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Plant regeneration through somatic embryogenesis in Pistacia vera

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ABSTRACT

TASKIN, T., D. COBANOGLU, G. COLAK, S. NAMLI, D. BASARAN & Th. GASPAR (1996). Plant regeneration through somatic embryogenesis in Pistacia vera. *Saussurea* 27: 59-65. In English, English and French abstracts.

Immature fruits of *Pistacia vera* L. cv Siirt were collected 12 to 14 weeks after pollination. Zygotic embryos and cotyledon explants were sampled and treated in *in vitro* liquid and solid cultures with different concentrations and combinations of BA, NAA, kinetin and 2,4-D with the aim to provoke somatic embryogenesis. Somatic embryos differentiated directly from cotyledon explant surfaces on solid MS medium containing NAA only, in about 30 days. These embryos could be developed into viable plantlets either using liquid MS medium with BA or solid MS medium with GA3 and L-glutamine.

RÉSUMÉ

TASKIN, T., D. COBANOGLU, G. COLAK, S. NAMLI, D. BASARAN & Th. GASPAR (1996). Multiplication par embryogenèse somatique chez Pistacia vera. *Saussurea* 27: 59-65. En anglais, résumés anglais et français.

Des fruits immatures de *Pistacia vera* L. cv Siirt sont cueillis 12 à 14 semaines après la pollinisation. Des embryons zygotiques et des explants cotylédonnaires en sont prélevés, et traités *in vitro* par différentes concentrations et combinaisons de BA, NAA, kinétine et 2,4-D dans le but de provoquer l'embryogenèse somatique. Des embryons somatiques se sont différenciés directement à la surface des cotylédons excisés en environ 30 jours, sur milieu MS solide contenant du NAA seul. Ces embryons ont pu être développés en plantules viables soit sur milieu MS liquide avec BA, soit sur milieu MS solide avec GA3 et L-glutamine.

Introduction

Pistacia vera L. cv Siirt is a perennial xerophyte plant species belonging to the Anacardiaceae family (ÖZEBEK & AYFER, 1953; CRANE, 1974). This plant from

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Middle Asia origin is mainly cultivated in countries like Turkey, Afghanistan and Iran (WHITEHOUSE, 1957; ANON, 1985). Because of the difficulty to root scions, reproduction of *Pistacia* is actually mainly based upon grafting of mature plant cuttings on young trees raised from seeds. Some attempts to multiply *Pistacia vera in vitro* have been made through classical micropropagation from young and mature plant material (HANSMANN & OWEN, 1986; BARGHCHI & ALDERSON, 1989; DOLCET-SANJUAN & CLAVERIA, 1995). Somatic embryogenesis by *Pistacia* species had not been extensively explored. AHMAD & al. (1992) have obtained cell suspension cultures of *Pistacia vera* and have obtained embryos from mature seed materials in liquid cultures. But no regeneration of whole plantlets from somatic embryos had been obtained from any Pistacia species. The present work reports on direct somatic embryogenesis from immature cotyledons of *Pistacia vera* L. cv Siirt, and on whole plant regeneration from these embryos.

Materials and methods

Plant material

12 to 14 weeks after beginning of pollination of *Pistacia vera* L. cv Siirt in April, immature fruits were collected from a production area nearby the campus of University of Dicle in Diyarbakir in the beginning of August. The immature fruits were first kept in 70% ethanol for 40 seconds, then were sterilized in 3% of NaOCl for 35 minutes. Later on they were rinsed in sterilized pure water five times for five minutes. The pericarp layers were opened in a sterilized way and the testas of the immature seeds were removed; embryos and cotyledon explants were isolated and cultured.

Culture medium

MURASHIGE AND SKOOG's (1962) nutrient medium was used as basal culture medium. Medium was modified with the following additions: nicotinic acid (4.06 µM), pyridoxine HCl (2.43 µM) and thiamine HCl (29.65 µM). Sucrose (35 g/l) was used as carbon source. The following growth regulators were used: BA(benzyladenine), NAA (α-naphthaleneacetic acid), 2,4-D (2,4-dichlorophenoxy acetic acid), GA_3 (gibberellic acid) and kinetin. The pH of the medium was adjusted to 5.6 or 5.8 with 1 N HCl or 1 N KOH and the medium was autoclaved and sterilized at 1 atmosphere (121°C) for 20 minutes.

Induction and development medium

Half of the explants were cultured on agar solidified (8 g/l) medium in test tubes of 2 × 18 cm. The other half was cultured in 25 ml liquid medium dispensed in 100 ml Erlenmeyer flasks agitated on a shaker at 100 rpm. All cultures were grown under 30 µmol m^2 s^{-1} delivered continuously by cool white fluorescent lamps (OSRAM L 40 W) with a temperature of 25 ± 2°C.

The addition of growth regulators to basal nutrient medium were in the following combinations:

a) 2 mg/l kinetin and 2 mg/l 2, 4-D

b) 2 mg/l 2,4-D

c) 0.5 mg/l BA and 1 mg/l NAA

d) 1.5 mg/l NAA

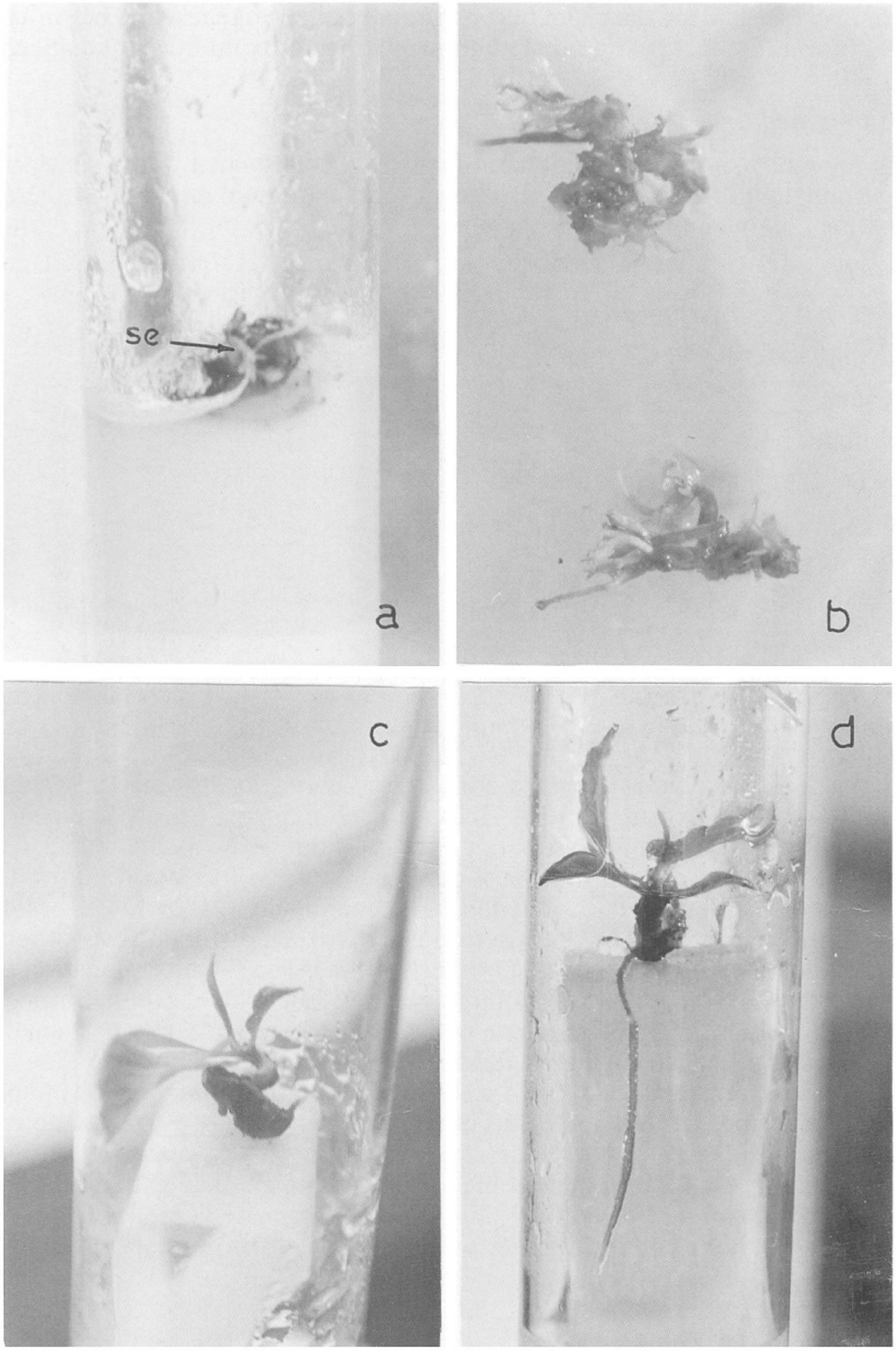

Fig. 1 – Somatic embryogenesis by *Pistacia vera* L. *in vitro*.

a. Somatic embryogenesis (se = somatic embryo) on immature cotyledons, after 33 days, on MS solid medium containing 1.5 mg/l NAA. **b.** Secondary embryogenesis on somatic embryos on MS medium without growth regulator. **c.** Germination of a somatic embryo on a MS medium containing 1.5 mg/l BA. **d.** A whole pistachio plantlet developed from a somatic embryo.

They were tested for the induction of somatic embryogenesis in both liquid and solid agar media. Explants in liquid medium were transferred in fresh medium each five days.

Germination medium

Somatic embryos from development medium were isolated. They were subcultured for germination in Murashige and Skoog's solid and liquid medium. In these media the following combinations were prepared:

Liquid media

a) BA (0.5,1,1.5,2 mg/l)

b) 1 mg/l kinetin and 0.1 mg/l 2,4-D

Solid media

a) without hormone

b) 2 mg/l BA

c) 1 mg/l GA_3 and 600 mg/l glutamine

Results

Explant reactions and somatic embryogenesis

The reactions of zygotic embryos and excised cotyledons as explants cultured in liquid medium are shown in Table 1. Because such explants released phenolics, the liquid medium was replaced by a fresh one every five days. The media with 2,4-D, supplemented or not with kinetin, did not induce any embryo formation but only a thin white callus at the surface of the cotyledons. The combination BA + NAA, besides also inducing callus (a green one) on the cotyledons, provoked adventitious shoot formation on 15% of them. These shoots were abnormally formed. The same combination allowed 53% of the zygotic embryos to develop into plantlets but 15% of others formed somatic embryos. NAA alone in solid medium, on the contrary, was the only hormonal condition favoring somatic embryogenesis from cotyledon explants (Table 2). The somatic embryos appeared at the end of about 4 weeks without intermediary callus but on cotyledons which had considerably increased in volume (Fig.1a). The first somatic embryos began to form at the edge of cotyledon explants and later they appeared on the other zones. These embryos produced new somatic embryos when their settled medium parts were taken and transferred to MS medium without hormone (Fig. 1b). However, on these media, growth of the embryo root only took place, the plumula zone remaining «quiescent».

Embryo germination

Germination of bipolar somatic embryos formed on the above NAA (alone) solid medium were tested for germination on paper bridges in liquid media (Table 3) and on solid media (Table 4). In the former culture type, BA alone at 1.5 mg/l was the only hormonal condition to allow embryo germination but by 20% only (Fig. 1c). In the latter condition, the combination GA_3 + glutamine allowed 30% germination in about 4 weeks. After this time, embryo shoots reached 3 cm long (Fig. 1d). Note that a solid medium without growth regulator (Table 4) allowed 82% of the embryos formed to

Table 1. – Responses of immature zygotic embryos and cotyledon explants in *in vitro* liquid MS medium in the presence of different growth regulators.

Liquid Medium (MS)	***Zygotic Embryo***			***Cotyledon Explant***			
	Callus (%)	Plantlet development (%)	Somatic embryo (%)	Callus (%)	Somatic embryo (%)	Shoot (%)	Root (%)
2 mg/l Kin + 2 mg/l 2,4-D	0	0	0	60	0	0	0
2 mg/l 2,4-D	0	0	0	20	0	0	0
0.5 mg/l BA + 1 mg/l NAA	32	53	15	80	0	30	0
1.5 mg/l NAA	0	44	0	0	0	0	0

Table 2. – Responses of immature zygotic embryos and cotyledon explants on *in vitro* solid MS medium in the presence of different growth regulators.

Agar Medium (MS)	***Zygotic Embryo***			***Cotyledon Explant***			
	Callus (%)	Plantlet development (%)	Somatic embryo (%)	Callus (%)	Somatic embryo (%)	Shoot (%)	Root (%)
2 mg/l Kin + 2 mg/l 2,4-D	35	0	0	63	0	0	0
2 mg/l 2,4-D	20	60	0	76	0	0	0
0.5 mg/l BA + 1 mg/l NAA	72	28	0	74	0	0	0
1.5 mg/l NAA	15	0	0	5	85	0	35

Table 3. – Responses of somatic embryos on liquid MS media with different growth regulators, tested as germination-development media.

Nutrient Medium MS (20 g/l sucrose)	Somatic embryo germination (%)	Callus (%)	Forming new somatic embryo (%)
0.5 mg/l BA	0	0	0
1 mg/l BA	0	0	15
1.5 mg/l BA	20	0	10
2 mg/l BA	0	0	0
1 mg/l Kin + 0.1 mg/l 2.4-D	0	40	0

Table 4. – Responses of somatic embryos on solid MS media with different growth regulators, tested as germination-development media.

Nutrient Medium MS (35 g/l sucrose)	Somatic embryo germination (%)	Forming new somatic embryo (%)
Without Hormone	0	82
1 mg/l GA_3 + 600 mg/l L-Glutamin	30	27
2 mg/l BA	0	0

continue secondary embryogenesis. The plantlets grown from somatic embryos could be acclimatized in soil where they continued their development into normal plants.

Discussion

The attempt of AHMAD & al. (1992) to regenerate *Pistacia vera* through somatic embryogenesis from cell suspension cultures of mature plants had been unsuccessful. We succeeded to get somatic embryos of this plant using zygotic embryos sampled from immature fruits and culturing explants in liquid and solid media. Conditions for secondary embryogenesis have been determined as well as culture media allowing up to 30% of embryo germination and development into normal plantlets *in vitro* and *ex vitro*. This is an important achievement for the multiplication of this hard-to-root species and with an expected guarantee of genetic stability because very few deviations from genetical conformity have been shown in regeneration through somatic embryogenesis (OZIAS-AKINS & VASIL, 1982; AMMIRATO, 1983; THOMAS & SCOTT, 1985; TABAEIZADEH & al., 1990).

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