# Biotechnological Interventions for Improvement of Guava (*Psidium guajava* L.)

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**Abstract**

**Although large number of varieties of guava has been selected, only few selections viz., Allahabad Safeda, Sardar, Pant Prabhat and Lalit are being exploited commercially. A medium tall less seeded, colored variety with good keeping quality and resistance to guava wilt disease is need of the day. Guava is being propagated through budding, stooling and grafting. Wilt infected mother plants of guava are playing major role in spreading the disease beyond leap and bounds. There is an urgent need to produce disease free planting material in guava. Biotechnology can help in solving some of the long-standing problems of guava cultivation. Research on guava biotechnology is still in its infancy. This paper describes current status and future need of biotechnological research for improvement of guava.**

## INTRODUCTION

Guava belongs to family Myrtacene. This is native to tropical South America, but it is cultivated in many tropical and subtropical part of the world. It is one of the 50 most well known edible tree fruits of the tropical and subtropical climate. In later years, it quickly reached most of the tropical and subtropical areas of the world. Guava is gaining popularity in India and it is being cultivated in Bihar, Uttar Pradesh, Madhya Pradesh, Karnataka, Gujarat and Andhra Pradesh. It is estimated that it is cultivated over an area of 1.5 lakh ha in India with 1710.6 Mt production. Although a large number of varieties of guava have been selected, only few selections viz., Allahabad Safeda, Sardar, Pant Prabhat and Lalit are being exploited commercially. A medium tall less seeded, colored variety with good keeping quality and resistance to guava wilt disease is still a far cry. Guava is being propagated through budding, stooling and grafting. Wilt infected mother plants of guava are playing a major role in spreading the disease beyond leap and bounds. There is an urgent need to produce disease free planting material in guava. Biotechnology can help in solving some of the long-standing problems of guava cultivation. Research on guava biotechnology is still in its infancy. This paper describes current status and future need of biotechnological research for improvement of guava.

## MOLECULAR CHARACTERIZATION

Guava is an allogamous fruit crop, which is highly heterozygous. Several guava (*Psidium guajava*) cultivars have emerged as a result of seedling selection and seedling of these cultivars are being commercially exploited through seed propagation which has indirectly given rise to several types which are not true to the commercial type and vary in several characters from the parent population. Morphological characters may not be reliable to discriminate between closely related guava genotypes although several morphological traits such as fruit colour, leaf shape and size may be useful but they lose their usefulness for assessment of genetic diversity between closely related guava genotype. Molecular markers can be gainfully employed to discriminate between species and cultivars of guava. Recently, few reports have appeared on use of RAPD marker for genetic assessment of guava. RAPD markers were used to estimate molecular diversity of 41 genotypes of guava consisting of five *Psidium* species, 23 varieties, 12 selections and a hybrid. Analysis suggests that Indian guava can be rated as low to moderate diversity and

also indicated that various triploid seedless cultivars of guava are not genetically identical and have independent origins. All the species used in their study could be subspecies of *P. guajava* (Prakash et al., 2002). RAPD analysis for discriminating 13 North Indian cultivars of guava revealed that Hisar Safeda and Allahabad Safeda were the closest pair of cultivars with a distance of 0.051 on scale of zero to one. Cultivars Pear Shaped and Red Supreme were most distantly placed in relation to each other with a distance of 0.423. Average similarity index among 13 cultivars was 0.604 and on an average 81.85 bands were amplified/cultivar (Dahiya et al., 2002). We used DAMD to discriminate between individuals in half sib population of *Psidium guajava* consisting of 6 half-sib progeny, Allahabad Safeda and 2 *Psidium* species. In order to detect adequate levels of polymorphism between these genotypes, a total of 6 minisatellite primers were screened and 4 primers that gave best results in terms of discrete and consistent band patterns were selected. Analysis of the profile obtained after amplification of the guava DNAs with four primers namely HVR, HBV, M13 and 33.6 (Fig. 1) depicted that the seedlings could be discriminated from Allahabad Safeda, *P. acutangula* and *P. guineense*. The genetic distance between the half sib progeny was less but they showed considerable distance from Allahabad Safeda and the two *Psidium* species studied.

## MOLECULAR AIDED BREEDING

Molecular markers are especially advantageous for improving agronomic traits in perennial fruit crops that is otherwise time consuming and difficult to tag the genes conferring resistance to pathogens, insects, nematodes, tolerance to abiotic stresses, quality parameter and quantitative traits. By using Marker Assisted selection, the breeder could select molecular markers that are tightly linked to resistant genes for identifying resistant genotypes. Unfortunately information pertaining to molecular assisted breeding is lacking in guava and there is an urgent need to develop genetic map of guava in days to come.

## IN VITRO CELLULAR SELECTION

Guava Wilt Disease (GWD) is a major problem faced by guava growers across the globe. Wilt affected trees are removed as control measure. It is reported to be caused by *Fusarium oxysporium, Glycadium roseum* (India) and *Penicillium* (Africa) fungus. It is of the utmost importance to develop wilt resistant guava cultivar in days to come otherwise guava industry may collapse. Biotechnological technique such as DNA recombinant technology, Molecular aided breeding and in vitro cellular selection can be used to develop a wilt resistant variety. There is hardly any information on these areas. However, a wilt resistant rootstock has been developed using in vitro cellular selection in South Africa. Cell free filtrates derived from *Penicillium vermoesenii* were used to screen thirty thousand guava seedlings in vitro. Ten promising selections were made and cloned in tissue culture. Three of the selections exhibited 100% tolerance or resistance to GWD. The major advantage of using this technique to screen for resistance is that the juvenile growth phase of the plants could be maintained. This facilitated the use of nodal and split-nodal cuttings from tissue culture derived rammets instead of the slow, conventional propagation techniques such as air-layering and hardwood cuttings. As a result, 25% of the trees lost to GWD in South Africa have been replaced by trees with tolerant rootstocks within a research period of five years (Vos et al.,1998). Work has also been initiated at CISH, Lucknow, to devise in vitro cellular selection system for production of wilt resistant rootstock. In vitro cellular selection has been developed in Indian guava. Somatic embryogenesis has been developed using fruit mesocarp tissue obtained from immature fruit (Chandra et al.,2004). The somatic seedlings developed are being screened against the fungal toxins.

## GENETIC ENGINEERING

Recently, genetic modification of plants based on transgenesis involving identification, isolation and mobilization of genes for traits of economic importance in

crop plants is emerging as an attractive and at times indispensable adjunct to conventional plant breeding. Guava suffers colossal losses due to guava wilt disease. It is a perishable fruit, which accounts for huge post harvest losses. DNA recombinant technology can be gainfully utilized for development of wilt resistant cultivar with regulated ripening. Not much headway has been made in this direction. However, an efficient in vitro regeneration system in guava using fruit mesocarp as explant has been developed. Mesocarp tissue excised from immature fruits were cultured on MS medium fortified with 2 mg/L 2,4-D, 100 mg/L ascorbic acid, 400 mg/L glutamin and 6% sucrose produced transluscent, mucilaginous small somatic embryos. Embryos easily germinated on plain MS medium (Chandra et al., 2004). Recently, genetic transformation of guava with CBF1, CBF2 and CBF3 cold hardy genes has been demonstrated at Fort Valley State University, USA. There is a need to develop efficient transformation system in guava so that suitable transgene could be transferred. There is a need to engineer genes controlling ethylene biosynthesis and ethylene sensitivity in guava for better shelf life. Insertion of genes encoding hydrolytic enzymes (which can degrade fungal cell wall) such as chitinase and glucanase could also be helpful for controlling diseases in guava.

**MICROPROPAGATION**

Micropropagation method could assist in rapid and mass production of clonal stock of newly released improved cultivars of guava. Leaching of phenol in culture, in vitro contamination and other regeneration problems impedes micropropagation of majority of woody perennial fruit trees particularly of guava. The phenolic exudation could be controlled by using different antioxidants, changing redox potential of medium, keeping cultures in dark or through etiolation of explant. Partial etiolation of stock plant of guava cv. Allahabad Safeda and Aneuploid No. 82 rootstocks before explant excision resulted in early bud sprouting and significant increase in explant survival. The lower degree of browning of culture medium was confirmed by recording the lesser amount of total phenol exudation in to medium. Agitation of guava explants in 0.5% polyvinylpyrrolidone and 2-3 changes of medium for the initial 10-15 days were essential for establishment of cultures. A method for establishing aseptic culture of guava from mature field grown stock plants for micropropagation through enhanced axillary branching technique has been worked out. The maximum aseptic explant with shoots was obtained by a combination of sterilizing agents involving hydrogen peroxide (10%), silver nitrate (0.25%) and mercuric chloride (0.05%) treatment. Khattak et al. (1990) reported that shoots of Allahabad Safeda taken from plastic wrapped on unwrapped branches of 10-year old-trees were surface sterilized with $HgCl_2$, NaOCl or ethanol at various concentrations for different durations. The best results were obtained by treating the seedling explants with 70% ethanol for 1 min followed by 5% NaOCl for 5 min, and treatment of shoots from plastic wrapped branches with 0.05% $HgCl_2$ for 5 min. Shoot tips (1-2 cm in length) were excised from these seedlings and shoots were placed in tubes on a medium comprising MS salts + 0.7% agar + 3% sucrose + 0.1 mg/L BA and cultured for 5 weeks. The apical portion (5-7 cm) of shoots from new growth on mature branches and from basal sprouts of a 15-year-old guava plant was cultured. After sterilization procedure 1.0-1.5 cm nodal explants were prepared aseptically and inoculated vertically on the media after a dip in solution of antioxidants (75 mg/L citric acid and 50 mg/L ascorbic acid prepared in distilled water). After 2-3 initial changes of medium, when phenolic exudation was checked, the explants were oriented horizontally. This facilitates rapid uptake of nutrients by the growing root tips. The earlier medium (MS + 4.5 m BA) reported by Amin (1986a) and Jaiswal and Amin (1986) worked well with axillary shoot buds also. In this medium explants responded by axillary bud enlargement and bursting shoot masses within 4-6 weeks. After proliferation started, the explants with proliferating shoot masses were subcultured in wider culture vessels with more medium to meet the increasing nutrients requirement and space. 3-6 shoots per culture vessel were reported at 12 weeks of incubation. The response of in vitro shoot nodal segments was better in comparison with the field grown tree and the probable reason given is due to the absence

of lag period between explanting and adaptation of explants to in vitro conditions. The inoculation was carried out throughout the year and the maximum response with minimum contamination and browning of explant and medium was obtained from explants cultured during April to June. (Amin, 1986b; Jaiswal and Amin, 1986). Amin and Jaiswal (1988) reported a micropropagation method for guava (*Psidium guajava* L.) 'Chittidar' using nodal explants of field-grown adult trees. Murashige and Skoog (1962) (MS) revised medium containing 1 mg/L benzyladenine, was found to be the best when the axillary buds grew out within 3-4 weeks. On transfer to fresh medium of the same composition, these shoots attained 3-5 cm in length and had 4-6 nodes after 4 weeks further culture. Nodal segments taken from in vitro proliferated shoots gave rise to 2-4 shoots by precocious axillary branching without an initial lag-period. By repeated sub-culture, a large number of shoots were built up with a shoot multiplication rate of 3 to 4 fold per sub-culture. Shoots obtained from the proliferation stage were rooted on half-strength MS medium containing indole butyric acid and alpha-naphthalene-acetic acid. Rooting rate was about 33% in the initial cultures and 70-90% in shoots of the 5th and subsequent sub-cultures. Regenerated plantlets were successfully established in soil under field conditions. In the absence of sucrose, cultures could not survive after 3 weeks of incubation. In the sucrose containing media, 30-45 g/L gave the best result. Complete inhibition of bud sprouting and shoot growth and death of the culture on sucrose free medium confirm the necessity of a carbon source in the medium. Increase of agar from 5 to 8 g/L remarkably increased the number of usable shoots and reduced the frequency of vitrified culture. The agar concentration more than 10 g/L yielded very poor result by producing spindly, dry and less number of usable shoots. The early bud breaking was noticed on medium with lowest agar concentration (5 g/L) can be attributed to the easy availability of nutrient elements like Ca, K, Mg and Mn in the soft gel medium (Debergh, 1983). However, the brittle, turgescent and water-soaked nature of cultures on lower agar concentration might be due to the changes in the matrix potential of media water (Debergh et al., 1981). On the other hand, reduced growth and of agar could be due to restricted diffusion of macronutrients (Romberger and Tabor, 1971) or reduced availability of organic matter and water (Skirvin, 1981; Debergh, 1983). It is evident from the results that concentration of agar in the medium can affect the culture growth in several ways. Therefore, the level of agar in the medium should be such that it minimizes the water loss and allows a good nutrient diffusion.

Success of in vitro propagation of mature guava plants (Amin and Jaiswal, 1987, 1988) opens up the possibility of producing massive number of clonal plants from a selected cultivar. However, before exploiting such a technique for commercial purposes, gathering information regarding the requirements for each of micropropagation and then developing an industrially practicable procedure for the best culture conditions is necessary. (Anderson, 1980; Lakshmi Sita et al., 1982; Murashige, 1974). Concentrations of sucrose and agar, and levels of pH alter the properties of the culture medium, which in turn influence the availability, and use of nutrient elements by the excised tissue. (Murashige, 1974; Skirvin, 1981; Bhojwani and Razdan, 1983). This can partially be explained by the easy diffusion and availability of mineral nutrients in soft gel media at pH 4.5 and 5.0. It is an interesting observation by Amin and Jaiswal (1989 a and b) that while soft gel medium resulted from more acidic pH favored in vitro growth of guava shoots, the soft gel medium resulted from lower amount of agar caused vitrification of culture, a morphological anomaly common in woody plant micropropagation. There might be more reasons other than easy diffusion and availability of nutrients in soft gel medium for this discrepancy. Amin and Jaiswal (1987, 1988, 1989) have reported that phloroglucinol inhibits in vitro rooting of guava microcuttings and it was also reported that phloroglucinol in combination with NAA is more inhibitory than IBA and phloroglucinol comparatively, less acidic pH medium (5.5-6.00) showed better rooting than more acidic pH medium (4.5-5.0). Papadatou et al. (1990) reported that the ability to clone individual seedlings at a young stage to provide genetically uniform plant material would be useful since many genetically uniform plants arising from each seedling could

be evaluated for the desired characteristics in a relatively short period of time. Blackening of the medium, which was a serious problem with cultivar `Chittidar' guava (Amin and Jaiswal, 1988), did not observed by Papadatou et al. (1990). This is probably because the young seedlings do not synthesize phenolic substances when they grow in a growth chamber. Proliferating shoot cultures were established from shoot tips excised from seedlings grown in a growth chamber and cultured on Rugini Olive medium (OM) supplemented with benzyladenine (BA). Maximum proliferation was achieved with 2 mg /L BA. The low multiplication rate may be due to low BA concentrations (1 mg/L). Shoot explants were easily rooted in vitro using OM with α-naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA) at 0.5 and 1 mg/L for both auxins. Rooted shoots were readily established in peat-based compost (Amin and Jaiswal, 1988). Prakash (1992), Prakash and Tiwari (1993) reported in vitro clonal propagation of guava. The explants (apical portion 5-10 cm) were collected from 12-year-old guava plants of cv. Sardar. The shoots were collected, from new flush growth of the plant, in distilled water containing 5 ml/L teepol + 2 mg/L Bavestin and streptomycin each + 75 mg/L ascorbic acid + 50 mg/L citric acid. The shoots were washed thoroughly under running tap water and then kept under running tap water for 30-40 min, then washed with autoclaved 1% KCl followed with 1% NaOCl for 5-7 min. The treated explants were washed 3-4 times with sterile distilled water. The shoot buds proliferated in MS medium supplemented with 3 mg/L BA + 0.4 mg/L IBA + 4 g/L PVP. Regenerated shoots were multiplied on MS medium supplemented with 1 mg/L BA + 0.2 mg/L IBA and for shoot elongation, 1 mg/L $GA_3$ was added. Half strength of MS medium + 0.2 mg/L IBA + 0.2 mg/L NAA + 0.1% charcoal + 2% sucrose + 0.7% agar was used for rooting of regenerated shoots. The regenerated plantlets were transferred to earthen pots filled with autoclaved FYM + sand (1:1) and covered with glass beaker to maintain humidity. The humidity was reduced gradually. Using this method they have produced hundreds of tissue culture plantlets and successfully transplanted in field. Tissue culture studies on the nodal explants of the glass house grown (2-3 months old) seedlings of guava plants have been conducted. They used 0.4% $HgCl_2$ for 10 minutes to obtain the axenic cultures. The tissue culture plantlets, which remain in the culture vessel, do not fulfill the requirement unless they are successfully established in the field conditions. Recently, complete micropropagation protocol using shoot bud in Allahabad Safeda cultivars of guava have been developed at CISH, Lucknow. Good shoot proliferation was reported on Ms medium fortified with 3 mg/L BAP. Proliferated shoots rooted on MS supplemented with 10 mg/L IBA. Rooted shoots were acclimatized on autoclaved coconut husk fortified with ½ MS nutrient solution+ 1mg/L paclobutrazol (Fig. 2). Many protocols for micropropagation of guava are available. However, low in vitro shoot proliferation and poor acclimatization are two major problems. In order to make this technology commercially viable, we have to achieve high shoot proliferation and plant survival efficiency in commercial guava cultivars. There is a need for scaling up of protocols.

## CONCLUSION

Guava (*Psidium guajava* L.) is considered the poor man's apple in India. Guava fruits are in demand as fresh fruit as well as in processing industry. However, some of the long standing problems such as guava wilt disease and lack of soft seeded, coloured variety requires urgent attention of researchers. There is a need to develop guava variety resistant to guava wilt disease with delayed ripening attribute. Conventional breeding has been of not much help so far. Biotechnological interventions may bring in desired traits in commercial guava variety. Recombinant DNA technology and marker-aided breeding could facilitate development of desired guava cultivars. There is a need to develop genetic map of guava. Work on transforming guava with cold hardiness gene (CBF1,CBF2 & CBF3) is going on. There is a need to engineer genes controlling ethylene biosynthesis and ethylene sensitivity in guava for better shelf life. Insertion of genes encoding hydrolytic enzymes (which can degrade fungal cell wall) such as chitinase and glucanase could also be helpful for controlling diseases in guava. Many groups have reported in

vitro regeneration protocol in guava using somatic embryogenesis and organogenesis pathway. Shoot bud culture is the most preferred pathway for clonal multiplication. Guava is a recalcitrant species and in vitro oxidative browning, in born contamination, low in vitro shoot proliferation and poor survival of micropropagated plants during acclimatization are some of the problems which makes micropropagation protocols of guava commercially unviable. There is a need to devise a more efficient and economic system of micropropagation of guava.

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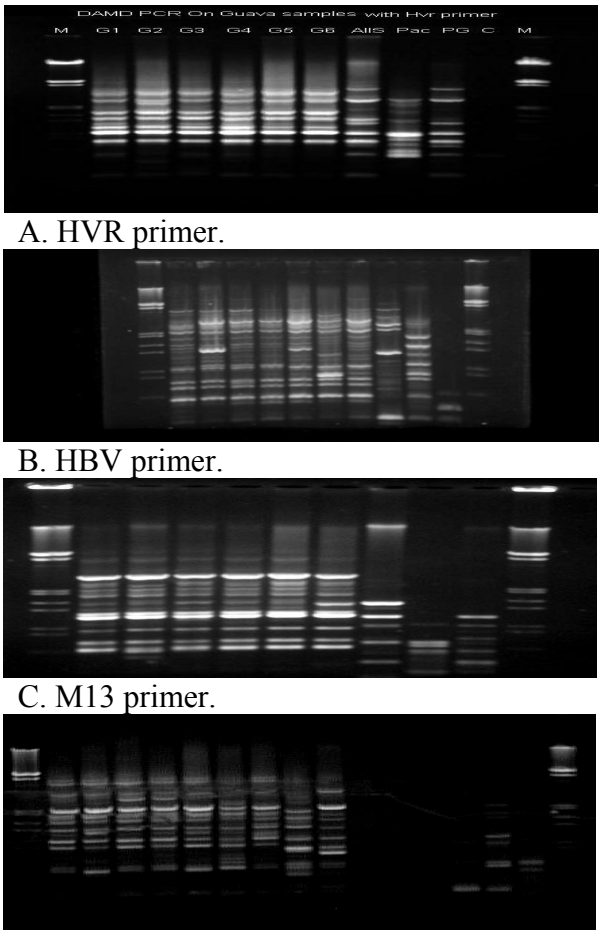
**Figures**




A. HVR primer.

B. HBV primer.

C. M13 primer.

D. 33.6 primer.

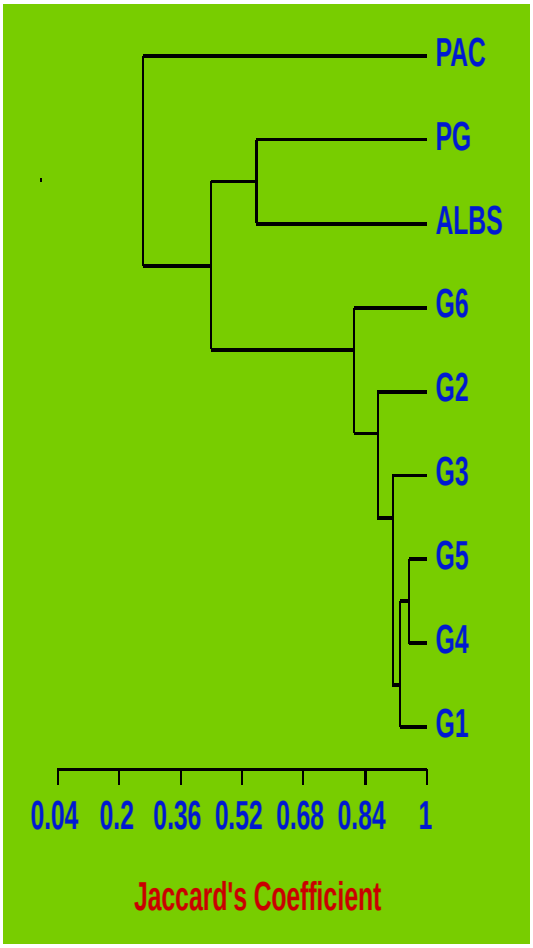



Fig. 1. Molecular and morphological discrimination in half sib population of *Psidium* species.

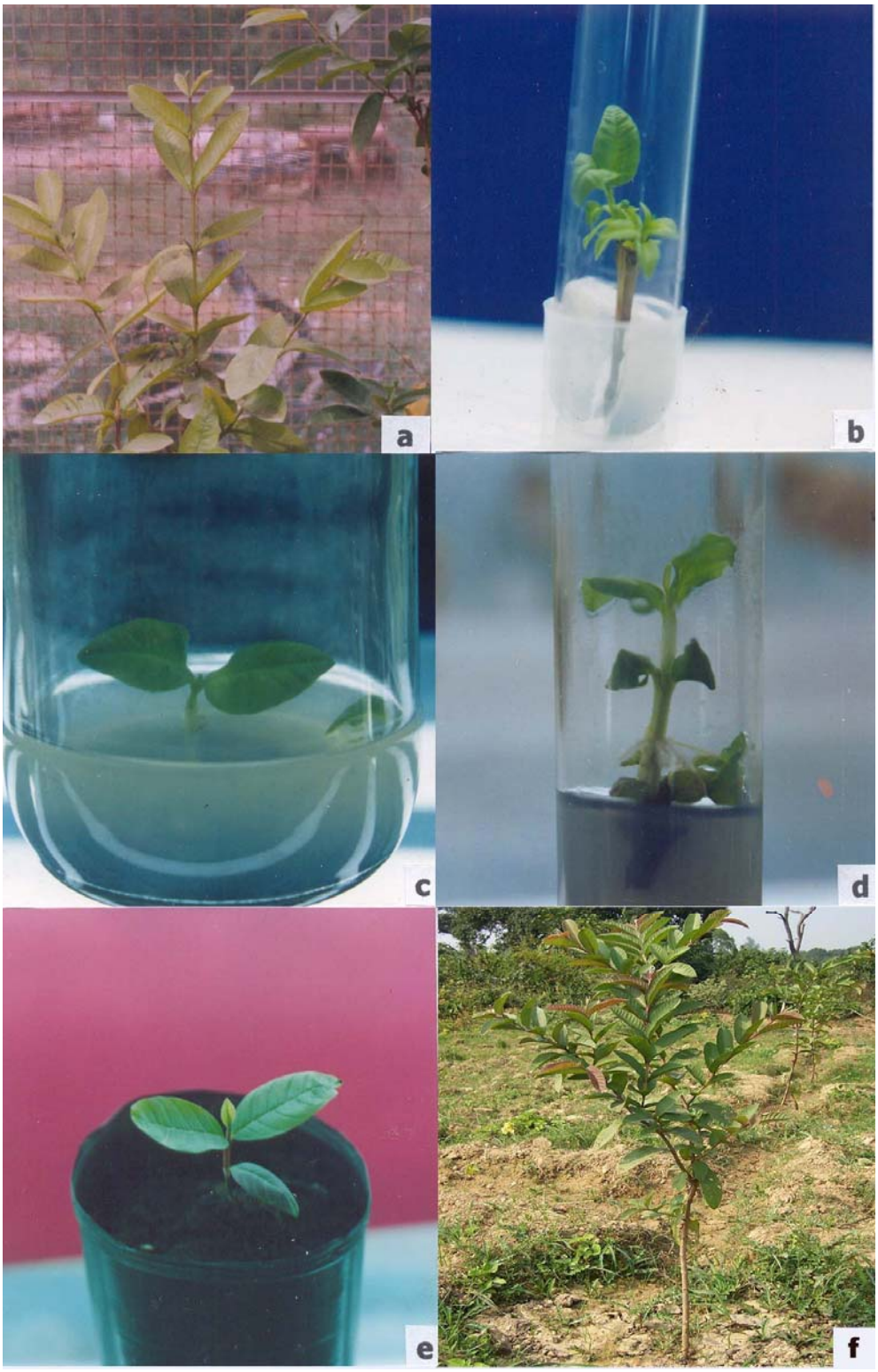



Fig. 2. Micropropagation of guava through shoot bud culture.