plus the prevailing winds that mainly blow from the west to the east may also cause the North Kaibab trees to function somewhat as an island population. It may be that the populations came from the same ancestors but after isolation, the North Kaibab population began to diverge slowly from the other 2 populations. If the three populations studied indeed represented 2 separate populations, we may need to develop separate tree improvement programs to fully exploit their potentials. Further sampling in all populations is needed to verify this hypothesis.

A small percentage of genetic diversity appeared to be due to differences between populations. Still, by choosing parent trees from a single population, all but 1.4% of the genetic variation in the population can generally be sampled. In achieving genetic gain and conserving a reasonably large superior genetic base, a trade-off usually occurs. This study indicated that there is minimum trade-off as the superior trees maintain a diverse genetic base. Phenotypic selection with natural regeneration should result in tree improvement without a significant loss in genetic diversity.

Acknowledgements

This research was funded by USDA McIntire-Stennis program and NAU Bureau of Forestry Research Program. We especially thank Dr. P. Keim for reviewing this manuscript. We also thank Dr. E. A. Kurmes, Dr. W. Groman and Brad Blake for assistance and guidance.

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Micropropagation of Ailanthus malabarica DC. Using Juvenile and Mature Tree Tissues

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(Received 2nd January 1992)

Summary

Multiple buds were induced from nodes of mature trees (NM) as well as from cotyledonary nodes (NC) of the seedlings of *Ailanthus malabarica* DC. The number of buds induced from NM was highest on Murashige and Skoog's (MS) medium supplemented with 50 mg · 1^{-1} activated charcoal, 175.29 mM sucrose and 133.2 μM BA while in case of NC, the number induced was highest on MS medium supplemented with 175.29 mM sucrose and 88.8 μM BA. Buds induced from NM failed to elongate and could not be rooted. Buds obtained from NC could be elongated to form microshoots on MS medium supplemented with 22.2 μM BA and 2.32 μM KN. The elongated microshoots were harvested at the end of each subculture. They were rooted *in vitro* through a sequential treatment involving an initial 15-day treatment on MS medium supplemented with 58.43 mM sucrose and 26.85 μM NAA followed by transfer of the microshoots to MS medium

*) For correspondence

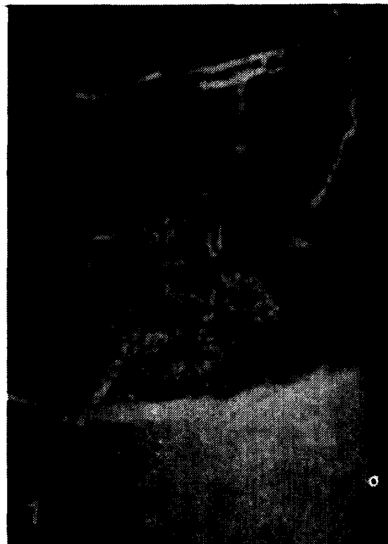
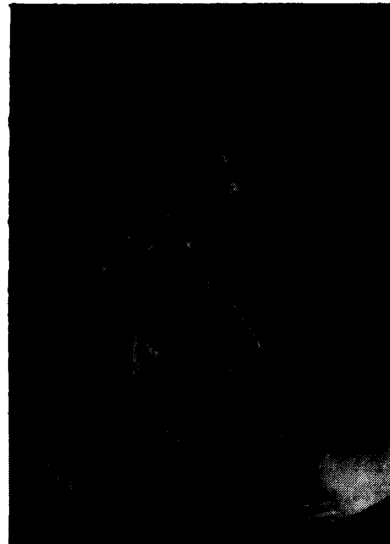

Figure 1 and 2. — Multiple buds induced from nodes of *Ailanthus malabarica* DC.
Figure 1. — Multiple buds induced from nodes of mature trees (NM).
Figure 2. — Multiple buds obtained from cotyledonary nodes (NC).

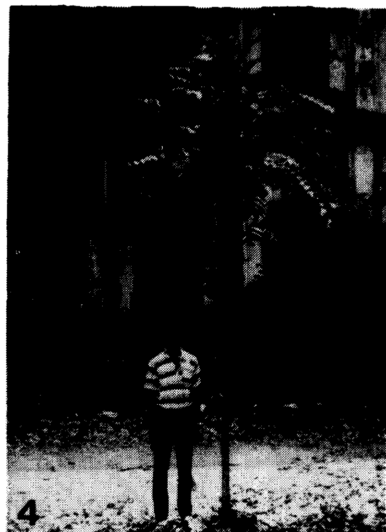

Figure 3 and 4. — Micropropagation of *Ailanthus malabarica* DC.
Figure 3. — A rooted shoot.
Figure 4. — A two-year old *in vitro* propagated tree.

supplemented with 58.43 mM sucrose, 5.37 μM NAA and 53.71 μM IAA. Plantlets have been transferred to soil and have been established in the field.

Key words: Ailanthus malabarica DC., matchwood tree, micropropagation, multiple buds, *in vitro*, afforestation, phenolics, coconut husk.

Introduction

Ailanthus malabarica DC., commonly known as the matchwood tree, is a stately tree which thrives well in the laterite soil of the west coast of India and is capable of surviving even on very hostile terrain. It is used as an avenue tree as well as in afforestation and social forestry programmes. The wood is used for match sticks and paper pulp. A decoction of the bark is of medicinal value while the timber is used in construction and in making furniture, fishing boats, packing cases and drums. The foliage is a good source of green manure and fodder.

A. malabarica is normally seed-propagated. Though the percentage of germination of fresh seed is high, the seeds loose their viability within a month. As a result, planting material is not readily available round the year. Moreover, seed-set is affected by the frequent attack by the webworm, *Eligma narcissus* on the foliage, terminal apices, inflorescence and fruits (VARMA, 1986). Micropropagation of *A. malabarica* can provide a continuous source of plants for large scale afforestation and social forestry programmes.

Cell culture studies (ANDERSON et al., 1987a, 1987b; JAZIRI, 1990; JAZIRI and HOMES, 1990), regeneration through organogenesis (CARUSO, 1974) and protoplast culture (PARK and LEE, 1990) have been reported for the temperate species *A. altissima*. There have been no reports on *in vitro* propagation of *A. malabarica*. This report deals

Table 1. — Mean number and mean length of buds initiated from nodes of mature trees (NM) and cotyledonary nodes (NC), at the end of 60 days and 21 days respectively, on MS medium supplemented with different concentrations of sucrose and BA.

Treatment number	BA (µM)	Sucrose (mM)	NM			NC		
			Response of explants (%)	Mean number of buds	Mean length of buds (cm)	Response of explants (%)	Mean number of buds	Mean length of buds (cm)
1	44.4	58.43	-	-	-	62.35	0.72	3.32
2	44.4	116.86	-	-	-	60.47	2.00	3.00
3	44.4	175.29	-	-	-	62.33	4.38	1.02
4	44.4	233.72	-	-	-	59.27	6.80	3.57
5	88.8	58.43	-	-	-	65.03	3.16	2.15
6	88.8	116.86	-	-	-	61.01	5.00	2.75
7	88.8	175.29	45.36	2.00	0.50	61.80	8.23	0.93
8	88.8	233.72	10.24	1.00	0.10	59.30	6.47	3.25
9	133.2	58.43	25.25	1.00	0.13	63.09	4.38	2.07
10	133.2	116.86	35.24	2.08	0.22	61.71	6.02	2.58
11	133.2	175.29	40.28	7.08	0.45	60.88	7.90	0.73
12	133.2	233.72	20.22	3.17	0.45	61.17	5.00	2.37
13	177.6	58.43	16.98	2.03	0.13	45.82	2.60	1.25
14	177.6	116.86	24.42	2.00	0.28	59.34	3.76	1.05
15	177.6	175.29	38.67	3.22	0.33	58.03	3.85	0.40
16	177.6	233.72	12.63	1.00	0.13	57.29	3.07	2.57
[a]								
F			(F ratio)	(F ratio)	(F ratio)	(F ratio)	(F ratio)	(F ratio)
BA (B)			23.51***	20.62***	1.11	13.70***	68.48***	42.20***
Lin			23.51***	20.62***	1.11	21.44***	0.24	124.80***
Quad			-	-	-	17.47***	204.81***	0.90
Cubic			-	-	-	2.20	0.42	1.20
Sucrose (S)			48.55***	28.11***	1.56	1.04	78.59***	101.40***
Lin			0.46	14.81**	0.37	0.35	173.24***	2.60
Quad			118.58***	41.17***	1.25	1.40	45.42***	122.70***
Cubic			26.60***	48.94***	1.20	1.15	17.12***	178.40***
B x S			1.78	21.91***	0.89	4.95**	18.02***	4.90**
Lin			1.25	28.57***	0.55	25.91***	113.88***	17.80***
Quad			0.52	6.28*	0.42	17.78***	46.12***	14.70***
Cubic			0.32	10.53**	0.12	0.85	2.12	11.60**

*) $P < 0.05$ **) $P < 0.01$ ***) $P < 0.001$

[a]) Statistical analysis of the two-factor experiment for treatments 7 to 16 in case of NM and treatments 1 to 16 in case of NC were carried out by ANOVA and by fitting response models. The experiments were repeated 3 times with 10 and 20 replications per treatment for NM and NC explants respectively.

with multiple bud induction from nodes of *A. malabarica*, rooting of the shoots and establishment of plantlets in the soil.

Materials and Methods

Nodes from mature trees (NM) as well as cotyledonary nodes from seedlings (NC) of *A. malabarica*, from Mangalore, South India were used for the experiments. Young twigs with 4 to 5 terminal nodes and compound leaves were collected from mature trees. The leaves were cut off and discarded. The stems were held under running tap water for an hour, surface-sterilized with 70% ethanol for 1 min. followed by an 8-min. treatment with a solution of 0.1% mercuric chloride and 0.1% sodium lauryl sulphate and finally rinsed three times in sterile distilled water. The nodal portions of the stem, 5 mm in length, were used as explants.

Seeds were separated from the samara fruits and were surface-sterilized in a solution of 0.1% mercuric chloride and 0.1% sodium lauryl sulphate for 3 mins. This was followed by three rinses in sterile distilled water. The seeds were germinated *in vitro* in sterilized screw-capped bottles on 0.7% plain agar medium. Seedlings obtained five days after inoculation of the seeds were used as a source of explants. The epicotyl was cut off just above the cotyledonary node while the hypocotyl was cut off 1 cm below the node. The cotyledonary nodes with the cotyledons intact were used as explants.

The MS medium of MURASHIGE and SKOOG (1962) supplemented with various concentrations of sucrose, 6-benzyladenine (BA) and kinetin (KN) was used for initiation and elongation of buds. Exudation of phenolics was a serious problem in case of NM explants. This was controlled by incubating the explants on media supplemented with 50 mg · l^{-1} activated charcoal and by subculturing every two days up to the seventh subculture. During this period the cultures were incubated in the dark. After the seventh subculture they were transferred to a 16-hour

photoperiod at a photon-flux density of 10 to 15 μmol m^{-2} s^{-1} produced from cool white daylight fluorescent tubes.

Microshoots which had attained a minimum length of 1 cm were rooted through a sequential treatment. This involved a 15-day incubation period on MS medium supplemented with 58.43 mM sucrose and different concentrations of naphthalene-1-acetic acid (NAA) followed by transfer to MS media incorporated with various auxins, viz. indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and NAA, singly and in combinations.

The pH of the media was adjusted to 5.9 prior to autoclaving at 121° C and 103.4 kPa for 20 mins. The media were gelled with 0.8% agar (Bacteriological Grade, Himedia Laboratories, Bombay). Explants were cultured in cotton-plugged test tubes containing 10 ml of medium. The cultures were maintained under the photoperiod mentioned above. The temperature and relative humidity of the culture room was maintained at 28 ± 2° C and 75 ± 5% RH. Subcultures were carried out periodically.

Rooted plantlets were transferred to various mixtures of sterilized soil, sand, soilrite (a mixture of expanded perlite, exfoliated vermiculite and peat moss) and powdered coconut husk in perforated polythene bags. The plantlets were kept in a humidity controlled glasshouse maintained at a relative humidity of 95% to 100% and were covered with polythene covers as an additional precautionary measure. Partial shading was provided by filtering sunlight through translucent fibre glass sheets. When the plants had shown signs of survival, as judged by the appearance of a new leaf, the polythene covers were removed. After two weeks the plants were transferred to pots with unsterilized soil and kept in a open glasshouse which received diffuse sunlight and had the relative humidity of the outside air. After 4 weeks of hardening, the plants were transferred to the field and protected against the sun for a few days with a cover of thatched coconut leaves.

All experiments were carried out in triplicate, each experiment having either 10 or 20 replications per treat-

Table 2. — Mean number of buds from cotyledonary nodal (NC) explants which elongated to a minimum shoot length of 1 cm at the end of each subculture on MS medium supplemented with 175.29 N/M sucrose and different concentrations of KN. Each treatment carried 20 replications and the experiment was repeated 3 times.

BA (µM)	KN (µM)	Mean number of buds > 1 cm in length						Total of mean number of buds harvested
		S1[a]	S2[a]	S3[a]	S4[a]	S5[a]	S6[a]	
88.81	0.46	1.34 (1.25)[b]	1.20 (0.95)	-	-	-	-	2.54
88.81	2.32	1.38 (1.37)	1.33 (1.03)	-	-	-	-	2.71
22.20	0.46	2.46 (1.94)	2.42 (1.65)	1.03 (0.87)	1.05 (0.75)	-	-	6.94
22.20	2.32	2.71 (1.56)	2.70 (1.38)	2.54 (1.25)	2.49 (0.95)	1.97 (0.62)	1.35 (1.05)	13.76
4.44	0.46	2.32 (1.98)	1.42 (1.30)	-	-	-	-	3.74
4.44	2.32	2.37 (1.25)	2.14 (1.59)	-	-	-	-	4.51
-	0.46	2.30 (1.75)	1.25 (1.05)	-	-	-	-	3.55
-	2.37	2.35 (2.00)	2.10 (1.70)	-	-	-	-	4.45

[a]) S1, S2, S3, S4, S5, S6 represent subculture numbers 1, 2, 3, 4, 5 and 6 respectively.
[b]) Values in parentheses within each column represent ± SD.

Table 3. — Mean number of roots per shoot and mean length of the roots induced through sequential culture on Medium I and II. The experiment was repeated 3 times with 20 replications per treatment.

Medium I NAA (μM)	Medium II NAA (μM)	Medium II IBA (μM)	Medium II IAA (μM)	Response of rooting (%)	Mean number of roots per shoot	Mean length of roots (cm)
53.71	53.71	-	-	-	-	-
53.71	5.37	-	-	35.50 (5.63)[a]	2.56 (1.35)[a]	2.97 (1.35)[a]
53.71	-	-	-	-	-	-
53.71	-	53.71	-	30.65 (3.60)	2.32 (1.75)	3.80 (2.00)
53.71	-	5.37	-	30.32 (4.75)	1.25 (1.00)	2.63 (1.75)
53.71	-	-	53.71	28.00 (3.25)	2.36 (1.50)	2.45 (1.35)
53.71	-	-	5.37	30.57 (6.95)	1.75 (1.05)	2.25 (1.97)
53.71	5.37	-	53.71	38.65 (7.32)	1.86 (0.95)	2.00 (1.87)
53.71	-	5.37	53.71	36.12 (5.67)	0.36 (0.32)	1.75 (1.39)
53.71	5.37	53.71	-	35.10 (8.75)	0.93 (0.67)	1.20 (1.65)
26.85	53.71	-	-	-	-	-
26.85	5.37	-	-	-	-	-
26.85	-	-	-	-	-	-
26.85	-	53.71	-	-	-	-
26.85	-	5.37	-	-	-	-
26.85	-	-	53.71	-	-	-
26.85	-	-	5.37	-	-	-
26.85	5.37	-	53.71	55.55 (9.32)	4.05 (2.35)	2.35 (1.35)
26.85	-	5.37	53.71	-	-	-
26.85	5.37	53.71	-	45.32 (4.75)	3.83 (1.85)	2.75 (1.50)

[a]) Values in parentheses within each column represent ± SD.

ment. The data was transformed and subjected to analysis of variance (ANOVA). The treatment means were examined using DUNCAN's multiple range test (DUNCAN, 1955) and fitting response models (SNEDECOR and COCHRAN, 1956), the selection of the appropriate technique being dependent on the nature of the treatments and the objectives of the experiments (MIZE and CHUN, 1988). In experiments which gave a "0" response, either the means and standard deviations were calculated or the F test was carried out for those treatments that gave a response.

Results

The highest mean number (*Table 1*) of buds (*Figure 1*) in case of NM explants were initiated on 175.29 mM sucrose and 133.2 μM BA, at the end of 60 days. There was a significant increase in the number of buds followed by a significant steep fall with increasing concentrations of sucrose at each level of BA. The B x S interaction for the length of the buds and the percentage of response did not differ significantly (*Table 1*). In case of NC explants (*Figure 2*), 88.8 μM BA and 175.29 mM sucrose induced the highest mean number of buds within 21 days of inoculation (*Table 1*).

Buds induced from NM explants were below 0.5 cm in length (*Table 1*) whereas buds induced from NC explants were longer. Transfer and incubation of the NM explants with the buds on various media did not promote elongation of the buds. However, transfer and incubation of

Table 4. — Percentage of survival of plants on soil mixtures.

Type of soil	Survival of plants (%)[1]
Sand : soil (1:1)	43.33 ac
Sand : soil : soilrite[2] (1:1:0.25)	23.33 bc
Sand : soil : powdered coconut husk (1:1:0.25)	61.67 a
F[3]	(F ratio) 6.89*

*) $P < 0.05$
[1]) Values followed by the same letters are not significantly different according to DUNCAN's multiple range test ($P < 0.05$).
[2]) A commercial product (mixture of expanded vermiculite, exfoliated perlite and peat moss) of Karnataka Explosives Ltd, Bangalore, India.
[3]) Statistical analysis of a single-factor experiment by ANOVA and by DUNCAN's multiple range test. The experiment was repeated 3 times with 20 replications per treatment.

NC explants with the buds onto 22.2 μM BA and 2.32 μM KN, favoured the elongation of an average of 1 to 3 buds at the end of each subculture comprising 21 days (*Table 2*).

Buds which had elongated to form microshoots 1 cm or more in length were harvested at each subculture. The harvested microshoots were inoculated onto rooting media. An initial 15-day incubation of shoots on 58.43 mM sucrose and 26.85 μM NAA followed by incubation on a lower concentration of NAA (5.37 μM) along with 53.71 μM IAA was the optimum treatment for inducing the best rooting (*Table 3*). A rooted plant is shown in *figure 3*.

The percentage of survival of rooted plantlets was highest on a mixture of sand, soil and coconut husk (1:1:0.25, v:v:v) (*Table 4*). Plants transferred to the field have established themselves in the soil and are growing well. At the end of 2 years some of these have attained a height of about 2.3 m and a girth of about 28 cms at the base (*Figure 4*).

Discussion

In *in vitro* culture, response from explants of mature trees is comparatively low when compared to that from seedling tissues (PIERIK, 1987). Cotyledonary nodes and tissues have been frequently used as explants for multiple bud initiation (RAO et al., 1981). In *A. malabarica*, though buds could be initiated from nodes of mature trees (NM), they failed to elongate and could not therefore be rooted. The elongation stage is considered by many to be a preparatory stage for rooting during which the influence of cytokinins is reduced (DRUART and GRUSELLE, 1986; QUOIRIN and LEPOIVRE, 1977). Buds initiated from cotyledonary nodes (NC) could be elongated to form microshoots by decreasing the concentration of BA in the medium and incorporation of KN at the elongation step. Reduction in the concentration of BA promotes elongation of buds as reported in mulberry (KIM et al., 1985).

A specific sequential treatment of the microshoots was found to be best for rooting. Serial cultures for rooting have been reported for *Dalbergia* (SANKARA RAO, 1986), *Eucalyptus* (SANKARA RAO, 1988) and apple (WELANDER, 1983).

Among the soil mixtures tried, a mixture of sand, soil and coconut husk gave the best percentage of survival of plantlets on transplantation. The heavy loss of plants on a sand, soil and soilrite mixture could be due to the susceptibility of peat moss (one of the components of soilrite) to pathogens. Association of peat moss with pathogens has been reported earlier by BLUHM (1978) and COYIER (1978). Besides, being a good transplantation substrate, the sand, soil and coconut husk mixture is economical.

Acknowledgements

ID thanks the University Grants Commission for a research fellowship. Thanks are due to the Department of Science and Technology, Government of India, for the grants; the Mangalore Jesuit Education Society for the facilities provided and JEMMI SOUZA for technical assistance.

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First Analysis on Allozyme Variation in Cedar Species (Cedrus sp.)[1][2]

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(Received 28th January 1992)

Summary

Isozyme variation among the four species of *Cedrus* genus was investigated in dormant vegetative buds of 25 trees of each species using starch gel electrophoresis. The most useful enzyme systems were LAP, MDH, 6PGD and PGI.

The results of this small study show that there is large heterozygosity in *Cedrus brevifolia, Cedrus libani* and *Cedrus atlantica*. In contrast, *Cedrus deodara* was fixed for the above studied enzymes. Moreover, it is shown that there are clear distinctions between *C. brevifolia, C. deodara* and the group of *C. atlantica-C. libani*.

Key words: Cedrus atlantica, Cedrus brevifolia, Cedrus libani, Cedrus deodara, isozyme variation, taxonomy.

Introduction

The genus *Cedrus*, according to a number of authors, includes four coniferous evergreen tree species, with geographically separated distributions. *Cedrus brevifolia* HENRY in Cyprus, *Cedrus atlantica* MANETTI in Algeria and Morocco, *Cedrus libani* A. RICH in Lebanon, Syria and Turkey, *Cedrus deodara* LOUDON in Afganistan and India (M'HIRIT, 1987; ARBEZ, 1987; DAVIS, 1965).

Cedrus has been successfully introduced in many countries outside of its natural distribution as ornamental and reafforestation species. According to TOTH (1980) and M'HIRIT (1987) it has, since the previous century, been introduced, to several European countries (France 1862, Italy 1866, Bulgaria 1890) and also into U. S. A. and Russia.

Due to the performance of its initial introductions, it soon became an important exotic species for Mediterranean and other countries with similar environmental conditions. In spite of the great interest in the genus, the limited information concerning the amount and pattern of its genetic variability, has been based mainly on provenance trials and studies of anatomical and morphological traits (FAO, 1989). On the other hand, in the best of our knowledge, there has been no publication, on enzyme system studies in the genus cedar.

Our objective was to determine the level of genetic variability among and within species, as well as the usefulness of isozymes for taxonomic classification of the genus cedar.

Material and Methods

Sample collection

Buds were collected from 25 randomly selected mature trees of each species and were stored in —20° C. The material for *C. brevifolia* came from a natural stand of cedar in Cyprus (Paphos forest), whereas that for the other three species came from a species and provenance plantation established in 1968, in the arboretum of Loutra Thermis — Thessaloniki.

Seeds were also used in order to carry out a segregation analysis for those trees of *Cedrus brevifolia*, which proved to be heterozygous for at least one locus from the bud-isozyme analysis.

Electrophoresis procedure

Four buds from each tree were homogenized with 0.3 ml vegetative extraction buffer pH 7 (CHELIAK and PITEL, 1984).

Megagametophytes dissected from germinated seeds (inhanced to germinate with cold stratification) with a radicle of 3 mm to 5 mm long, were homogenized separately in 0.4 ml seed extraction buffer, pH 7.6. The extraction buffer consisted of 0.1 M Tris, 3% (W/V) PVP-40, and 5 drops of b-mercaptoethanol. pH was adjusted to 7.6.

The homogenates were analyzed for: acid phosphatase (ACP E.C. 3.1.3.2.), aspartate aminotransferase (AAT or GOT E.C. 2.6.1.1.), diaphorase (DIA E.C. 1.6.4.3.), leucine aminopeptidase (LAP E.C. 3.4.11.1.), malate dehydrogenase (MDH E.C. 1.1.1.37.), peroxidase (PER E.C. 1.11.1.7.), 6-phosphogluconate dehydrogenase (6PGD E.C. 1.1.1.44.) and phosphoglucose isomerase (PGI E.C. 5.3.1.9.), in horizontal electrophoresis system with 11.5% (W/V) starch. Gels were prepared from a mixture (9:3 W/V) of MERK and CONNAUGHT starch.

[1]) Part of this research was submitted as partial fulfillment of the M. Sc. degree of A. CHRISTOU in Forest Genetics at the Dep. of Renewable Natural Resources, Forestry Section, of the Mediterranean Agronomic Institute of Chania, Greece.

[2]) This work was financially supported by the E. E. C. in the framework of the project "Mediterranean Firs and Cedars" No MA2B 0008 C(EDB).