Advances in Guava Micropropagation

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Guava (Psidium guajava L.) belongs to Myrtaceae family. Place of origin of guava is tropical America. It is utilized as fresh fruit consumption in India along with other Asian sub-continental countries. Genus Psidium have about 150 species, most of which are fruit bearing trees. It is native to tropical America, stretching from Mexico to Peru. It is widely adopted and tolerate in frost, drought and saline conditions. It has good amount of ascorbic acid, dietary fibres, vitamin-A (about 250 IU/100 g), pectin, calcium, phosphorus, iron and other nutrients. The roots, bark, leaves and green fruits are used as medicine for gastrointestinal problems, diarrhoea and dysentery. Salad, guava jelly and pudding are made from the of ripe fruits. Besides that, Guava juice and guava pulp wines are also prepared from guava fruit. It also contains high number of antioxidants like, lycopene, carotenoids and polyphenols. Guava has the chemicals which help to reduce the incidence of diseases like arthritis, arteriosclerosis, diabetes, cancer, heart disease, inflammation and brain dysfunction. Antioxidants reported to retard aging, high concentrations of pectin content in guava fruit reduces the cholesterol of human body. Production of Guava is overlaying the agronomical and horticultural problems including susceptibility to many pathogens, wilting, low fruit growth, short life, higher seed content and stress sensitivity. Improving guava species by traditional methods of breeding are limited because plants generally have long juvenile growth periods, and are heterozygous in nature. While, seed originated guava plantlets do not maintain the genetic purity of the variety due to the segregation and mixing of genetic characters during sexual reproduction. In addition, high internal fungi, bacterial contamination and phenolics compounds exudation tend to limit *in vitro* cultures of the guava plant. Genetic engineering technique reducing the breeding period. Due to this approach micro-propagation and regeneration produce large numbers of rooted plants from unique plants. Clonal propagation can reduce plant-to-plant variation and ensuring the uniform populations of clones.

In vitro proliferation of guava (Psidium guajava L). (A) stock guava plants in the greenhouse; (B) nodal section became browning after sterilization; (C) new shoots break out from healthy nodal sections; (D) shoots proliferated; (E) elongated shoots; (F) rooted shoots by medium method (medium with IBA); (G) rooted shoots by dipping method; (H) guava plantlets acclimatized into the soil for 2 weeks; (I) guava plantlets acclimatized into the soil for 10 weeks

Micropropagation

Micropropagation refers to *in vitro* plant propagation method. The advantage of micropropagation over conventional propagation technique is to reduce the time needed for achieving large scale propagation of plants which are true to the type and disease free. There are four stages of micropropagation i.e. establishment, multiplication, rooting and acclimatization. Micropropagation is now a well-established technology which has made significant contributions to the propagation and improvement of agricultural crops in general and applicable to various crops.

Explants

Totipotency is the ability of plant tissues/parts (excepting bark) to regeneration *in vitro*. Juvenile explants, comparatively gives batter results. New vegetative growths in *Psidium guajava* L. have been reported to be reliable source of explant. The majority

of workers have used actively growing shoot tips or nodal segments as explant.

Time of collection and size of explants

The explants of guava collected from the base of the main stem when vegetative growth vigorously establish. The early spring collection of explants show less contamination in compare to late autumn and summer. Explants collection in spring gives best culture establishment and profuse sprouting. Generally, the size of explants best suited for *in vitro* propagation of guava is 1.0-3.0 cm.

Phenolics

Guava exude the phenolic compounds into the culture media makes difficulty in regeneration process. *Psidium guajava* L. is a recalcitrant species having high phenolic exudation that kills explants from sources outside the laboratory. Establishment of *in vitro* cultures of woody plants is greatly hampered by the browning of the explant and culture medium. Browning of explant is due to oxidation of the phenolic compounds, released from the cut ends of the explants. *In vitro* establishment of the guava explants was very difficult due to the exudation of phenolic compounds into cultures, by which the media turned brown or black within 12-24 hrs and most of the explants died within 2 days of inoculation.

Plant growth regulators

Plant Growth regulator are such organic compounds occurring naturally in the plants as well as synthetic and promote, inhibit or modify any physiological process in small amount. The concentration of plant growth regulator varied from species to species for *in vitro* culture of guava, and type of growth to be initiated i.e. callus formation, shoot proliferation, rooting, etc. In order to support good growth of tissue and organs, it is generally required to add one or more PGR's such as auxin, cytokinin and gibberellins in the medium. Cytokinin levels especially have been shown to be critical for multiplication of many tropical fruit trees. BA has been the most common cytokinin used for guava propagation.

Rooting

Among the various auxins *viz.*, IBA, IAA, NAA etc. used for *in vitro* rooting, IBA is the most commonly used auxin.

Hardening of rooted plantlets

The success of any micropropagation research depends on the success of plantlet transferring technique, where shoot or plantlets that have been growing heterotrophically under an aseptic environment of test tube (having very high humidity) become autotrophic and grows under condition of moderate to low humidity. For acclimatization rooted plantlets of guava were taken out of culture tubes, washed thoroughly to remove any remaining medium and planted in small plastic pots filled with garden soil and compost (1:1). During first 7-10 days, the potted plantlets were covered with glass beakers to provide high humidity. The plantlets were kept outdoor under 80 per cent shade for about a week, after which they were transplanted in pots with soil. More than 90 per cent of the plantlets survived after transplantation to soil.

Genetic fidelity

The commercial multiplication of a large number of diverse plants species represents one of the major success stories of utilizing tissue culture technology profitably. However, a major problem often encountered with the use of tissue culture techniques such as SE is the occurrence of soma clonal variation, which is often heritable as it represents induced genetic changes. Thus, genetic fidelity testing is an important prerequisite for *in vitro* regeneration protocols of many crop species, particularly if the resultant plants are to be transplanted to the field. Several strategies have been employed to assess the genetic fidelity of regenerated plants, each with their own advantages and disadvantages. Molecular markers facilitate the screening of SE regenerated plants with high precision, and since these markers are unaffected by environmental factors (that can alter phenotypes), they produce reliable and reproducible resu-lts. However, for an effective analysis of the genetic stability of *in vitro* regenerated plantlets, a combination of markers that amplify different regions of the genome should be used.

Overall, *in vitro* clonal propagation protocols for different guava spp. and varieties have been developed in all over the world. For extending the guava production in tropical and subtropical areas a rapid and efficient method for clonal propagation of elite mature genotype is necessary. During past few decades, transpiring biological techniques for micropropagation and tissue culture of predominant guava cultivars have been discussed by several researchers are effective. However, there are several problems related with *in vitro* cultures of these explants including browning or blackening of culture medium due to leaching of phenolics, microbial contamination, and *in vitro* tissue recalcitrance which require to be given more attention. Understanding of the biological processes that permit the manipulation of *in vitro* morphogenesis and investigations on various physiological, biochemical and molecular aspects of plant hormones will highly improve our understanding and gives details that will necessary for addressing the problems of *in vitro* recalcitrance or *in vitro* plant growth and development.

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