CHAPTER III: HARDENING AND ACCLIMATIZATION OF IN VITRO RAISED PLANTLETS OF MANTISIA SPATHULATA AND MANTISIA WENGERI

INTRODUCTION

Plantlets often die during the transfer from in vitro to ex vitro conditions (Pospisilova et al., 1999). The overall success of in vitro raised plantlets depends on successful hardening and transplantation in the field. Under controlled culture conditions the anatomical and morphological conditions of in vitro plantlets such as development of cuticle, hairs, opened stomata photosynthetic ability and conducting tissues etc. required for the growth and development of plantlets remains non functional. The in vitro plantlets are very delicate and therefore wilt rapidly on direct transfer to normal green house or field condition. Rapid loss of water through transpiration (Grout and Aston, 1977) may lead to high mortality rate unless plantlets are transferred gradually from initial high humidity to reduced humidity and increased light intensity (George and Sherrington, 1984). However, acclimatization of in vitro raised plantlets prior to transfer helps the plants to adapt to the environmental changes (Brainerd and Fuchiagam, 1981; Roy, 1994; Baruah, 1996). Bhojwani and Razdan (1983) have stressed on high humidity conditions during the initial days for successful transplantation. Therefore, for successful transplantation the first and foremost requirement is the maintenance of plantlets under very high humidity conditions (90 -
100%) for the initial 10 - 15 days followed by gradual reduction of humidity (70 - 60%) and temperature (28 - 38°C) in the glasshouse (Vij et al., 1995). Temperature is also very crucial for higher survival rate and growth of transplanted plants. During summer, plants are exposed to high irradiance and temperature (30 - 40°C) and low humidity. Thus, careful step wise procedure is needed when *in vitro* plants are transferred to pots or field conditions.

For hardening of *in vitro* plantlets, various compositions of substrates such as mixtures of sand, charcoal, brick pieces, dry cow dung etc. in different ratios have been tried out for good drainage and sufficient aeration of roots in wide range of plants including many orchids (Kumaria and Tandon, 1994; Kumaria et al. 2005). However, the available literature shows that most of the species of zingibers are easily hardened in soil and sand mixtures (Nadgauda *et al*., 1978; Borthakur *et al*., 1999; Prathanturarug *et al*., 2004) and some times farmyard manure proves to be beneficial for higher survivability and better growth of the transferred plantlets (Shirin *et al*., 2000). Proper drainage prevents plantlets from the fungal infection and, aeration provides formation of cuticle and waxes over the roots thereby increasing the capacity of roots for nutrient uptake from the potting substrates. Different types of pots have been used for acclimatization of plantlets, but the glazed pots are not suitable, as they do not allow sufficient aeration of the roots and the compost. Mukherjee (1983) suggested the use of clay pots for many epiphytic orchids like *Cattleya, Epidendrum, Dendrobium* etc. To facilitate drainage and aeration, the plastic pots are poked for small holes. High humidity is generally maintained by covering the transferred plantlets with perforated polybags which are removed after few days resulting in
decrease in humidity leading to the gradual acclimatization of plantlets within 3 - 4 weeks of transfer (Bisht et al., 1988; Palini et al., 1994; Vyas et al., 1999).

Preconditioning of *in vitro* cultured plantlets has been useful for successful acclimatization of plantlets in the field. Hazarika *et al.* (2000, 2001) reported that *in vitro* preconditioning of citrus microshoots with sucrose concentrations of 3% was optimum for subsequent *ex vitro* survival and growth. Similarly, Nagaraju and Mani (2005) reported an *in vitro* prehardening of *Zygopetelum intermedium* in medium containing paclobutrazol and activated charcoal for its high rate *ex vitro* survival and growth of plantlets.

**MATERIALS AND METHODS**

Healthy *in vitro*-raised complete plantlets of both *M. spathulata* and *M. wengeri* were taken for hardening and establishment. Eight weeks-old plantlets measuring 2.5 - 3cm in height were taken out from the culture tubes/flasks by means of long handled spoon along with a small amount of the adhering agar. The agar medium sticking to the roots was removed slowly with a soft brush. The plantlets were washed with sterile water taking due care to avoid damage to the roots. These were then transferred to clean 8cm long thermocol/plastic pots containing different mixtures of composts viz., (i) soil, sand and compost (dry cow dung) (1: 0.5: 0.5), (ii) charcoal, brick pieces and sand (1:1:1) and (iii) soil and compost (1:1).

Thermocol/plastic pots were thoroughly washed with distilled water and dried to minimize the spread of disease or infections. Small holes were made in the pots to provide aeration and drainage of the water. The pots were filled 3/4th with compost and watered as planting in the moistened compost is easier. The washed plantlets were
picked up with the help of forceps and the roots were carefully placed into the crevices of the compost. Single plantlet was potted in each pot. The pots along with the plantlets were covered with perforated polythene bags and were carefully sprayed with water and shifted to the glass house for hardening of the plantlets. The minimum and maximum temperatures of the glass house at the time of transplantation were 18°C and 32°C respectively. The relative humidity of the glass house was around 70 - 80%. The plantlets were watered in the evening on alternate days and fed with MS nutrient salt solutions (diluted 10 times) fortnightly for about a month. Readings were recorded after 60 days of hardening and subsequently the plantlets were made ready for field transfer.

RESULTS

Of the various compost used, the combination of soil and compost in 1: 1 ratio was found to be the best substratum for the survival and healthy growth of both *M. spathulata* and *M. wengeri*. Around 90.7% of *M. spathulata* (Table 3.1; Fig. 7 A) and 84% *M. wengeri* (Table 3.2; Fig. 7 C) survived within 4 weeks of transfer to pots containing soil and compost in equal ratio. However, the survival percentage of the plantlets in all the different compost tried was lower. On the other hand, the height of the plantlets transferred to pots differed significantly in the various composts. The plantlets of *M. spathulata* and *M. wengeri* attained a maximum height of around 8.9cm and 4.4cm respectively in the substratum containing soil and compost. A significantly increased height of 8cm was attained for the plantlets of *M. wengeri* in soil, sand and compost mixtures. Complete healthy green plantlets of both *M. wengeri*.
Table 3.1: Hardening and acclimatization of *M. spathulata* plantlets

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival* (%)</th>
<th>Height (cm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil, sand and compost (dry cow dung) (1:0.5:0.5)</td>
<td>84.1±4.6</td>
<td>6.7±0.4</td>
</tr>
<tr>
<td>Charcoal, brick pieces and sand (1:1:1)</td>
<td>76.0±6.1</td>
<td>7.6±0.6</td>
</tr>
<tr>
<td>Soil and compost (1:1)</td>
<td>90.7±2.7</td>
<td>8.9±0.8</td>
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<tr>
<td><strong>NS</strong></td>
<td><strong>NS</strong></td>
<td><strong>NS</strong></td>
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‘*’ indicates mean average values of three repeated experiments with standard error (±SE)

NS: Non significant

ANOVA at 5% level of significance shows that all the parameters of growth are non-significant

Data recorded after 60 days
Table 3.2: Hardening and acclimatization of *M. wengeri* plantlets

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival * (%)</th>
<th>Height* (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil, sand and compost (dry cow dung) (1:0.5:0.5)</td>
<td>80±2.3</td>
<td>8.0±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Charcoal, brick pieces and sand (1:1:1)</td>
<td>74.7±8.1</td>
<td>5.3±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soil and compost (1:1)</td>
<td>84±8.3</td>
<td>4.4±0.5&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>

<sup>*</sup> indicates mean average values of three repeated experiments with standard error (±SE)
NS: Non significant
ANOVA at 5% level of significance shows that all the parameters of growth are highly significant. Means followed by the same letters (a, b, c) are not significantly different according to Turkey's test (p = 0.05)
Data recorded after 60 days
spathulata and M. wengeri got established well in the substratum within 60 days of transfer (Fig. 7 B, D).

DISCUSSION

Successful hardening and acclimatization depends on suitable size of the plantlets and their state of growth in vitro. The type of plant materials viz. herbaceous, aquatic or woody, epiphytic etc. also determines the successful transfer of plant materials. Most of the orchids often require suitable substratum for higher survivability and non availability of suitable growth conditions may drastically decrease the survival percentage of transferred plantlets (Yadav et al., 1988; Cribb, 1990; Robbins and Bell, 1990). However, most of the zingibers get efficiently hardened in normal soil and sand mixtures after transferring to the normal environmental conditions (Borthakur et al., 1999; Koul et al., 2005). Hardening in soil and sand at equal ratios resulted in 85% survivability of Hedychium spicatum (Koul et al., 2005). Similarly, more than 80% of the transferred plantlets of Alpinia galanga survived in the potted 1:1 ratio of soil and sand mixture (Borthakur et al., 1999). Also, a very high survivability and better growth of transferred plantlets of Zingiberaceae plants like Curcuma zedoaria and Zingiber zerumbet has been reported (Stanly and Keng, 2007). Besides soil and sand, addition of farm-yard manure to the substratum was found to enhance the acclimatization of Zingiber officinale (Sharma and Singh, 1997) and Kaempferia galanga (Shirin et al., 2000). In the present study, the mixture of soil and sand in equal ratios resulted in very high percentage of survivability and healthier growth of plantlets of both M. spathulata and M. wengeri. Profuse rooting from the rhizomes allowed better adherence and efficient nutrient
uptake from the soil leading to healthier growth of the plantlets. Survival of transferred plantlets depends on their ability to carry out photosynthesis and withstand water loss. *In vitro* plantlets have the characteristics of less or no photosynthetic pigment, malfunctioning of stomata and marked decreased in epicuticular waxes that leads to the death of transplanted plants (Bhojwani and Dhawan, 1989). Many studies have shown that inclusion of triazoles e.g. paclobutrazol in the rooting media is promising in the protection against different stresses such as chilling, heat shock, water-logging and drought stress (Kraus and Fletcher, 1994; Gilley and Fletcher, 1997; Panaia *et al.*, 2000). Supply of diluted nutrient medium during hardening has been found to be beneficial for healthy growth of many orchids (Kumaria and Tandon, 1994). As the plants are perennial herbs the appropriate natural temperature and humidity during monsoon also favoured the higher survivability of the plantlets during post hardening period.