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Short communication

In vitro induction of tetraploids in *Colophospermum mopane* by colchicine

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Abstract

Colophospermum mopane is an ecologically and economically important tree in subtropical southern Africa with ethnobotanical and medicinal uses. The effect of colchicine on the polyploidisation of seeds of *C. mopane* was studied *in vitro*. Seeds of *C. mopane* were imbibed in colchicine solutions of 0.05, 0.1 and 1.0% (w/v) for 24, 48 and 96 h before transfer to 1/8 MS basal medium. Ploidy levels of the surviving *in vitro* grown seedlings were determined using flow cytometry. Of a total of 45 surviving seedlings, 44% were found to be tetraploids, one was a chimera while the remainder were diploids. Tetraploid induction occurred in seedlings derived from seeds imbibed for 48 h at 0.05% and 0.1% (w/v) colchicine solutions. In comparison to the control plants, tetraploids showed morphological differences such as an earlier induction of enhanced growth of lateral branches and smaller leaflets and multiple shoots per single cultured seed. Treatments with colchicine concentrations greater than 0.1% and exposure times of more than 48 h were severely detrimental to growth and survival.

Keywords: Colchicine; Flow cytometry; *In vitro*; Polyploidy; Tetraploid

Colophospermum mopane Kirk ex J. Leonard is one of the most ecologically and economically important leguminous trees in subtropical southern Africa. Leaves of *C mopane* are the main source of food for mopane worms, *Imbrasia belina* as well as providing browsing for herbivores (Timberlake, 1995). Medicinally, different parts of *C. mopane* are used for the treatment of a variety of ailments and other conditions throughout southern Africa (Watt and Breyer-Brandwijk, 1962).

Polyploids have played an important role in higher plant evolution, as most flowering plants are tetraploids (Leitch and Bennett, 1997). Polyploidy has been used in horticulture as a breeding tool to enhance ornamental characteristics such as plant size, leaf thickness, increased width-to-length ratio of leaves and flower size (Shao et al., 2003). The use of colchicine as a chromosome doubling agent *in vitro* has been reported, amongst others, for *Citrus* spp. (Wu and Mooney, 2002), *Acacia dealbata* and *Acacia mangium* (Blakesley et al., 2002), *Alocasia* spp. (Thao et al., 2003), *Punica granatum* (Shao et al., 2003).

This study was aimed at testing the effectiveness of colchicine as an antimitotic agent for the induction of *C. mopane* polyploids with improved leaf characteristics since they serve as food source for mopane worms, herbivores and medicinal uses.

Fruits of *C. mopane* were collected from Musina experimental farm in the Limpopo province of South Africa. Seeds were manually removed from fruit and were surface sterilised in 50% (v/v) commercial bleach JIK® (3.5% (w/v) active chlorine) containing a few drops of Tween 20, for 10 min before being rinsed 4 times with sterile distilled water. Sterile seeds, 20 per treatment, were imbibed in sterile solutions of colchicine at concentrations of 0.05, 0.1 and 1.0% (w/v) for 24, 48 and 96 h on a shaker at room temperature. Seeds imbibed in sterile distilled water served as a control. Imbibed seeds from the different treatments were cultured on hormone free 1/8 MS

Abbreviations: MS, Murashige and Skoog medium (1962); Tween 20, polyoxyethylenesorbitan monolaurate; IBA, Indole-3-butyric acid.

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basal medium (Murashige and Skoog, 1962) supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar. Culture media, (pH 5.8) colchicine solutions and distilled water were sterilised by autoclaving at 121 °C for 20 min. Cultures were maintained for 3 months at 26±2 °C in a 16 h photoperiod. Leaf samples from three month old seedlings were analysed for polyploidy by flow cytometry using PA Ploidy Analyzer, Partec. Pieces from control plants and experimental samples subjected to the different colchicine treatments were cut with a sharp razor blade in a 55 mm plastic Petri dish containing 400 μL of nuclei extraction buffer (Partec High Resolution Staining Kit), filtered through a 50 μm Celltrics disposable filter, and mixed with 1.6 ml of DAPI staining solution (4′, 6,-Diamido-2-phenylindole) for flow cytometric analysis. *In vitro* rooting was also tested on stem cuttings excised from tetraploid seedlings. *In vitro* rooting of single nodal sections excised from tetraploid seedlings was also tested on 1/2 MS medium (Murashige and Skoog, 1962) supplemented with concentrations of 5 mg l^{-1} IBA, 1 g l^{-1} PVP, 3% (w/v) sucrose and 100 mg l^{-1} myoinositol. All i*n vitro* produced seedlings and plantlets, after being transferred in pots covered with plastic bags, were acclimatised for 3 months under controlled growth conditions (26±2 °C in a 16 h photoperiod) by gradual increasing perforations.

The flow cytometric results of this study indicated that colchicine-induced polyploidy in seedlings of *C. mopane*. The histogram results presented in Fig. 1B are a representative fluorescence profile for nuclei from plants of normal ploidy in

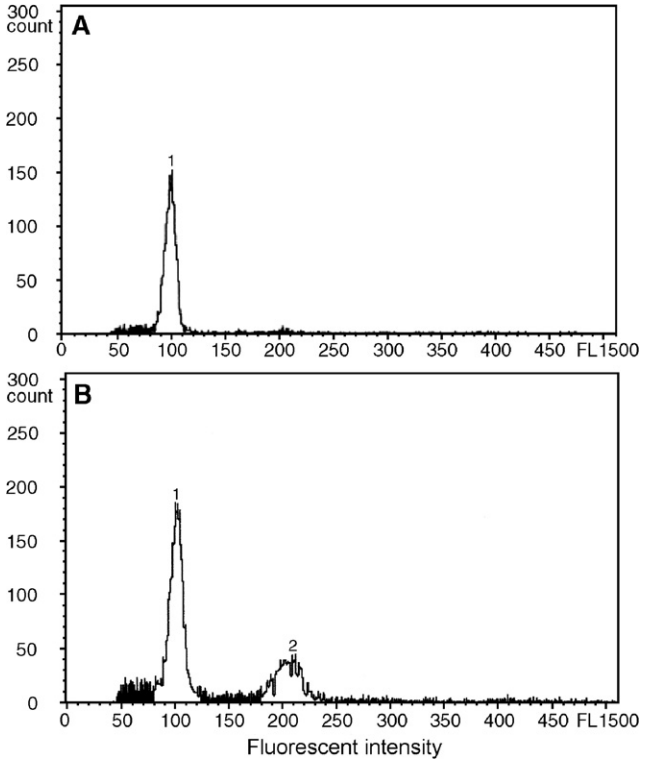

Fig. 1. Flow cytometric histograms of: (A) a control, normal diploid plants (2×) showing a single peak (B) a mixed ploidy plant sample with diploid (1) and tetraploid (2) nuclei present.

Table 1
The effect of colchicine treatments on polyploidy induction of *Colophospermum mopane*

Colchicine treatment		Ploidy levels %			
Concentration in % (w/v)	Duration	Surviving seedlings %	2×	2×+4×	4×
Control	24	30	100	0	0
0	48	45	100	0	0
	96	10	100	0	0
0.05	24	25	20	20	60
	48	35	14	0	86
	96	10	0	0	100
0.1	24	35	71	0	29
	48	25	0	0	100
	96	5	0	0	100
1.0	24	5	0	0	100
	48	0	0	0	0
	96	0	0	0	0

comparison to tetraploids from colchicine-treated plants. The concentration of colchicine, and the duration of seed imbibition, influenced the level of polyploidisation of *C. mopane*. Colchicine treatments for 48 h at concentration 0.05% and 0.1% (w/v) resulted in 86% and 100% tetraploids respectively while the 24 h treatment was less effective (Table 1). No surviving seedlings were recorded from seeds of *C. mopane* imbibed in 1.0% (w/v) colchicine for 48 and 96 h (Table 1). Blakesley et al. (2002) also reported the negative effect of high colchicine concentrations and increased length of exposure on *Acacia dealbata* and *Acacia mangium*. Exposure of *C. mopane* seeds to 1.0% (w/v) colchicine concentrations, but for a shorter period of time (24 h) resulted in much lower survival rate of seedlings than the treatments with lower colchicine concentrations although the remaining survivor proved to be a tetraploid.

In comparison with the control (Fig. 2A), tetraploids of *C. mopane* showed some morphological changes such as induction of multiple shoots from single seeds cultured on hormone free media (Fig. 2 C) or the formation of lateral shoots with more leaves per plant (Fig. 2 B). Although the leaves of tetraploids were typically a darker green, they were often narrower and shorter and exhibited similar characteristics as tetraploids of *Citrus spp*. as described by Wu and Mooney (2002). High concentrations of colchicine coupled with a long exposure time were detrimental to subsequent seedling growth and produced tetraploids with undesirable characteristics such as stunted growth. The characteristics observed *in vitro* were maintained when plants were hardened off and planted in potting soil. After 6 months, the growth form of the tetraploids was markedly different from controls (Fig. 3). The stunting observed with high concentrations of colchicine persisted also under natural growing conditions.

Nodal explants from colchicine-induced multiple shoots of tetraploid seedlings had 50% success rate of *in vitro* rooting on 1/2 MS (Murashige and Skoog, 1962) supplemented with 5 mg l^{-1} IBA. This suggests their potential use as explants for *in vitro* vegetative multiplication of tetraploids. Tetraploids varieties of

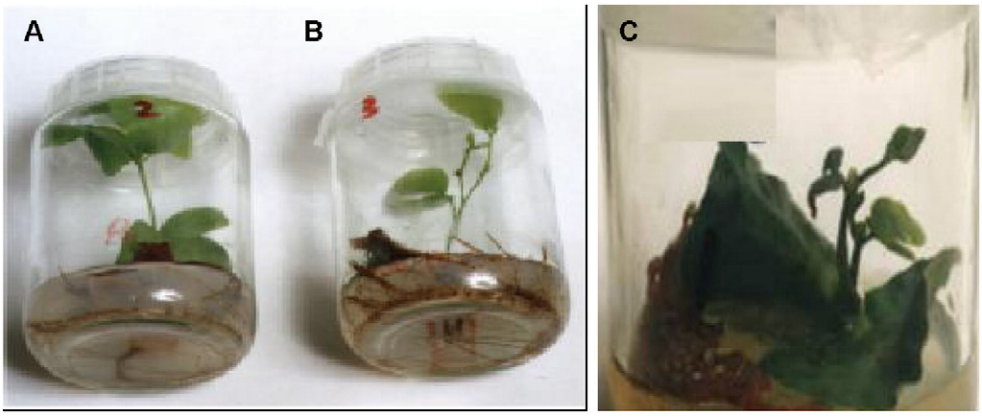

Fig. 2. Two months old seedlings of *C. mopane* grown *in vitro* from seeds imbibed in different concentrations of colchicine: (A) control; (B) in 0.05% colchicine for 24 h, and (C) in 0.1% for 96 h. Increasing the concentration of colchicine and the time of imbibition resulted in induction of lateral branching (B) or development of multiple shoots from a single seed (C).

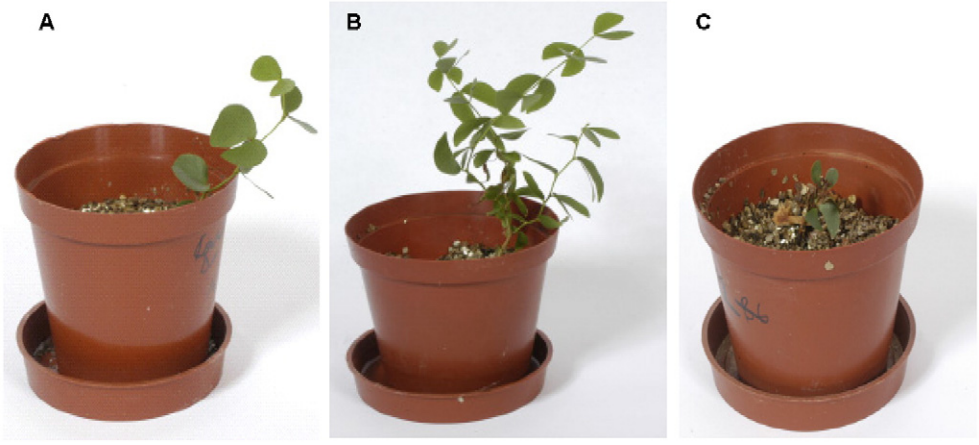

Fig. 3. Six months old, *in vitro* produced and acclimatised plants of *C. mopane:* (A) Control plant with single shoot and broader leaves; (B) Induction of axillary shoots from a tetraploid plant treated with 0.1% (w/v) colchicine for 48 h; (C) Stunted tetraploid plant treated with 0.1% (w/v) colchicine for 96 h.

C. mopane with increased lateral growth may have potential value as a food source, providing larger biomass, for herbivores and mopane worms.

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