In Vitro Culture of Guayule Using Pretreated Seeds

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Abstract. Multiple shoot and callus formation was achieved with pretreated guayule (Parthenium argentatum Gray) seeds using a Murashige and Skoog basal medium. With BA, only shoots developed; NAA stimulated rooting. As guayule plants display a high degree of apomixis, this technique provides an ideal system for genetic manipulation. Chemical names used: N-[(phenylmethyl)-1H-purin-6-amine (benzyladenine, BA); 2-(1-naphthyl)acetic acid (NAA).

Guayule, a perennial shrub indigenous to north-central Mexico and southwestern Texas, is an economically important plant that produces rubber similar in quality to that of Hevea brasiliensis. Guayule is most easily and economically propagated by seed. However, cuttings and in vitro culture may facilitate rapid propagation of selected clones. Guayule plants can reproduce asexually by apomixis, an important feature for specialized plant breeding (LaBreque, 1980). Parthenium plants have been grown in culture using a variety of explants: seedlings (Zavala et al., 1981; Staba and Nygaard, 1983), shoot tips (Dastoor et al., 1981), and cotyledons, stems, leaves, petioles, roots, and flowers (Radin et al., 1982; Subramanian and Rao, 1980; Wickham et al., 1980). We used seeds as explants in our study because of ease of sterilization and the importance of apomictic seed for breeding.

Seeds at the following developmental stages were used for experimentation: 1) immediately after anthesis, 2) before pericarp hardening, and 3) fully mature. Seeds from stages 2 and 3 were either pretreated using a water soak, sodium hypochlorite, and GA\textsubscript{4} (Hurly et al., 1989), or left untreated. The seeds were sterilized for 5 min in 1\% NaOCl containing 0.01\% Tween 20 and washed three times in sterile distilled water. The pericarp was removed on one-half of the seeds, and the seeds then inoculated on a half-strength Murashige and Skoog medium (Murashige and Skoog, 1962) supplemented (per liter) with 30 g sucrose, 8 g agar (No. 1 Oxoid), 0.1 g myo-inositol, and 0.5 g polyvinylpyrrolidone (PVP). The PVP was used to absorb the phenolics released by the covering structures of the seeds. Thirty seeds of two local strains of guayule (W8 and W10) were used per treatment. They were placed in culture tubes (10 × 90 mm) containing 10 ml of medium and covered with alumina Cap-O-Test lids. The cultures were incubated in a growth room held at a light intensity of 0.5 μmol·s\textsuperscript{-1}·m\textsuperscript{-2} and 25 ± 2°C.

The pretreated seeds germinated more readily than the nontreated seeds (Table 1). This difference is attributed to the stimulatory effects of the combined treatments of the 4-hr water soak and the NaOCl, which may facilitate GA entry or breaking of dormancy, and the promotive effects of the GA\textsubscript{4} mixture (Hurly et al., 1989).

Callus and shoots were formed mainly on the cotyledons of the germinated seeds (Fig. 1 a and b), as reported by Wickham et al. (1980). About 10\% of the explants with the pericarps removed became vitrified, probably due to mechanical damage during culture. Cultures transferred from a light intensity of 0.5 μmol·s\textsuperscript{-1}·m\textsuperscript{-2} to a growth room held at 25 μmol·s\textsuperscript{-1}·m\textsuperscript{-2} resulted in the greening of the shoots; however, the callus turned brown and senesced due to the presumed production of a “self-destructive toxin” (Wickham et al., 1980; Radin et al., 1982; Staba and Nygaard, 1983). Shoots were removed and placed on a rooting medium that contained 0.1 mg NAA/liter and were kept in the light (Subramanian and Rao, 1980). Callus retained at the low light intensity remained creamy-beige, continuously producing multiple shoots. Shoot production was

Fig. 1. In vitro growth of guayule seeds: (a) Callus, root, and shoot formation on cotyledons at 1 mg NAA/liter; (b) callus formation on cotyledons and shoot multiplication; (c) shoot differentiation from callus at 1 mg BA/liter.

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achieved on either 0.1 or 1 mg BA/liter (Fig. 1c), while rooting only occurred on NAA. This result is in contrast to the low BA requirement for rooting found by Staba and Nygaard (1983).

The simple two-step seed culture technique used in this experiment eliminates the necessity of subculturing seedlings and other explants, and reduces contamination to 0% to 5%. Callus browning, a major problem with guayule (Wickham et al., 1980; Radin et al., 1982; Staba and Nygaard, 1983), is overcome by maintaining the cultures under a low light intensity. The use of seeds as explants, especially from cultivars with a high rate of apomixis, allows the rapid production of many plants similar to the parents.

**Table 1. In vitro germination of treated and nontreated guayule seeds.**

<table>
<thead>
<tr>
<th>Seed development stage</th>
<th>Pretreated with pericarp</th>
<th>Without pericarp</th>
<th>Nontreated with pericarp</th>
<th>Without pericarp</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>40 ± 5</td>
<td>50 ± 7</td>
<td>5 ± 2</td>
<td>15 ± 5</td>
</tr>
<tr>
<td>3</td>
<td>90 ± 10</td>
<td>95 ± 10</td>
<td>20 ± 9</td>
<td>20 ± 3</td>
</tr>
</tbody>
</table>

*1 = Immediately after anthesis; 2 = before pericarp hardening; 3 = fully mature seeds.

**Literature Cited**


**Inheritance Patterns for Juice Vesicle Branching in the Citrinae (Aurantioidae)**

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**Abstract.** Some *Citrus* spp. and cultivars exhibit juice vesicle branching. In this study, we determined that the branching trait is inheritable. The mode of inheritance of this trait was analyzed in progenies from various 2x x 2x and 2x x 4x citrus crosses. No consistent model for inheritance of branching has been found, although some crosses do suggest simple inheritance. We found that if one parent is a pummelo, even if this parent does not exhibit the branching trait itself, branching may be inherited by a substantial portion of the progeny, suggesting that more than one locus is involved in this trait.

Citrus vesicles have been described in the literature as solitary stalked structures (Bain, 1958; Swingle and Reece, 1967). Recently, Tisserat et al. (1988) found some citrus species that exhibit vesicle branching. The branching trait was especially prominent in the pummelo (*Citrus maxima* (J. Burm.) Merrill) and grapefruit (*C. paradisi* Macf.) cultivars, while it was only occasionally expressed in some *C. reticulata* Blanco (mandarin) cultivars (Tisserat et al., 1988). Sometimes (e.g., pummelo), these branches are numerous and become so enlarged they almost appear as distinct vesicles. In contrast, vesicle branching was absent in other Citrinae (subtribe of Citreae in the subfamily Aurantioidae) genera, including: *Fortunella*, *Eremocitrus*, *Microcitrus*, *Poncirus*, and *Severinia*. This study was conducted to determine if the vesicle branching trait was genetic in origin and, if so, how it is expressed in progeny from crossing parents with and without the trait. This paper surveys various parents and hybrid progenies with respect to the branching trait.

Mature fruit was examined for vesicle morphology in Oct. 1987 from parents and progeny grown in the Citrus Variety Collection and in field plots on the Univ. of California, Riverside, campus. Six diploid (2x = 18) zygotic pistillate cultivars that produce at least some zygotic progeny ['Fortune' mandarin (*C. reticulata*), 'King' mandarin hybrid? (*C. reticulata*?), 'Siamese Acidless' pummelo (*C. maxima*), 'Sukenga' (*C. paradisi* x *C. sinensis* (L.) Osbeck), trifoliate orange (*Poncirus trifoliate* (L.) Raf.); 'Wilking' mandarin (*C. reticulata*) were crossed with eight diploid pollen parents ['Prun' mandarin (*C. reticulata*), Ichang papeda (*C. ianchengensis* Swingle), 'Kao Panne' pummelo (*C. maxima*), 'Lima' orange (*C. sinensis*), 'Paper Rind' orange (*C. sinensis*), 'Parson's Special' mandarin (*C. reticulata*), 'Siamese Acidless' pummelo (*C. maxima*), and 'Wilking' mandarin (*C. reticulata*) and four tetraploid (4x = 36) pollen parents ['Hall' grapefruit (*C. paradisi* Macf.), 'Paper Rind' orange (*C. sinensis*), 'Ruby' orange (*C. sinensis*), and 'Seedy Marsh' grapefruit (*C. paradisi*)]. Table 1 presents the crosses made with these parents.

To determine if the branching trait was uniform in a single clone, samples consisting of two fruits from each of 10 randomly selected trees in a commercial 'Pink Marsh' grapefruit (*C. paradisi*) orchard were collected and analyzed in Dec. 1987. Thirty vesicles from five segments for each fruit