Effect of concentration and duration of colchicine treatment on polyploidy induction in *Dendrobium scabrinerve* Lindl. *in vitro*

Presented by Suputtra Sarathum
**Dendrobium scabrilingue** Lindl.

**Plant:**
This is one of the smaller species, the pseudo bulbs are between 10-14 cm.

**Flower:**
The petals are white, the labellum is yellow, 2.5-2.8 cm. in size and fragrant.

**Flowering Period:**
Dec-Feb

**Habitat:**
Grows epiphytically on trees, from 1,000-1,200 m.
The reason of an extinction of wild orchids
Her majesty Queen Sirikit

Re-introduction of Native Thai orchid to the forest, (RENTOP)

Production of *D. scabrilingue* Lindl. for Natural Fragrance Industry.
Induction of polyploidy & Hybridization

Diploidy needs a very specific type of habitat.  

\[2n \quad 3n \quad 4n\]

Triploid plant generally have bigger and longer blooms, blooming time is increased and they are easier to cultivate as has been reported for *Dendrobium* (Kamemoto et al., 1972), *Phalaenopsis* (Griesbach, 1981) and *Paphiopedilum* (Watrous and Wimber, 1988).

Introduction 5
The objective

To determine an effective concentration and duration of colchicine treatment to induce the multiplication of the chromosome number in PLBs of *Dendrobium scabrilingue* Lindl.
Material and method

**Colchicine treatments**
Concentration: 0.0, 0.025, 0.05, 0.075 and 0.10 %
Duration: 3, 7, 14 and 21 days

- Lacking colchicine medium
- One month later
- Chromosome No. and DNA content
- Eight months later
The functional principle of a flow cytometer

1) Flow chamber
2) Laser-source
3) Light detector
4) The calculation unit
Table 1  Effect of \textit{in vitro} colchicine treatment on the percentage of surviving plbs of \textit{D. scabrilingue} Lindl. after being cultured for one month.

<table>
<thead>
<tr>
<th>Colchicine Conc. (%)</th>
<th>Survival (%)(^\dagger)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment duration (days)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>0.000</td>
<td>89.2 a</td>
<td>86.8 ab</td>
</tr>
<tr>
<td>0.025</td>
<td>80.9 bcd</td>
<td>80.5 cd</td>
</tr>
<tr>
<td>0.050</td>
<td>62.7 g</td>
<td>56.7 h</td>
</tr>
<tr>
<td>0.075</td>
<td>42.5 i</td>
<td>39.5 ij</td>
</tr>
<tr>
<td>0.100</td>
<td>36.3 ij</td>
<td>38.1 ji</td>
</tr>
<tr>
<td>Mean</td>
<td>62.3</td>
<td>60.3</td>
</tr>
</tbody>
</table>

\(\dagger\) Results followed by a different letter differ significantly at the 0.05 level using Duncan’s Multiple Range Test.
Table 2  Effect of *in vitro* colchicine treatment on the percentage of polyploid plantlets of *D. scabrilingue* Lindl. after being cultured for eight months.

<table>
<thead>
<tr>
<th>Colchicine Conc. (%)</th>
<th>Polyploidy induction (%)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment duration (days)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>0.000</td>
<td>0.00 k</td>
<td>0.00 k</td>
</tr>
<tr>
<td>0.025</td>
<td>9.20 ij</td>
<td>10.00 i</td>
</tr>
<tr>
<td>0.050</td>
<td>6.55 ij</td>
<td>21.51 h</td>
</tr>
<tr>
<td>0.075</td>
<td>6.08 j</td>
<td>39.13 e</td>
</tr>
<tr>
<td>0.100</td>
<td>5.88 j</td>
<td>52.25 c</td>
</tr>
<tr>
<td>Mean</td>
<td>5.54 B</td>
<td>24.58 AB</td>
</tr>
</tbody>
</table>

Result 2
The percentage of tetraploid plantlets of *D. scabrilingue* Lindl. after being cultured for eight months.
Flow cytometric histograms show the amount of DNA in each cell (DNA content) of diploid plant, 2n (a) and tetraploid plant, 4n (b) of *D. scabrilingue* Lindl.

Morphology of diploid plant, 2n (a); polyploid plant, 4n (b); 6n (c) and 8n (d) of *D. scabrilingue* Lindl. after being cultured for eight months.
Result 6
CONCLUSIONS

The most effective treatment was 0.075 % colchicine for 14 days which resulted in a high percentage of surviving plbs and the highest percentage of tetraploid plantlets.

From the results obtained so far, it is expected that the ongoing selection process will provide an improved and easier to cultivate plant material of *D. scabrilingue* Lindl.
The further work

Cloned plants of *D. scabrilingue* Lindl. were initially cultivated in the greenhouse for two years.
Acknowledgments

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Yen orchids farm
Thank you for your attention