EMBRYO CULTURE OF LUISIA ABRAHAMI VATSALA; ORCHIDACEAE

A.N. PYATI AND H.N. MURTHY
Orchid Laboratory, Department of Botany, Karnatak University, Dharwad-580003, India.
(Accepted April, 1997)

The germination response of Luisia abrahami embryos (seeds) on Vacin and Went (VW), Knudson 'C' (KC), Burgeff N$_3$f (N$_3$), and Murashige and Skoog (MS) media was tested. KC medium proved the best for obtaining better crop of protocorms; it favoured rapid proliferation of the embryos and accelerated differentiation of shoot and roots. Addition of growth adjuncts such as coconut water, sugar cane juice and yeast extract, promoted and banana extract, kinetin, naphthalene acetic acid, gibberellic acid inhibited embryo germination.

Key Words: Embryo culture, Luisia abrahami orchid.

The orchid seeds possess undifferentiated embryos and require an appropriate fungal association for germination in vivo. Their ability to germinate asymbiotically in vitro, demonstrated for the first time by Knudson (1922), has been successfully employed to propagate several species of commercial value (Arditti and Earnst, 1984; Waes and Debergh, 1986; Linden, 1992; Arditti and Ernst, 1992). This communication briefly reports the in vitro asymbiotic germination response of embryos of Luisia abrahami, which is a fast receding species and call for urgent conservation and multiplication measures.

MATERIALS AND METHODS

Capsules of Luisia abrahami were washed with detergent and surface sterilized with 5% sodium hypochlorite solution. These were then split opened under aseptic conditions, and the embryo scooped out and cultured. Four culture media, namely Vacin and Went (VW) (1949), Knudson C (KC) (1946), Burgeff (N$_3$) (1936), and Murashige and Skoog (MS) (1962), were used as source of nutrition. The cultures were incubated at 22±2°C under 12 h photoperiod (2000 lx). In another set of experiments the individual or collective effect of naphthalene acetic acid (NAA), kinetin (KN), gibberellic acid (GA$_3$), coconut water (CW), sugar cane juice (CJ), banana extract (BE), and yeast extract (YE) were also tested in KC medium.

OBSERVATIONS AND CONCLUSIONS

The embryos of Luisia abrahami responded to all the four basal media, indicating their wider nutritional amplitude. Their germination frequency and subsequent morphogenetic changes, leading to seedling development, however, varied with nutritional pool (Table 1). On MS and N$_3$f media, the germination frequency was significantly impaired (28-39%) whereas moderate germination observed on VW medium (52%) but protocorms failed to develop further. On KC medium, on the other hand 66% embryos developed into darkgreen protocorms within 6-8 wks from culture (Fig. 1). The first leaf appeared in 10-12 wks from culture, the roots appeared 4 wks later, and the seedlings were well formed in about 20 wks from cultures (Fig. 2). The frequency with which the embryos were activated to develop into protocorms and subsequently into seedlings there from were selectively affected when growth adjuncts were used singly or in combination in KC medium (Table - 2). The germination frequency and development of leaves and roots from the protocorm were enhanced when CW (10%), CJ (10%), YE (1000 mg l$^{-1}$) were used in the medium. Addition of banana extract retarded germination. Combination of growth adjuncts was not beneficial in enhancing germination frequency and development of protocorm . The 35 to 40 wks old seedlings were successfully transferred to pots.

Embryos of Luisia abrahami germinated better (66%) in KC medium due probably to their specific nutrient requirements. An increased germination frequency (70-80%) in medium supplemented with CW or CJ or YE would suggest that the growth adjuncts probably invoke germination by satisfying nutritional complexities, since orchid embryos are generally at different stages of development. Similarly promoting effect of complex additives on germination frequency is already on record in orchid culture (Nimoto and Sagawa, 1961). Banana extract drastically reduced
Table 1. Effects of basal media on germination of embryos and plantlet formation in Luisia abrahami.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Percentage response</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>KC</td>
<td>66</td>
<td>Germination frequency, chlorophyll development and rhizogenesis enhanced, seedlings growth improved.</td>
</tr>
<tr>
<td>N_f</td>
<td>39</td>
<td>Chlorophyll development impaired, rhizogenesis delayed, seedlings growth poor.</td>
</tr>
<tr>
<td>VW</td>
<td>52</td>
<td>Germination frequency and differentiation enhanced, chlorophyll development impaired.</td>
</tr>
<tr>
<td>MS</td>
<td>28</td>
<td>Differentiation eluded.</td>
</tr>
</tbody>
</table>

Figures 1, 2. Fig. 1 Profusion of Protocorms at various stages of development. Fig. 2 Flask culture showing abundant growth of the seedlings.

germination frequency in Luisia abrahami, similar observations were made in Paphiopedilum species (Fast, 1971; Pierik et al., 1988). The effect of vitamins during orchid seed germination, varies from promotion (Muralidhar and Mehta, 1986) to inhibitory (Bahme, 1949), in our studies vitamins have enhanced the germination frequency and development of protocorms. Our observations on the deleterious effect of an increased level of NAA, KN, GA, in Luisia abrahami cultures corroborates earlier findings (Vij et al., 1981; Nath et al., 1991).

Financial support from University Grants Commission, New Delhi, India to the project “Orchids of Karnataka: Collection, Conservation and Improvement” is gratefully acknowledged.

REFERENCES

Embryo culture of *Luisia abrahami* vatsala; orchidaceae


Burgeff H 1936 *Somenkamung der orchideen* Fisher verlag, Jena.


Knudson L 1922 Non symbiotic germination of orchid seeds. *Bot Gaz* 73 1-25.


Muralidhar C E & A R Mehta 1986 Tissue culture studies on *Cymbidium longifolium* Don - *in vitro* seed germination and sequential stage of histomorphological changes from embryo to PLBs. In *Biology conservation and Culture of orchids* (Vij S P) Affiliated East West Press, New Delhi p 413-422.


