**Ornithogalum**: from Diploid to Tetraploid

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**Abstract**

*Ornithogalum* L. is a popular cut flower genus of the Hyacinthaceae family. Only a few southern African species are used in breeding programs. Polyploidization can solve some incompatibility problems existing in breeding with *O. thyrsoides* and *O. dubium*: the two species commonly used. Polyploids could also contribute more variation. Since *Ornithogalum* (2n=12) is a bulbous plant, different in vitro colchicine treatments were evaluated when meristematic tissue is more exposed. Explants were exposed to different colchicine concentrations (0.1, 0.2 and 0.3%) at various exposure times (24, 48 and 72h). In vitro colchicine treatments proved to be very successful: 54-89% tetraploids. The treatment that applied 0.2% colchicine for 48h was the most effective. Various *Ornithogalum* hybrids that were treated resulted in 17-100% tetraploids in the regenerated plants. Therefore in vitro colchicine treatment of *Ornithogalum* can be seen as a very effective method to produce tetraploids.

**INTRODUCTION**

*Ornithogalum* L. is a popular cut flower genus of the Hyacinthaceae family that hosts about 200 species found in Europe, Africa and western Asia (Obermeyer, 1978). Species found in the south-western Cape are used extensively in breeding programs in South Africa, USA, China and Israel (Littlejohn, personal communication). *O. thyrsoides* Jacq. has been utilized for many years as a long lasting cut flower with a long straight stem ending in a raceme of white flowers. *O. dubium* Houtt. is the species with the colored specimens: yellow to orange, buff or white. The one aim of the Agricultural Research Council’s Ornithogalum breeding program is the production of a bright yellow or orange cut flower with a long straight stem. The colored progeny resulting from crosses within *O. dubium* or between *O. thyrsoides* and *O. dubium* is often pale in color or lack height. *O. thyrsoides* and *O. dubium* have a small number of chromosomes (2n=12) (Pienaar, 1963). Polyploidization can maybe help in overcoming some breeding incompatibility between species and solve the sterility in F1 hybrids (Pienaar, 1963 and Roos and Pienaar, 1966). Colchicine could result directly in more variation.

The reason for using polyploidy in lilium breeding are the larger flowers, the stronger stems and in interspecific hybridization the restoration of F1-sterility at the tetraploid level (Van Tuyl, et al., 1992). Polyploidization significantly deepened the yellow colored cyclamens (Takamura and Miyajima, 1996). An increase in size of organs and plant height was also reported in tetraploid *Anemone* (Jacob et al., 1997).

Colchicine is a natural alkaloid with an antimitotic activity, obtained from the wild *Colchicum autumnale*. Since Blakeslee and Avery (1937) discovered the chromosome doubling effect of colchicine it is widely applied in the induction of polyploids (Tambong, et al., 1998) for the induction of variance.

Traditional colchicine treatments of seedlings often result in the formation of chimeric plants (Chen et al., 1994). In vitro exposure of *Brassica napus* microspores for 24h to 1000µM colchicine resulted in the formation of 94% embryos with doubled...
chromosome number (Hansen and Andersen, 1996). Van Tuyl et al (1992) also used in vitro colchicine procedures to obtain tetraploid Lilium. Le Nard (personal communication) also reported that an in vitro ploidy conversion is the best method when working with bulbous plants. This higher efficiency of in vitro colchicine treatment has extensively been reported in a variety of plants (Griesbach, 1981, Espino and Vazquez, 1981 and Takamura and Miyajima, 1996)

The aim of this study was to determine the most efficient colchicine concentration and the duration of exposure that will produce the highest number of tetraploids in Ornithogalum.

MATERIAL AND METHODS

In Vitro Production of Material

A pale yellow Ornithogalum cross between O. dubium (O. leipoltii L. according to Leighton (1944)) and O. dubium was selected for the experiment to determine the most suitable procedure. An additional wide range of hybrids was also treated. Explants (10mm x 10mm) were cut from the leaves and were placed on a MS (Murashige and Skoog 1962) medium containing 2mg.l⁻¹ benzyladenine purine (BAP), 0.5mg.l⁻¹ naphtaleneacetic acid (NAA) and 30g.liter⁻¹ sucrose (Hussey 1976 and Nel 1981). The pH of the medium was adjust to 5.8 and solidified with 7g.liter⁻¹ agar. The medium was dispensed into glass vials and sterilized at 121°C for 15 minutes. The leaves used were surface sterilized by soaking them in 70% ethanol for two minutes, followed by 1.5% NaOCl for 15 minutes and rinsing in sterile water. The explants were incubated in a growth room at a temperature of 23-25°C and a 16/8h light/dark photoperiod.

In Vitro Colchicine Treatment

The material was treated with colchicine when buds were clearly visible, after about four weeks. Three colchicine concentrations (0.1, 0.2 and 0.3%) were tested at different times of exposure (24, 48 and 72h). The additional hybrids were only treated with 0.2% colchicine for 48h. The dissolved colchicine was added to the vials using 0.22 µm filter sterilization. Enough colchicine was added to cover all new growth. The material was incubated in the growth room for the duration of the treatment. After exposure the material was transferred to an MS medium containing 30g.liter⁻¹ sucrose at pH 5.8 and 7g.liter⁻¹ agar. The material was given a cold treatment at 6°C in the dark for 24h before further incubation until bulbs were transplantable to soil medium.

Determining the Chromosome Number

Transplanted bulbs were allowed to form real roots. The root tips were then cut and prepared for chromosome number determination using a refined root tip squash technique compiled by Pienaar (personal communication) for wheat. Quick growing roots were cut and placed in cold distilled water at 4°C for 24h. The roots were then fixated in a freshly prepared fixative consisting of four parts methanol and one part propionic acid for at least three hours, but preferably 42 hours. The fixative was replaced with distilled water and left for 30 minutes. The roots were hydrolyzed in 1N hydrochloric (HCl) at 60°C for 6.5 minutes. Transferring the roots to distilled water for two minutes stopped hydrolyzation. The water was replaced with Feulgen stain (Darlington and La Cour 1960) and stained for 30 minutes to one hour. The roots were then rinsed twice in distilled water for two to five minutes in each rinse. The distilled water was replaced with a pH4.5 sodium acetate buffer (3.16g sodium acetate and 3.47ml glacial acetic acid in one liter distilled water; adjust pH to 4.5) for two to five minutes. The buffer solution was drained off and one milliliter freshly prepared pectinase solution (0.4 g pectinase and 0.2g peptone in 20ml pH4.5 sodium acetate buffer) was added. Roots were incubated at 37°C for 15 minutes. These prepared roots were placed on a clean microscope slide and the stained root tip was cut off in a small drop of 1% aceto carmine (Rosner formulation: 1g carmine in 55ml distilled water to which 45ml glacial acetic acid was added after five
minutes. Boiled in a flask fitted to a reflux condenser for four hours and filtered.). The cells of the root-tip were very gently tapped out with the blunt end of a hard wood (ebony) needle holder. The drop containing the cells was covered with a cover glass and placed in the fold of a folded filter paper. It was pressed very gently to remove the excess aceto carmine. The slide was gently heated over a spirit flame and returned to the folded filter paper. It was firmly pressed with the thumb to spread the cells apart and getting the chromosomes in the same focal plane. The chromosomes were then counted using a light microscope.

RESULTS AND DISCUSSIONS

In general the material with dominating O. thyrsoides characteristics reacted more vigorously in vitro. Ornithogalum explants induce new plants through various pathways: adventitious buds, shoots and bulbils can be induced either indirectly following the initiation and proliferation of callus or directly without an intermediate callus phase. Nodules of meristematic tissue are the origin of the differentiated structures in both pathways (De Villiers and Borman, 1999). Thus with using the in vitro approach the colchicine has easy access to the meristematic area: just a few cell layers.

The results must only be seen as preliminary, but 54% to 100% of the regenerated plants were tetraploid. The most effective treatment seems to be the 48h exposure to 0.2% colchicine (89% tetraploid); although the 24h exposure to 0.1% colchicine resulted in 85% tetraploids. The 72h exposure treatments were the least effective: 54% to 62% tetraploids. Plants were found with diploid and tetraploid cells in the root tips. Only one octaploid (2n=48) was found with the 0.2% colchicine for 48h treatment.

The other hybrids that were treated produced 17% to 100% tetraploids in the regenerated plants (Table 1). Unfortunately, the treated plants are not in flower yet. During the 2000/2001 flowering season it would be possible to start evaluating the morphological effects of the colchicine treatments.

Literature Cited


Table 1. Results following the treatment of *Ornithogalum* explants *in vitro* with 0.2% colchicine for 48h.

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Parent species (dominant flower color)</th>
<th>Percentage of plants tetraploid</th>
</tr>
</thead>
<tbody>
<tr>
<td>92WR161</td>
<td>O. dubium (yellow) x O. dubium (white)</td>
<td>99</td>
</tr>
<tr>
<td>E.389</td>
<td>O. dubium (1-17A)</td>
<td>100</td>
</tr>
<tr>
<td>92RR22</td>
<td>O. thyrsoides (white) x O. thyrsoides (white)</td>
<td>94</td>
</tr>
<tr>
<td>E.214</td>
<td>O. dubium (1-16A)</td>
<td>100</td>
</tr>
<tr>
<td>E.377</td>
<td>O. dubium (1-14A)</td>
<td>53</td>
</tr>
<tr>
<td>E.57-3</td>
<td>O. dubium (1-23A)</td>
<td>17</td>
</tr>
</tbody>
</table>