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Somatic Embryo Induction and Germination in Quercus suber L.[1])

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Abstract

Embryogenic callus was formed on the hypocotyl of immature zygotic embryos under the influence of 2,4-dichlorophenoxyacetic acid (2,4-D) in liquid as well as in agar medium. Germination was induced by chilling but not by desiccation treatments. Epicotyl dormancy was overcome by placing the embryos with elongated radicles on medium with 6-benzyl-adenine (BA). Normally developed plantlets were transferred to soil, and acclimated in a greenhouse.

Key words: cork oak, germination, plant regeneration, *Quercus suber*, somatic embryogenesis, tissue culture.

Introduction

Cork oak (*Quercus suber* L.) is a forest species present in many countries of the Mediterranean basin, where it is exploited for cork production. Cork oak is mainly propagated by seed. However, seedling production from acorns is sometimes not advisable because of the high heterozygosity due to wind pollination. Therefore, the preservation of some characteristics can only be obtained by vegetative propagation. Nevertheless, conventional techniques, such as grafting or cutting, have proven particularly difficult for oaks, and this has encouraged us to look for alternative propagation methods.

Micropropagation of juvenile and adult material was reported previously (PARDOS, 1981; MANZANERA and PARDOS, 1990). However, a higher amount of explants can be produced by somatic embryogenesis.

Somatic embryogenesis has been little studied in this species (EL MAATAOUI and ESPAGNAC, 1987), and plant regeneration has not been achieved until now.

Material and Methods

Plant material

Samples were collected every two weeks during the period of fruit development, from June 25 until September 19, 1990. Pollination took place in May. Eight trees were selected for their good cork and fruit production in "La Herguijuela" (Cáceres, Spain). Fifty open pollinated acorns were taken per tree, per collection date. In June, only a few immature embryos were visible. In July, embryos were at the heartshaped phase. In August, the cotyledons grew and filled the ovule cavity. In September, the embryos were mature. Acorns were sterilized for 20 min with 2% NaOCl plus a few drops of Tween 20, followed by three rinses in sterile destilled water for 10 min each. Complete embryos were extracted from the ovule and cultured.

Culture medium

The basal culture medium comprised macronutrients of SOMMER et al. (1975) and micronutrients of MURASHIGE and SKOOG (1962), with the following additions: ascorbic acid (11.3 μM), nicotinic acid (8.1 μM), glutamine (3.4 mM), calcium pantothenate (4.2 μM), pyridoxine · HCl (4.9 μM) and thiamine · HCl (3 μM). Sucrose (87.6 mM) was used as carbon source. The following growth regulators were used: 6-benzyl-adenine (BA) and 2,4-dichlorophenoxyacetic

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acid (2,4-D). The pH of the medium was adjusted to 5.6±0.1 with 0.5 M NaOH or 0.1 M HCl, and the medium was autoclaved at 0.7 atmospheres (115°C) for 20 min, except for glutamine, which was filter-sterilized and added after autoclaving.

Induction media

Half of the explants were cultured on agar-solidified (8 g/l) medium in petri dishes. The other half were cultured in 25 ml or 50 ml liquid medium, dispensed in 100 ml or 250 ml erlenmeyer flasks, and agitated on an orbital shaker at 100 rpm.

Four concentrations of 2,4-D (2.3 μM, 4.5 μM, 22.6 μM and 45.2 μM) were tested for induction of somatic embryogenesis, in both agar and liquid medium. The treatments lasted 30 days, after which explants were transferred to growth regulator-free medium. Five explants were used per treatment, tree and sampling date. A total of 1005 explants were used in the experiment in an incomplete block design, due to the lack of a sufficient amount of embryos in the first samples and to losses by contamination.

Germination media

Germination was attempted following 2 different procedures: desiccation and chilling.

For the desiccation experiment, the following treatments were tested: basal solid medium as control, medium with sorbitol (0.3 M) for 30 days, medium with sorbitol (0.7 M) for 30 days, air drying inside sealed petri dishes containing a cellulose filter moistened with sterile destilled water for 2 weeks, and air drying inside empty sealed petri dishes for 2 weeks. The embryos were then transferred to basal medium. Sixty somatic embryos belonging to 6 half-sib families were used per treatment.

In the chilling experiment, 20 to 30 somatic embryos were subjected to one of the following treatments: cold storage at 5° C in darkness for either 10 weeks, 4 weeks or 2 weeks, storage in darkness at 2° C for 2 weeks, and the control at 25° C in petri dishes with basal medium.

All cultures were incubated in a climate chamber under a 16 h photoperiod, and a photon flux density of 100 μmol m^{-2} s^{-1} provided by Osram cool-white 18 W fluorescent lamps. Day temperature was 25° C ±2, and night temperature was 20° C ±2.

Statistical Analysis

For statistical calculations, a log-linear model was fitted to all experiments using a Chi-square test. Asymptotic standard errors of the parameter estimates were computed by the "delta" method (Lee, 1977). The ratio of the log-linear parameter estimate to its standard error was used to obtain the frequency significance level.

Results

Somatic embryo induction

Callus was formed on the hypocotyl of zygotic embryos at all the 2,4-D concentrations used. In the solid medium the higher concentrations of 2,4-D (22.6 μM and 45.2 μM) provided a lower percentage of embryogenic tissue, but the differences were not statistically significant (*Fig. 1*). There were no significant differences in embryogenic potential among explants from different mother trees either. Somatic embryos were formed either directly on the zygotic embryo or in the callus during the second and third week after the beginning of the 2,4-D

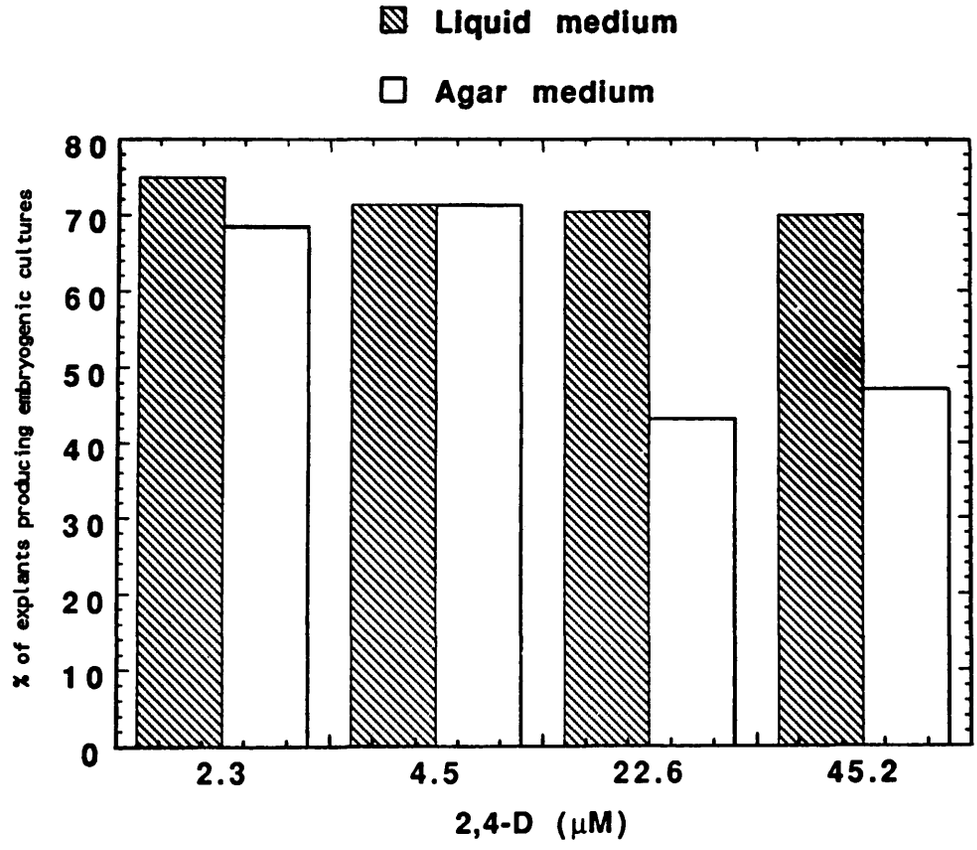

Figure 1. — Percentage of embryogenic cultures induced by 2,4-D action.

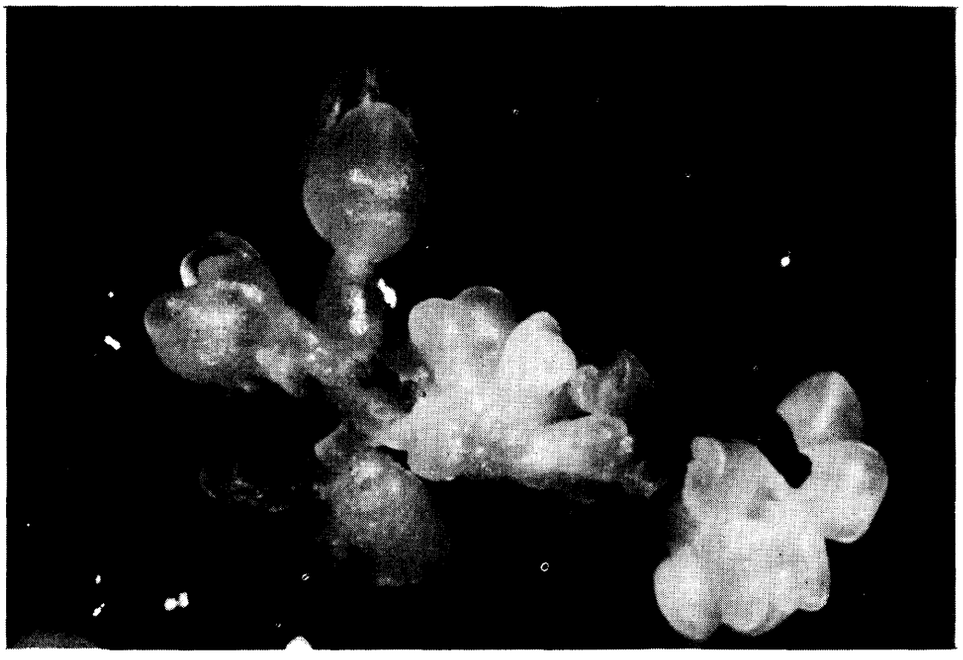

Figure 2. — Somatic embryos formed after 2,4-D treatments.

treatment (*Fig. 2*). At day 30, the explants were transferred to growth regulator-free medium for somatic embryo development.

Germination

Desiccation treatments by the addition of an osmoticum (sorbitol, 0.3 M or 0.7 M) to the medium impeded germination almost completely. Air-drying had little effect. Desiccation at high relative humidity inside sealed petri dishes with a wet cellulose filter had no effect on embryo germination either.

In contrast, cold storage treatments, i. e., 10 weeks at 5^0 C and 2 weeks at 2^0 C, promoted radicle growth (*Fig. 3*). Epicotyl dormancy was overcome by placing the somatic embryos that had developed only a radicle, on paper bridges, in test tubes containing 10 ml of the medium of Sommer et al. (0.4 μM BA). Thirty plantlets with normally developed shoots were transferred to soil, and acclimated in a mist tunnel at high relative humidity inside the greenhouse (*Fig. 4*).

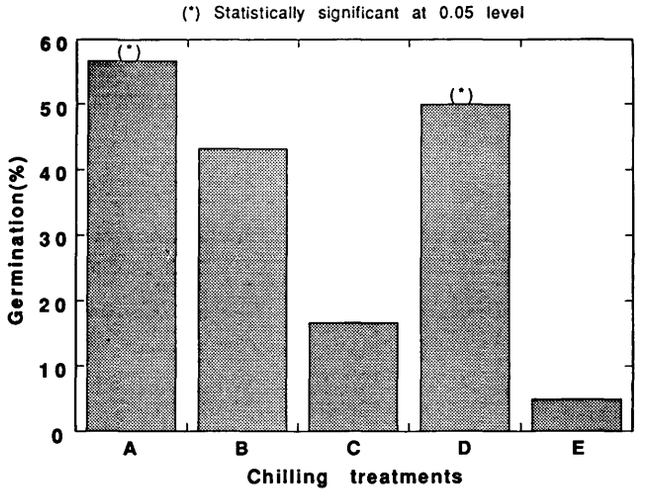

Figure 3. — Effect of chilling treatments on somatic embryo germination. A= 10 weeks at 4^0C; B= 4 weeks at 4^0C; C= 2 weeks at 4^0C; D= 2 weeks at 2^0C; E= control; *= significantly different from all of the other treatments.

Discussion

Zygotic embryos proved to be somatically embryogenetic at all the 2,4-D concentrations tested, but particularly at 2.3 μM and 4.5 μM. It was also the best plant growth

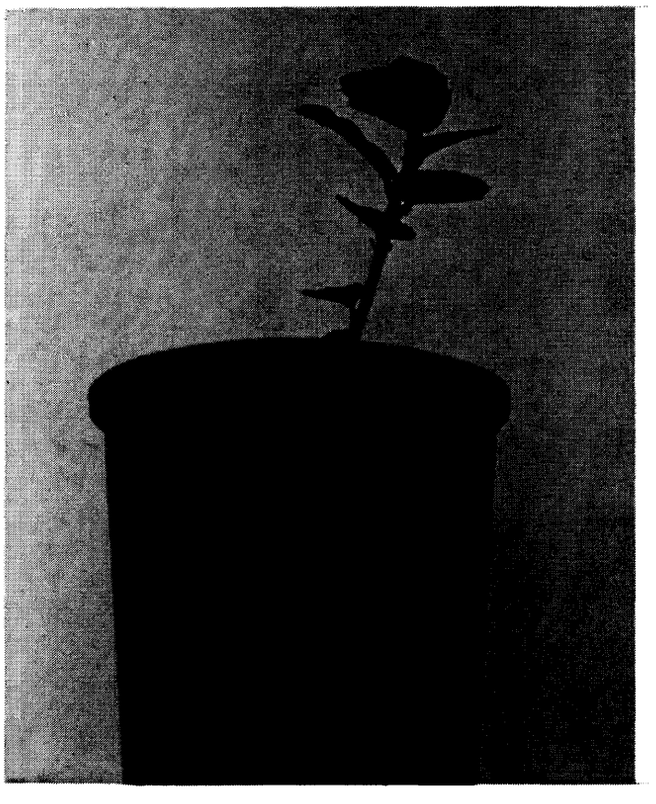

Figure 4. — Regenerated plantlet.

regulator in experiments with *Q. robur* reported earlier (MANZANERA, 1992), as compared with combinations of BA and α-naphthaleneacetic acid (NAA). Other authors (LELU and BORNMAN, 1990; SUNG et al., 1988) have observed the role of 2,4-D in cell division and embryogenic potential. However, 2,4-D is also an inhibitor of further embryo development (HALPERIN, 1970). In contrast with our results, CHALUPA (1990) obtained embryogenesis in *Q. robur* embryos using BA, alone or combined with gibberellic acid (GA_3), whereas 2,4-D was inefficient.

The effect of desiccation by the addition of sorbitol or by air-drying on the germination of cork oak somatic embryos was negligible in our case. On the contrary, a high concentration of osmoticum produced an effect similar to that of abscisic acid (ABA) in rapeseed (FINKELSTEIN and CROUCH, 1986). In interior spruce, a pulse of mannitol in combination with ABA doubled the production of mature somatic embryos compared with the standard ABA treatment (ROBERTS, 1991). GINGAS and LINEBERGER (1989) obtained germination of red oak somatic embryos by desiccation with either sorbitol or by air-drying. In pendunculate oak, somatic embryo germination was also stimulated after desiccation with 6% sorbitol (CHALUPA, 1990), but was hampered in olive (RUGINI, 1988). In interior spruce, partial drying at high humidity promoted germination of the somatic embryos up to 90% (ROBERTS et al., 1990).

Cold storage of somatic embryos matured "in vitro" at 5° C for 10 weeks and at 2° C for 2 weeks were the best treatments for breaking dormancy. These conditions are similar to those necessary for zygotic embryos of other *Quercus* species to break dormancy.

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Genotypic Differences in the Ability of Embryogenic Abies nordmanniana Cultures to Survive Cryopreservation[1])

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Abstract

Embryogenic cultures of *Abies nordmanniana* were cryopreserved after preculture in 0.4 M sorbitol and pretreatment with 5% DMSO (dimethyl sulfoximide). After cryopreservation, only a few meristematic cells in the embryo heads survived. Following an initial lag-phase, growth resumed and complete embryos were formed by the surviving cells. Regrowth capacity depended strongly on genotype as determined by frequency of survival, length of the lag-phase, and growth rate after cryopreservation. Only one of five genotypes resumed growth at same rate as before cryopreservation. Possible causes of differential crytolerance among genotypes are discussed.

Key words: Abies nordmanniana, cryopreservation, somatic embryogenesis, tissue culture.

Introduction

In conifers the use of somatic embryogenesis for plant propagation purposes is limited since it can only be induced from juvenile material with unknown genetic properties. Maintenance of stock cultures in the laboratory during field evaluation of the clones is laborious, and prolonged subculture also increases the risk of losing clones due to microbial contamination and mislabelling.

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