipotency. The technique originated at 20th century by Gottlieb Haberlandt who proposed concept of *in vitro* cell culture and recognized as the father of plant tissue culture. It is now used for functional gene studies, for developing transgenic plants and also used with the emerging technologies such as the genome editing. The application of tissue culture with *Agrobacterium tumefaciens* infection play important role in genetic manipulation of plants with desirable traits. The first true plant tissue cultures were obtained by Gautheret from cambial tissue of *Acer pseudoplatanus*. The various techniques used in tissue culture were callus culture, anther culture, embryo culture, ovary culture, cell suspension culture and protoplast culture. The important tissue culture techniques used in tropical tuber crops are meristem culture, micropropagation, and somatic embryogenesis.

Meristem culture

It is a technique widely used in potato, cassava, yam and sweet potato for producing virus free plants. Meristems are the actively dividing tissues at the shoot tip. Meristem culture with thermotherapy is an effective method for producing virus free plants in cassava. Heat treatment (thermotherapy) is often done to cassava plants before meristem culture. This will helps to reduce viral load and increase the chances of successful virus elimination from the meristem. The meristematic tip with one or more leaves was cultured to the medium with growth regulators. The method is widely used in potato for producing virus free plants. The technique along can be used for eliminating the sweet potato virus complex from sweet potato.

Micropropagation

A technique used to rapidly multiply plants from small tissue samples, including tuber crops like potatoes. Single node cuttings were used for the propagation of virus tested clones of cassava, yams and sweet potato. Single node cuttings were placed in liquid culture medium for one month to induce multiple shoot formation and subsequently sub culturing the node cuttings in solid media. Ng and Hahn 1985, Ng 1988b). The method is used for producing disease-free seed potatoes and for conserving germplasm. Micropropagation involves several stages, including the initiation and multiplication of shoots, rooting, and acclimatization of plantlets. The technique is used for the quick production of a large number of plants from a single explant, which can be used for meeting the producing disease free planting material on large scale. It is widely used for producing potato, cassava, and yam propagation, ensuring a consistent supply of high-quality planting material. It is also used for conserving the germplasm on large scale.

Somatic embryogenesis

The first report of somatic embryo from cultured cells of *Daucus carota* is reported by Reinert 1958 and Steward et al., 1958. Since then the somatic embryogenesis of a number of plant species were reported and the clonal propagation of many genotypes on large scale is possible through this method. The main four steps of embryogenesis is induction of embyogenesis, embryo development, embryo maturation and conversion. Among them the first two steps deals with inducing the cells for the formation and development of embryos. The third step is preparing the embryo for germination and fourth refers to formation of somatic seedlings and growth. The same technique where plant embryos are developed from somatic (non-reproductive) cells, is used for propagating tuber crops like potatoes, yams, and cassava. Emerging technique for cassava and other tuber crops. The regeneration of sweetpotato through somatic embryogenesis has been reported from 1980s in several cultivars (Karamian and Ebrahimzadeh

2001; Sivparsad and Gubba). Similarly in the case of cassava various explants sources like axillary buds, protoplasts, shoot apices, were used for somatic embryogenesis.

Sweetpotato plants developed from apical bud method

In vitro microtuberization

Tuberization is a physiological process that is influenced by various factors, such as concentration and carbohydrate source, light intensity and plant growth regulators (Kerbauy and Fisiologia 2019). In tuberous plants microtuberization can be induced by *in vitro* methods from explants originated from apical and nodal parts of plants (Ovono et al., 2010). Eventually these can be used as propagative materials for pest and disease free plant materials. Particularly useful for potatoes and other tuber crops especially yams, which allows the efficient storage and distribution of good quality planting materials. The vegetative propagation of yams has limitations such as low sprouting index, dormancy, contamination of pathogens and pest infestation. In order to overcome these challenges the alternative methods to improve production of yam seedlings is induction of *in vitro* tuberization and the microtubers obtained from this methods can be used for the propagation of this crop. It is also used as an effective method for the international distribution of virus tested clonal germplasm in yams.

Anther culture

Anther culture is practiced in cassava for producing haploid plants which will be subsequently used for producing homozygous diploids. It is one of the technique where immature haploid male gametophytes are developed to haploid or doubled-haploid embryos. It is cost effective method with minimum input requirements compared to other microspore culture techniques. Callus was obtained from the anther culture from B5 medium supplemented with sucrose, coconut water and gelrite. The calluses will be transferred to the MS medium with sucrose, kinetin, IAA and gelrite. The MS medium without growth hormones will develop into protocom like structures and roots (Ng 1992). The anther culture was reported in cassava for generating homozygous plants (Perera et al., 2014a; Perera et al., 2014b).

Embryo/ovule culture

Cassava being an allotetraploid (2n=4x=36) and is a highly heterozygous crop (Fregene et al., 1997). The hybridization between cassava and its related wild Manihot sp. is done for developing pest and disease resistant varieties. But the germination percentage of seeds from wild species plays an important role and was low in some cases. So embryo culture was used in cassava to germinate embryos isolated from wild seeds and first reported in cassava by (Biggs et al., 1986; Fregene et al., 1999). Even immature embryo culture is also a technique that can also be used to rescue the hybrids which are difficult to germinate by conventional methods. Various methods have been developed for the treatment of mature seeds and for culture of mature seeds in cassava (Ng 1989).

Conclusion

The biotechnological research for producing disease – pest free cassava, sweet potato and yam, tissue culture plants by invitro culture is important for the rapid multiplication. Since more research needs to be done with molecular markers is useful for the rapid identification of offtype plants.

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