Embryogenesis and plant regeneration from mesocarp of \textit{Psidium guajava} L. (guava)

R Chandra*, A Bajpai, Soni Gupta and R K Tiwari
Biotechnology Laboratory, Crop Improvement and Production Division
Central Institute for Subtropical Horticulture, Rehmankhera, Lucknow 227 017, India

A protocol has been developed for the induction, maturation and germination of somatic embryos from mesocarp tissue of \textit{Psidium guajava} L. (guava). Explants were cultured in modified MS medium fortified with 2, 4-D (2.0 mg/l), ascorbic acid (100 mg/l), L-glutamine (400 mg/l) and sucrose (6%). Embryogenic proliferating tissue was induced, which was translucent, mucilaginous and differentiated into many small somatic embryos. The somatic embryos were retained in the same medium, where simultaneously differentiation of new somatic embryos and their conversion into plantlets was observed. Thus, the embryogenesis in guava could be perpetuated, which would be used in future for carrying out cellular selection against wilt causing organism.

\textbf{Keywords}: \textit{Psidium guajava}, guava, mesocarp, somatic embryogenesis, repetitive embryogenesis

\textbf{IPC Code}: Int. Cl. A 01 H 4/00, 5/00

\section*{Introduction}
The \textit{Psidium guajava} L. (guava) is an important fruit tree that attains a height of 10 m and grows well on all types of soils. It is commonly propagated through cuttings, grafting, budding, stooling or air layering, but these methods are cumbersome and slow\cite{1}. On the other hand, plant regeneration via tissue culture has immense scope, because it is a reliable method for fast multiplication of true to type plantlets.

Presently, the uniform elite planting material is always in short supply. Shoot bud culture has been fairly successful in guava\cite{2,3}, but its economic feasibility is still in doubt. In addition to shoot bud culture, somatic embryogenesis also offers the possibility of mass multiplication of elite planting material of guava. Besides being an efficient method, it facilitates scaling up operations, encapsulation, etc. It has additional importance for developing a system for somatic cell genetics, transgenics and \textit{in vitro} selection. The \textit{in vitro} selection assumes special significance in the present day scenario because of high rate of mortality due to wilt disease in guava orchards.

\section*{Materials and Methods}
Experiments were conducted with the uniform plantation of \textit{Psidium guajava} L var. Sardar. The explants (floral parts and mesocarp of the fruit) were collected from the rainy season crop. The cultures were maintained on MS medium\cite{4} and that of Gamborg \textit{et al} (B\textsubscript{5})\cite{5} supplemented with various plant growth hormones. The pH of medium was adjusted to 5.7 before autoclaving (121°C, 20 min). The plant material was subcultured into fresh medium at 20 day intervals and cultures were initially incubated in dark for 3 days and thereafter under light intensity of 3,000 lux of 16 hrs photoperiod provided by Philips cool-white 40 W fluorescent tubes and photosynthesis active radiation lamps (PAR) (28±0.5°C). Ten replications were set-up for each treatment.

\section*{Results and Discussion}
Of the different explants, which included fruit mesocarp, petals and anthers, the mesocarp of fruits responded most favourably to the culture manipulations. In both rainy and winter season crop (Table 1), the period for embryogenic proliferating tissue induction was limited to 3.4 and 3 days, respectively, when best response could be obtained. Even though callus induction frequency was good in anthers, the amount of callus induced was small in comparison to mesocarp. Thus, only mesocarp of fruits was taken as explant in further experiments. It was found that tissue derived from immature fruits was especially amenable to induce tissue proliferation (Table 1) as compared to mature explants. While incubation of explants in dark resulted in meristematic tissue formation, its proliferation continued in light.

---

*Author for correspondence:
Tel: 0522-2841023 extn 551; Fax: 0522-2841025
E-mail: docrchandra@hotmail.com
When the primary explant was cultured on medium containing auxin, 2, 4-D or NAA and cytokinins, BA or Kn, three different kinds of tissues were formed.

i. An embryogenic tissue, which was translucent, mucilaginous and differentiated into many small somatic embryos (Fig. 1a).

ii. Brown nodular tissue with meristemoids that under suitable conditions developed further into adventitious buds (Fig. 1b).

iii. A non-embryogenic tissue, which was fast growing, but lacked organogenetic potential

Specific role of plant growth regulators was assessed for getting desired response of in vitro proliferating tissue growth (Table 2). Shoot differentiation was also observed from proliferating tissue derived from mesocarp explants in P-9 media, suggesting that plant regeneration via organogenesis may also take place besides embryogenesis.

The first sign of differentiation of embryogenic structures was observed on explants within 4-6 weeks of culture in GW medium. The embryogenic and non-embryogenic calli were quite different from each other. Somatic embryogenesis occurred concomitantly with the initiation of tissue proliferation and its further growth. It may also be said to be indirect, as the meristematic tissue intervened in between the original explant and somatic embryo formation 6. The somatic embryos, when retained in the same medium (induction medium), instead of proceeding to next stage of its development, gave rise to new somatic embryos (Fig. 1c). Thus in guava, this is the first report of repetitive embryogenesis where the ability to perpetuate the embryogenic state indefinitely was realised as in eucalyptus, peach and nectarine 7-9. This kind of sustained somatic embryogenesis is suitable to be exploited for diverse goals, such as, mass propagation and production of transgenics.

Another most important fact was a very good recovery of complete plantlets by conversion of somatic embryos into plantlets (> 85%). Particularly striking was the phenomenon of continued induction and conversion of somatic embryos occurring simultaneously in the GW medium. (Fig. 1d)

Thus, the embryogenic system obtained in guava could be perpetuated via repetitive embryogenesis. This makes the system potentially attractive for mass production of clonal plantlets. The high multiplication rate obtained from embryogenic cultures, offers many advantages over other conventional and tissue culture propagation systems. Unlike other in vitro methods, there is no requirement for separate shoot growth and rooting steps. Another advantage offered by the system is that the in vitro mutation rate may be lower than
that occurring in organogenic regeneration system and hence, the genetic fidelity among the regenerant will be maintained.

The standardisation of plant regeneration methodology via somatic embryogenesis can advantageously be used in carrying out cellular selection against wilt causing pathogen under in vitro conditions, which will go a long way to ameliorate the potential danger of the guava industry from the disease.

Acknowledgement
The authors are grateful to Prof. R K Pathak, Director, CISH, Lucknow, for providing facilities to carry out the work. The work was supported by the grant of UPDASP/UPCAR-sponsored networking project on guava wilt (Biotechnology component).

References

<table>
<thead>
<tr>
<th>Media code</th>
<th>Description (conc in mg/l)</th>
<th>Days to tissue growth</th>
<th>Tissue characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>P&lt;sub&gt;0&lt;/sub&gt;</td>
<td>MS + BA 0.5 + NAA 0.1 + Adenine sulphate 150</td>
<td>7.0</td>
<td>Pale yellow with nodular structures</td>
</tr>
<tr>
<td>R&lt;sub&gt;1&lt;/sub&gt;</td>
<td>MS + Kn 10.0 + NAA 2.0</td>
<td>5.0</td>
<td>White, soft, fast growing</td>
</tr>
<tr>
<td>GC&lt;sub&gt;6&lt;/sub&gt;</td>
<td>B5 major salts + MS minor salts + vitamins + 2, 4D 1.0 + NAA 0.1 + BA 0.05 + Ascorbic acid 100</td>
<td>30.0</td>
<td>Compact slow growing with few globular structures</td>
</tr>
<tr>
<td>GW</td>
<td>B5 major salts + MS minor salts + vitamins 2,4D 2.0 + Ascorbic acid 100 + L-Glutamine 400</td>
<td>28.0</td>
<td>Compact, globular structures with embryogenetic potential</td>
</tr>
</tbody>
</table>