IN VITRO PROPAGATION OF JUVENILE AND ADULT CLONES OF *CUNNINGHAMIA LANCEOLATA* (LAMB.) HOOK.

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Abstract

A method for vegetative propagation *in vitro* of *Cunninghamia lanceolata* is described. 15 to 20 rooted plantlets have been obtained by axillary budding from stem explants by a year, but, even after 10 repetitive subcultures of initial explants, plagiotropic habit of mature clone has not been overcome.

1. Introduction

Of common occurrence in central and southern Asia, *Cunninghamia lanceolata* is mostly used in China for reforestation because of its rapid growth and quality of wood.

It is also one of the rare species of gymnosperms to produce side-shoots from the base of the trunk. In Europe, only few specimens are available in arboretum. However, this species has ornamental quality too, but requires appropriate ecological conditions.

This experiment has for purpose to test in comparison the ability for propagation *in vitro* of mature (over 50 years) and juvenile (6 months) genotypes by axillary budding method and has been carried out over 3 years. Loss of rooting potential and maintenance of a plagiotropic growth are generally associated with maturity ; it is necessary to insure rejuvenilization either by *in vitro* method (de la Goublaye de Nantois, 1980) or by other methods as micrografting, hedging,... (Franclet, 1981 ; Libby and Hood, 1976) prior to propagate mature genotype.

2. Material and methods

Primary explants have been taken from the main stem (20 needles nodes) for juvenile and from the basal side-shoots for the adult genotype and disinfected during 20 to 30 min. by calcium hypochlorite (100 g l^{-1}) + Tween 80 1 °/₀₀ (after one min. in alcohol 90°), then rinsed two times by distilled water. Basal medium (BM) was constituted by macro and microelements of Murashige and Skoog (1962), completed by vitamins (thiamine HCl 10 mg l^{-1} and inositol 100 mg l^{-1}) Fe EDTA, sucrose (30 g l^{-1}) and solidified by Bacto-agar (7 g l^{-1}). Active charcoal (Merck 2186) has been added in some experiments (2 g l^{-1}), Auxins and cytokinins, incorporated before autoclaving (110° C). Tubes and jars have been put in growth chamber where the following conditions prevail : 16 h lighting (40 Wm^{-2}, mixed 40 W fluorescent tubes Phillips TL, true lite Durotest, Sylvania Grolux), 26° C ± 1° C day temp. and 22° C ± 1° C night temp. Subcultures were performed at 4, 6, 8 weeks intervals according to the experiment.

Acclimatization was carried out at 20° C $\pm$ 2° C, 90 % R.H. in small plastic 'miniserres' on pine bark (1/2) - enriched peat TKS_2 type (1/2) or pine bark (3/5) - perlite (1/5) - enriched peat (1/5) during 4 to 6 weeks (attenuate natural lighting completed by Photoclaude lamps 400 W, 40 Wm^{-2} during 6 hours). Plantlets are later transfered into containers on the same substrate with a slow release fertilizer (osmocote 15-12-15) or liquid fertilizer type Coïc and Lesaint (1979).

3. Results and discussion

After establishment of mother plants *in vitro* from primary explants on BM supplemented with IBA (3 mg l^{-1}) or IAA (2 mg l^{-1}) with charcoal (2 g l^{-1}), explants have been excised from the main stem and axillary budding firstly stimulated on BM supplemented with BAP (1 mg l^{-1}) plus NAA (0.03 mg l^{-1}).

Maintenance of shoot production (figure 1) has been obtained on BM completed with charcoal (2 g l^{-1}) and IAA (2 mg l^{-1}) in subculturing initial explants each 6 weeks during one year without differences between mature and juvenile clone (15 to 20 shoots/explant/year).

Shoots have been rooted (figure 2) on BM (or $\frac{BM}{4}$) supplemented with IBA (3 mg l^{-1}) or IAA (5 mg l^{-1}). Rhizogenesis potential have reached 70 % (7 experiments), without any difference between mature and juvenile genotype (1 to 20 roots/explant), but required sometimes one or two subcultures in rooting medium. Direct rooting in substrate has been possible for juvenile clone after dipping in IBA (3 mg l^{-1}) during 5 hours followed by a one day storage in moist chamber before planting.

Based on ten experiments (1 000 plantlets), acclimatization has been successful for nearly 80 % of rooted shoots (figures 3 and 4) and transplantation has been performed without difficulty in greenhouse or outdoors.

Orthotropy has been mainly noticed (93 %) for the juvenile clone (figures 4 a and 5) and growth comparable with control seedlings but plagiotropic habit has been preserved for all plants issued from the mature genotype (figure 4 b) ; however, a temporary orthotropic growth has been recorded by darkness treatment (6 to 8 weeks), but shoots have quickly recovered their plagiotropic growth after exposure to light (4 to 8 weeks) and are then comparable with the control cultured in continuous lighting.

In conclusion, *in vitro* propagation of *Cunninghamia lanceolata* is feasible through axillary budding. Only twenty gymnosperms species have been studied *in vitro* culture (Thorpe and Biondi, 1984), but very few are able to lead to an effective true-to-type mass propagation (i.e. *Sequoia sempervirens* ; Boulay, 1979). It has not been possible to overcome plagiotropic habit by repetitive subcultures of mature genotype, but noticeable is the darkness effect on orthotropy recovery, more elaborate studies have to be started to determine if phytochrome is involved in the reversion towards plagiotropy by lighting.

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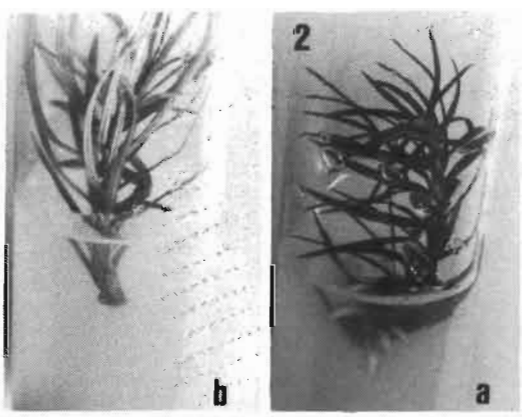


Figure 1 : shoots production from stem explants (BM ; IAA 2 mg l^{-1} ; 6 weeks).

Figure 2 : rhizogenesis *in vitro* (a : IBA 3 mg l^{-1} ; b : control).

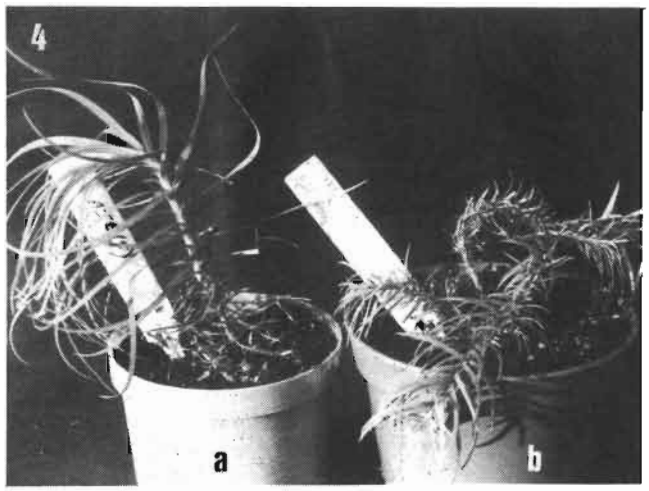
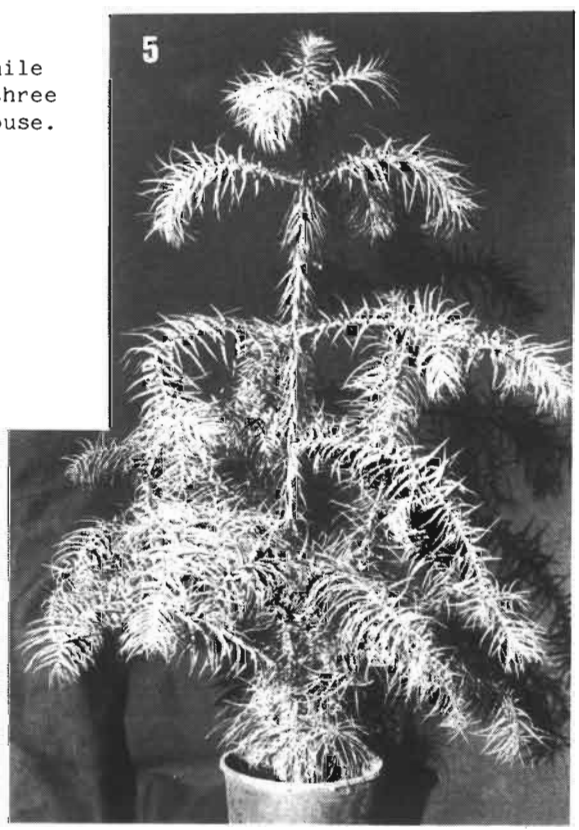


Figure 3 : rooted plantlets before acclimatization.

Figure 4 : acclimatization after 3 months (a : juvenile clone ; b : mature clone).

Figure 5 : plant from juvenile genotype after three years in greenhouse.



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