

supplemental mass-pollination. *For. Sci.* **29**: 423–432 (1983). — KANG, K. S., HARJU, A. M., LINDGREN, D., NIKKANEN, T., ALMQVIST, C. and SUH, G. U.: Variation in effective number of clones in seed orchards. *New For.* **21**: 17–33 (2001). — KANG, K. S. and LINDGREN, D.: Fertility variation and its effect on the relatedness of seeds in *Pinus densiflora*, *Pinus thunbergii* and *Pinus koraiensis* clonal seed orchards. *Silvae Genet.* **47**: 196–201 (1998). — KANG, K. S. and LINDGREN, D.: Fertility variation among clones of Korean pine (*Pinus koraiensis* S. et Z.) and its implications on seed orchard management. *For. Genet.* **6**: 191–200 (1999). — KÄRKKÄINEN, K. and SAVOLAINEN, O.: The degree of early inbreeding depression determines the selfing rate at the stage model and results from *Pinus sylvestris* (Scots pine). *Heredity*, **71**: 160–166 (1993). — KJÆR, E. D.: Estimation of effective population number in a *Picea abies* (KARST.) seed orchard based on flowering assessment. *Scan. J. For. Res.* **11**: 111–121 (1996). — LINDGREN, D., GEA, L. D. and JEFFERSON, P. A.: Loss of genetic diversity monitored by status number. *Silvae Genet.* **45**: 52–59 (1996). — LINDGREN, D. and KANG, K. S.: Status number – a useful tool for tree breeding. *Res.Rep., For.Gen.Res.Inst. Korea* **33**: 154–165 (1997). — LINDGREN, D. and MULLIN, T. J.: Relatedness and status number in seed orchard crops. *Can. J. For. Res.* **28**: 276–283 (1998). — MATZIRIS, D.: Variation in cone production in a clonal seed orchard of Black pine. *Silvae Genet.* **42**: 136–141 (1993). — MÜLLER-STARCK, G.: Reproductive systems in conifer seed orchard. 1. Mating probabilities in

a seed orchard of *Pinus sylvestris* L. *Silvae Genet.* **31**: 188–197 (1982). — ROBERTSON, A.: Inbreeding in artificial selection programmes. *Genet. Res.* **2**: 189–194 (1961). — RUOTSALAINEN, S. and NIKKANEN, T.: Variation in flowering of north Finnish clones in a Norway spruce seed orchard in central Finland. *Pro. IUFRO working party, S2.02–11, 1988, Sweden.* Eds.) STENER, L. G. and WERNER, M.: Norway spruce; Provenance, breeding and genetic conservation: 176–188 (1989). — SCHOEN, D. J. and STEWART, S. C.: Variation in male fertilities and pairwise mating probabilities in *Picea glauca*. *Genetics* **116**: 141–152 (1987). — SIEGISMUND, H. R., KJÆR, E. D. and NIELSEN, U. B.: Mating system estimates and effective population numbers for an isolated noble fir (*Abies procera*) clonal seed orchard in Denmark. *Can. J. For. Res.* **26**: 1135–1141 (1996). — STOEHR, M. U., ORVAR, B. L., VO, T. M., GAWLEY, J. R., WEBBER, J. E. and NEWTON, C. H.: Application of a chloroplast DNA marker in seed orchard management evaluations of Douglas-fir. *Can. J. For. Res.* **28**: 187–195 (1998). — WEI, R.-P.: Predicting genetic diversity and optimizing selection in breeding programmes. Ph.D. thesis. Swedish University of Agricultural Sciences, Umeå, Sweden. (1995). — WHEELER, N. C. and JECH, J. S.: The use of electrophoretic markers in seed orchard research. *New For.* **6**: 311–328 (1992). — XIE, C. Y., WOODS, J. and STOEHR, M.: Effects of seed orchard inputs on estimating effective population size of seedlots – a computer simulation. *Silvae Genet.* **43**: 145–154 (1994).

## Effect of Storage Conditions and Seed Treatment on Germination of *Cedrus deodara* LOUD. and *C. libani* A. RICH.

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### Abstract

The seeds of *Cedrus deodara* and *C. libani* species were stored during the winter at various temperatures. The storage of the seeds (with initial moisture contents of 19.3% and 18.7% respectively) was made in airtight PVC boxes at temperatures of +5°C, –10°C and –20°C, as well as in a basement at fluctuating temperatures of +10°C/20°C. Cones were also stored during the winter in a basement inside common linen sacks. The following spring, the effect of the storage temperatures as well as the effect of the seed treatment (cold stratification and soaking in water) on germination percentage and germination value were investigated. Storage in airtight boxes and at temperatures range +5°C to –10°C, were effective short-term storage methods for both of the species. It must be pointed out that during storage, the seeds became dormant that was successfully broken by cold stratification at +5°C±1°C for 15 days. The common storage conditions (10°C/20°C) as well as temperatures lower than –10°C had a negative effect on germination of both species. The cone storage of *C. libani* during the winter in the basement was the best method of wintering, because the seeds did not become dormant. On the contrary, cone storage of *C. deodara* in the basement (10°C/20°C) during winter is not recommended. The soaking of the seeds in water for 3 hours and the cold stratification at +5°C±1°C for 15 or 30 days resulted in a higher seed germination value.

*Key words:* *Cedrus deodara*, *Cedrus libani*, stratification, germination percentage, germination value, seed storage, water soaking.

### Introduction

According to many references, fresh seeds of *Cedrus* are not normally dormant and thus do not require treatment in order

to germinate (DIRR and HEUSER, 1987; TAKOS and MEROU, 1995; HARTMANN *et al.*, 1997). However, it is possible for dormancy to develop in some seed lots whose germination, without treatment, can be irregular. In such cases, if the seeds are treated with cold stratification (+4°C) for 2 months, then they germinate readily in 4 to 7 days (FORTHAM and SPRAKER, 1977; DIRR and HEUSER, 1987).

The main factors that affect seed viability during the storage are moisture content and temperature (BRADBEER, 1988; BONNER, 1990; GORDON, 1992; TAKOS, 1999a). Storage of many species seems to induce dormancy so that further treatment is necessary (WILLEMSSEN, 1975). CHANDRA and RAM (1980) referred to dormancy in stored seeds of *C. deodara*, which was broken after stratification for 15 or 30 days at +4.4°C. The resulting germination percentages were 16% and 45% respectively, whereas the control (untreated) germination percentage was 11%. THAPLIYAL and GUPTA (1980) also found improvement in the germination percentage, in 6 different seed lots of *C. deodara*, after stratification of the seeds for one week. They also noted that stratification for more than one week (up to four weeks) did not result in any statistically significant difference. According to the same researchers, the best results

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were accomplished at +9°C stratification temperature. On the contrary, FORTHAM and SPRAKER (1977) did not find any improvement in the germination percentage of *Cedrus* seeds after cold stratification. STRUCK and WHITCOMB (1977) proposed the soaking of *Cedrus* seeds for 2 or 3 hours as an alternative method for breaking of dormancy.

Therefore, although *Cedrus* seeds do not possess primary dormancy, it is later induced, especially the long-term storage (3 to 6 years) (YOUNG and YOUNG, 1992). Even short-term storage, from the time of the collection in the autumn till the sowing in the spring, can affect germination negatively. In many species a decrease in germination capacity, appears during the few first months after collection, due to inappropriate storage conditions (ROMANAS, 1991). KRÜSSMANN (1981) suggested that *Cedrus* seeds should remain in the cones during winter, because their germination percentage was better. The same storage method was proposed by YOUNG and YOUNG (1992) for *C. atlantica*, *C. libani* and *C. brevifolia*, however *C. deodara* was not studied.

The aim of the present study is to investigate the effect of a) the short-term storage conditions and b) the treatment (water soaking or cold stratification) on the germination percentage as well as the germination value (speed of germination) of the seeds of *C. deodara* and *C. libani*.

## Material and Methods

### Collection and extraction of seeds

According to common practice, cones were collected as soon as the carpel scales from the lower part of the cone became detached (KRÜSSMANN, 1981). Collections were made in early November, from trees approximately 40 years old which were growing at the nursery of the Institute of Forest Research in Hrisopigi (Greece), at an altitude of 605 m (41° 10' N, 23° 34' E). Cones were put in the sun for two weeks, until they were fully open. The extraction and dewinging of seeds was done manually. The moisture content of the extracted seeds was measured with the Aquasearch 600 (Kett industry). This method was chosen, instead of the drying at 103°C for 17 hours (ISTA, 1999), because the *Cedrus* seeds have a high content of oils and resins which evaporate at such high temperatures resulting in an incorrect moisture value (MATZIRIS, 1995). Then the seeds were put in airtight plastic (PVC) boxes. The storage of the extracted seeds, during winter and until the start of the experiment, was a) in a basement in which the temperature was fluctuating between +10°C and 20°C and b) in refrigerators with constant temperatures of +5°C, -10°C and -20°C.

A small number of cones were not extracted, but soon after collection were put in common linen sacks. The storage of the cones was made in the above-mentioned basement (+10°C/20°C).

### Germination test

In the following spring, 4 lots of 200 seeds from each storage method were counted (total 4 lots x 4 treatments = 16 lots). During the seed counting the empty, misshapen and shrunken seeds were removed. One lot, from every storage method served as the control and was immediately germinated. The second lot was put in static, tap water for 3 hours and after drainage it was also germinated. The two remaining lots were stratified, between two wet filter papers in glass petri dishes (diameter 20 cm), in the refrigerator with temperatures of +5°C ±1°C for 15 or 30 days. The filters were kept moist during the stratification. After 15 days for the first lot, and 30 days for the second, seeds were removed from the refrigerator and germinated.

In the germination tests the seeds of each lot (4 replicates of 50 seeds each) were put in petri dishes of 20 cm diameter on a moist filter and the petri dishes were put in a germination chamber. The temperature in the germination chamber was set at 25°C for 16 hours with 1000 lux light coming from cold light bulbs and at 20°C for 8 hours in the dark. The seeds were kept moist during the germination test. The first count was made on the 7<sup>th</sup> day and then every day till the 21<sup>st</sup> day (ISTA, 1999). The appearance of a 2 mm radicle long was the criterion for germination. The total germination percentage was counted as the average of the four replicates. The germination value was calculated using the equation  $GV = PV \times MDG$  (CZABATOR, 1962), where *GV* is the germination value, *PV* is the peak value, which is calculated as the quotient of the highest value of the cumulative germination percentage, divided by the number of days from the beginning of the test, and *MDG* is the average daily germination.

An ANOVA was carried out to determine the effect of the storage conditions, as well as the effect of the treatments on the germination percentage and the germination value. A DUNCAN test ( $p=0.05$ ) was further used for the comparison of the means in the SPSS statistical program (NORUSIS, 1997).

## Results

The initial (before storage) seed moisture content (on wet weight) was in *C. deodara* 19.3% and in *C. libani* 18.7%. After storage, the moisture content was measured again. It was the same in the seeds inside of the PVC boxes but had decreased in the seeds, which were stored in the cones (15.8% in *C. deodara* and 14.9% in *C. libani*).

### Germination in controls

The germination percentage of the control of *C. deodara* was severely affected by the storage conditions as is shown in table 1. Seeds that were stored at temperatures of +5°C and -10°C had the highest germination percentages (56% and 54% respectively) but were not statistically different. The germination percentage of the seeds that were stored in the basement under fluctuating temperatures (+10°C/20°C) was about 10% lower. Even lower, at 30%, were the seeds that were stored at -20°C, as well as the seeds that remained in the cones.

The seeds of *C. libani* in the controls acted differently (Table 1). The highest percentage was observed in the seeds that were stored in the cones in the basement. The value was significantly higher than the corresponding percentages for seeds, which were stored differently. However, the seeds, which were stored at +5°C and -10°C, gave satisfactory percentages (their values did not differ statistically), but the percentages were lower (approximately 40% and 50% respectively). The percentage of the seeds stored in PVC boxes in the basement was also very low, while the seeds stored at -20°C was zero.

### Germination from seeds after soaking in water

The soaking of the two species of the seeds did not improve the total germination percentage in any storage temperature as compared to the controls. The small differences in the values did not differ significantly (Table 1).

### Germination of seeds after stratification

Stratification, either for 15 or 30 days, improved the germination percentages in both species in all storage conditions, with the exception of the deep freezing (-20°C) (Table 1). The improvement in germination percentage of seeds stored in the basement in PVC boxes reached almost 50% for *C. deodara* and 80% for *C. libani*. The corresponding improvement in the

Table 1. – Effect of storage conditions and treatment on germination percentages of *Cedrus deodara* (C.d.) and *C. libani* (C.l.).

Storage conditions		Controls	Treatment		
			Soaking	Cold stratification	
				in water for 3 h	15 days
Basement 10/20°C (PVC)	C.d.	45,0 aA*	42,5 aA	60,0 aB	65,0 aB
	C.l.	22,5 aA	25,0 aA	41,0 aB	39,5 aB
Basement 10/20°C (cones)	C.d.	30,0 bA	25,5 bA	40,0 aB	39,5 bB
	C.l.	71,5 bA	75,0 bA	78,5 bAB	79,0 bB
Refrigerator 5°C (PVC)	C.d.	56,0 cA	50,5 acA	65,0 aB	64,0 aB
	C.l.	50,0 cA	50,0 cA	61,0 cB	63,5 cB
Freezer -10°C (PVC)	C.d.	54,0 cA	58,0 cA	75,0 bB	77,5 cB
	C.l.	48,0 cA	45,0 cA	78,5 bB	79,5 bB
Freezer -20°C (PVC)	C.d.	30,0 bA	25,0 bA	28,0 cA	26,5 dA
	C.l.	0,0 dA	0,0 dA	0,0 dA	0,0 dA

\*) Values of each species within the same column followed by the same small letter and within the same row followed by the same capitals are not significantly different at  $p = 0.05$ , DUNCAN test.

seeds stored inside the cones was 30% and 10% respectively; in the seeds stored at +5°C was 15% and 25%, and in the seeds stored at -10°C was 40% and 90%. The lengthening of the stratification time, from 15 to 30 days, did not result in any further improvement in germination percentages.

#### Germination value

The germination values of the soaked seeds in both species and in all storage conditions were positively affected, because they were significant statistically differences as compared to the control (Table 2). The germination values were even higher in the stratified seeds although the 30 day stratification was not, in all cases, significantly different compared to 15 day stratification.

Table 2. – Effect of treatment on germination value (germination speed) of *Cedrus deodara* (C.d.) and *C. libani* (C.l.).

Storage conditions		Controls	Treatment		
			Soaking	Cold stratification	
				in water for 3 h	15 days
Basement 10/20°C (PVC)	C.d.	3,07a*	9,59b	12,28bc	16,02c
	C.l.	1,32a	11,83b	14,45b	26,72c
Basement 10/20°C (cones)	C.d.	3,21a	9,99b	10,19bc	14,88c
	C.l.	6,04a	26,85b	32,63b	50,22c
Refrigerator 5°C (PVC)	C.d.	4,52a	22,90bc	30,95c	41,61d
	C.l.	4,29a	34,11b	38,67b	62,73c
Freezer -10°C (PVC)	C.d.	5,28a	28,04b	30,95bc	41,61c
	C.l.	2,77a	4,11b	10,81c	17,61d
Freezer -20°C (PVC)	C.d.	2,71a	12,04b	14,04b	14,26b
	C.l.	0,0a	0,0a	0,0a	0,0a

\*) Values within the same row followed by the same letter are not significantly different at  $p = 0.05$ , DUNCAN test.

## Discussion – Conclusions

In the present study, in which the seeds were stored at their initial moisture contents (19.3% for *C. deodara* and 18.7% for *C. libani*) it is confirmed that the storage temperature significantly affects the seed germination capacity (BRADBEER, 1988; BONNER, 1990). The most suitable storage temperatures, in both species, were +5°C and -10°C. The results agree with MATZIRIS (1995), who noted that temperatures between -4°C and -15°C, and moisture contents ranging between 12% to 13%, are better for the storage of *Cedrus* seeds for 1 to 5 years. According to the present study, short-term storage can be made without reducing the initial seed moisture content when using the above-mentioned range of temperatures (+5°C, -10°C). Higher temperatures and specifically the ones of the basement (alternating temperatures +10°C/20°C) reduced the seed germination viability. YOUNG and YOUNG (1992) also reported the unsuitability of this storage method because they pointed out that the *Cedrus* seeds are not well suited for storage under these conditions because of their high oil content. In the present study the deep frozen temperature (-20°C) was also found to be unsuitable. At this temperature the germination percentages of seeds of *C. deodara* were approximately 50% lower as compared to that of +5°C and -10°C. At the same temperatures the seeds of *C. libani* were totally frozen, a result that agrees with MATZIRIS (1995), who reported that the *Cedrus* seeds can keep their viability at such low temperatures only if their moisture content is very low.

The germination percentage of *C. libani* was very high in the seed stored inside the cones through winter, as noted by KRÜSSMANN (1981) and YOUNG and YOUNG (1992). On the contrary, in *C. deodara* the results of this storage method were disappointingly low. This result contradicts KRÜSSMANN (1981) who proposed that, for all the *Cedrus* species, the seeds should remain in the cones till the sowing. The above difference in storage requirements between *C. libani* and *C. deodara* can not be explained with any certainty in the present study. In order to decide if this storage method is adequate or not for *C. deodara*, it must be examined, if it causes deeper seed dormancy and for this reason a longer than 30 days cold stratification period is required to break dormancy. Additionally, the seed oil content could affect the storage requirements (YOUNG and YOUNG, 1992), which is also a topic of future research.

#### Dormancy-treatment effect on germination percentage and germination value

The present study showed that after short-term storage and depending upon the storage conditions, the seeds acquire some degree of dormancy. RUDOLF (1974), DIRR and HEUSER (1987), YOUNG and YOUNG (1992), HARTMANN *et al.* (1997) and TAKOS (1999b) also refer to this and this is the reason why they suggested a short period of stratification before the spring sowing. In the present study, the germination percentages after 15 or 30 days of stratification, did not show statistically significant differences in both species. This was also reported by THAPLIYAL and GUPTA (1980).

The germination percentages after the soaking of the seeds did not show statistically significant differences compared to controls. Thus, the positive effect of soaking referred to by STUCK and WHICOMB (1977) and HARTMANN *et al.* (1997) was not confirmed in this study. On the contrary, the soaking had a positive effect on the germination value as FORDHAM and SPRAKER (1977) and DIRR and HEUSER (1987) reported, however, the germination value after stratification was significant higher compared to controls, but not in every case after soaking. As far as the relationship between the stratification length and

germination value is concerned, significant differences between 15 and 30 day lengths were not found in all cases as also reported by THAPLIYAL and GUPTA (1980) for the *C. deodara*. However, the germination value was always higher in the 30 day stratification.

### Literatur

BONNER, F. T.: Storage of seeds: Potential and limitations for germplasm conservation. *For. Eco. and Man.* **35**: 35–43 (1990). — BRADBEER, W. J.: Seed dormancy and germination. Blackie Academic and Professional, Glasgow. 146 pp. (1988). — CHANDRA, J. P. and RAM, A.: Studies on depth of sowing deodar (*Cedrus deodara*) seed. *Indian Forester* **106** (12): 852–855 (1980). — CZABATOR, F. J.: Germination value: An index combining speed and completeness of pine seed germination. *For. Sci.* **8**: 386–396 (1962). — DIRR, M. A. and HEUSER, CH. W. Jr.: The Reference Manual of Woody Plant Propagation: From Seed to Tissue Culture. Varsity Press Inc., Athens, Georgia. 239 pp. (1987). — FORDHAM, A. J. and SPRAKER, L. S.: Propagation manual of selected gymnosperms. *Arnoldia* **37**: 1–88 (1977). — GORDON, G. A.: Seed Manual for Forest Trees. Published by Forestry Commission, Bulletin No. **83**. London. 132 pp. (1992). — HARTMANN, H. T., KESTER, D. E., DAVIES, F. T. Jr. and GENEVE, R. L.: Plant Propagation: Principles and practices. Prentice-Hall International Inc., Englewood Cliffs, New Jersey. 770 pp. (1997). — ISTA (International Seed Testing Association): International Rules for Seed Testing. *Seed Sci. Techn.* **27**, (Supplement) Rules 1999. 333 pp. (1999). — KRÜSSMANN, G.: Die Baumschule. Verlag Paul Parey, Berlin,

Hamburg. 656 pp. (1981). — MATZIRIS, D.: The storage of forest seeds. In: Proc. of 6th Conference of the Panhellenic Forestry Assoc. pp. 269–278. Panhellenic Forestry Association, Thessaloniki (1995). — NORUIS, M. J.: SPSS: SPSS 6.1 Guide to data analysis. Prentice-Hall, Englewood Cliffs, NJ. 582 pp. (1997). — ROMANAS, L.: Physiology of forest seeds. II. Effect of cold stratification on the germination of seeds. Edited by the National Agricultural Research Foundation (N.AG.RE.F.), Forest Research Intitute, Thessaloniki. 20 pp. (1991). — RUDOLF, P. O.: *Cedrus*. In: Seeds of Woody Plants of the United States. Forest Service, USA, Washington, DC. pp. 291–294 (1974). — STRUCK, D. and WHITCOMB, C. E.: Effects of nutrition on germination and growth of *Cedrus deodara* seedlings. *Oklahoma Agr. Exp. Sta. Res. Rpt.* **760**: 32–34 (1977). — TAKOS, I.: Electronics bank of woody plant seeds. In: RADOGLU, K. and RAFTOYANIS, I. (Eds.): Planting stock of woody species. pp. 93–109. National Agricultural Research Foundation (N.AG.RE.F.), Forest Research Intitute, Thessaloniki (1999a). — TAKOS, I.: Seeds of woody plants (CD-ROM). Edited by the Technological Education Intitute of Kavala. Agrosilva Corp., Thessaloniki (1999b). — TAKOS, I. and MEROU, TH.: Technology of woody plants seeds. Edited by the Technological Education Institute of Kavala. Art of Text, Thessaloniki. 181 pp. (1995). — THAPLIYAL, R. C. and GUPTA, B. N.: Effect of seeds source and stratification on the germination of deodar seeds. *Seed Sci. Techn.* **8**: 145–150 (1980). — WILLEMSEN, R. W.: Effect of stratification temperature and germination temperature on germination and the induction of secondary dormancy in common ragweed seeds. *Amer. Jour. Bot.* **62** (1): 1–5 (1975). — YOUNG, J. A. and YOUNG, Ch. G.: Seeds of woody plants in North America. Dioscorides Press, Portland, Oregon. 407 pp. (1992).

## Studies on *in vitro* Clonal Propagation of *Paulownia tomentosa* STEUD. and Evaluation of Genetic Fidelity through RAPD Marker

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### Abstract

Nodal explants were used for clonal propagation of *Paulownia tomentosa* by manipulating the cytokinin and auxin. Bud proliferation and multiple shoots were achieved from nodal explants derived from greenhouse grown plants of *Paulownia tomentosa* on Murashige and Skoog (MS) medium supplemented with 2.22–6.66  $\mu\text{M}$  BA. Inclusion of NAA (0.53–1.34  $\mu\text{M}$ ) in the culture medium enhanced the rate of multiplication. The shoot length was attained 3–4 cm on MS medium supplemented with 4.44  $\mu\text{M}$  BA + 0.53  $\mu\text{M}$  NAA + 3% (w/v) sucrose within 4 weeks of culture. The rate of multiplication was maintain in subsequent subculture. Excised shoots were rooted on half-strength MS medium supplemented with 0.49  $\mu\text{M}$  IBA after 2 weeks of culture. On an average of 81 plantlets were obtained from each nodal explant within 12-week of subculture. Rooted propagules were acclimatized and successfully transferred to greenhouse. The micropropagated plantlets appeared morphologically similar with the mother plants. No variation was detected among the micropropagated plants by the use of Randomly Amplified Polymorphic DNA (RAPD) markers.

**Key words:** Growth regulators, nodal explants, RAPD, shoot multiplication, woody tree.

**Abbreviations:** Ads – adenine sulfate; BA – 6-benzylaminopurine; Kn – kinetin, IAA – indole-3-acetic acid, IBA – indole-3-butyric acid, NAA – 1-naphthaleneacetic acid, MS medium – MURASHIGE and SKOOG (1962) medium.

### Introduction

Micropropagation of tree species offers a rapid means of producing clonal planting stock for afforestation, woody biomass production and an effective way to capture genetic gain (PARK and BONGA, 1992). Micropropagation has been applied to many tree species (BONGA and VON ADERKAS, 1992). *Paulownia tomentosa* (Scrophulariaceae) is a fast growing deciduous tree, growing for afforestation and mine site reclamation (ZHU et al., 1988). The wood and bark were reported to have astringent properties. The charcoal made from this wood is used in high class fire works and in the preparation of gun powder (Anonymous, 1988). It is propagated through seed or by root cuttings. Conventional methods of propagation through seed is unreliable because of disease and pest problem, poor germination and also slow growth than root cuttings (BERGMANN and MOON, 1997; BERGMANN, 1998). Application of *in vitro* culture techniques have great potential for clonal propagation of this important forest species. *In vitro* propagation of *Paulownia tomentosa* was achieved through shoot bud regeneration direct-

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