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In vitro embryo germination and proliferation of pistachio (*Pistacia vera* L.)

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ABSTRACT

In vitro development of isolated embryos and axillary bud proliferation were studied in *Pistacia vera* L. Different regulator-free nutrient media were compared to determine the effects of the mineral solution, agar and sucrose concentrations on seedling development from mature embryos. Nutrient-rich MS [Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tabacco tissue cultures. Physiol. Plant. 15, 473–479] and DKW [Driver, J.A., Kuniyuki, A.M., 1984. *In vitro* propagation of *Paradox walnut* rootstock. HortScience 19, 507–509] mineral solutions were more efficient for the development of aerial parts than nutrient-poor KN [Knop, W., 1884. Bereitung einer concentrierten nährstofflosung für pflanzen. Landwersuhssat 30, 292–294] and WT [Withe, P.R., 1936. Plant tissue cultures. Bot. Rev. 2, 419–437] solutions. Reducing the agar concentration enhanced fresh matter production and balanced seedling development. When tested at different concentrations, sucrose was found to orient mature embryo development, with the best results obtained at concentrations of 2–4%, whereas high concentrations (6 and 12%) mainly inhibited elongation of the aerial parts. Plantlets obtained previously from *in vitro* cultured embryos were propagated by axillary budding. High bud proliferation (six shoots per explant) was achieved when using 17.8 μM benzyladenine (BA) combined with 0.5 μM indole-3-butyric acid (IBA). The addition of 2.9 μM gibberellic acid (GA_3) to the propagation medium did not improve axillary shoot yields and on average, media with GA_3 produced 2.3–2.6 elongated stems per cultured explant. Shoots were rooted *in vitro* in half-strength MS medium containing 12.3 μM IBA.

1. Introduction

Pistachio (*Pistacia vera* L.) is a very economically and ecologically important fruit species. Due to its morphological qualities and physiological capacities, this drought-resistant multiuse tree species grows well in arid and semiarid areas of Algeria. It is grown for its fruit but also for its high-value forage, which generates income for local farmers.

Pistachio trees were introduced in Algeria at the beginning of the 1980s. Demonstration orchards were set up in different regions with the aim of promoting this crop. However, high quality plant production techniques must be implemented to ensure the successful extension of this species. Several authors have investigated ways to overcome problems encountered with conventional multiplication (grafting and cuttings) by focusing on developing more suitable propagation techniques. *In vitro* pistachio propagation techniques have been tested as an alternative to conventional propagation (Abousalim et al., 1991; Yang and Lüdders, 1993; Onay, 2000; Can et al., 2006). Some problems were encountered, however, especially involving contamination, tissue browning and a lack of reaction of the mature plant material.

Production of young shoots by the *in vitro* zygotic embryo germination technique should increase the success rate and markedly reduce the risks of *in vitro* contamination and phenolic oxidation often noted when micropropagating woody species.

An appropriate mineral composition in the culture medium (Tombolato and Monet, 1984; Sánchez-Zamora et al., 2006) and tailored sucrose supplies (Jay-Allemand and Cornu, 1986; Garcia et al., 2002) are essential for balanced complete *in vitro* embryo development. Good absorption of water and metabolites from the nutrient medium are necessary for this development. The concentration of agar, i.e. the most commonly used gelling agent, seems to have a direct effect on the nutrition of *in vitro* explant cultures (Gürel and Gülsen, 1998; Casanova et al., 2008).

The present study focused on assessing the effects of the mineral composition of the culture medium, its agar and sucrose

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concentration on *in vitro* development of isolated *P. vera* L. embryos.

The effects of different benzyladenine (BA) concentrations combined with indole-3-butyric acid (IBA) or gibberellic acid (GA_3) were tested on the *in vitro* induction and proliferation of axillary buds. We also report first results concerning *in vitro* rooting using a medium defined by Abousalim et al. (1991), for *P. vera* L.

2. Materials and methods

2.1. Plant material and sterilization methods

Naturally dried epicarp-free *P. vera* L. seeds were harvested in September 2007 in the El Fehoul orchards (Tlemcen, Algeria) and stored at room temperature (22–25 °C).

Seed coats were removed and the seeds were disinfected by soaking them for 2 min in 70% ethanol (v/v H_2O) and then for 10 min in an aqueous mercury chloride ($HgCl_2$) solution at 0.1% (w/v). Two 10 min rinses in a calcium chloride ($CaCl_2$) solution at 0.3% (w/v) were carried out in order to reduce toxicity. The seeds were then rinsed three times in sterile distilled water and left in the last rinse water overnight to increase cotyledon imbibition and facilitate embryonic axis removal.

Embryos were removed by opening the seeds with forceps and detaching the embryo axis from the cotyledon while taking care not to damage the embryo. Embryo axes were cultured in the different tested media.

2.2. Culture media and conditions

The studied culture media (MS, DKW, KN, and WT) differed with respect to their macroelement compositions, but they all contained the microelements and iron from Murashige and Skoog solution (1962), B_5 vitamins (Gamborg et al., 1968), myoinositol (100 mg l^{-1}), and casein hydrolysate (acid hydrolysed, Merck 2245, 500 mg l^{-1}).

These growth regulator-free nutrient solutions were compared in semisolid medium at 0.4% (w/v) or in solid medium at 0.8% (w/v) Difco Bacto-Agar. The sucrose concentration was modified according to the experimental conditions, with 3% (w/v) as the control. The culture medium pH was adjusted to 5.7 with KOH 0.1N prior to autoclaving at 113 °C for 20 min.

The embryos were sown in glass tubes (22 mm × 150 mm) containing 15 ml nutrient medium and placed in a culture room at 22 °C, with a 16:8 h (L/D) photoperiod at a light intensity of 90 μmol m^{-2} s^{-1} PAR (photosynthetic active radiation). After 30 days of culture, the following parameters were measured: germination, stem and taproot length, fresh weight of aerial parts and root system, and the mean number of leaves per developed embryo.

The shoots were then propagated by axillary budding. Previously obtained plantlets were cut into nodal segments each

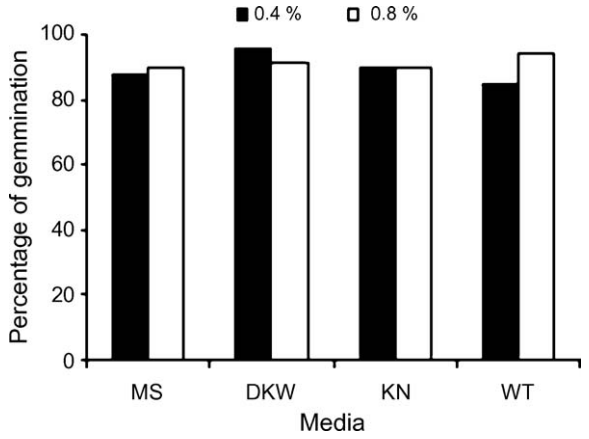

Fig. 1. Effect of the culture medium and its agar concentration (0.4 and 0.8%) on the percentage of germinated embryos after 1 month of culture ($n = 48$ per each treatment).

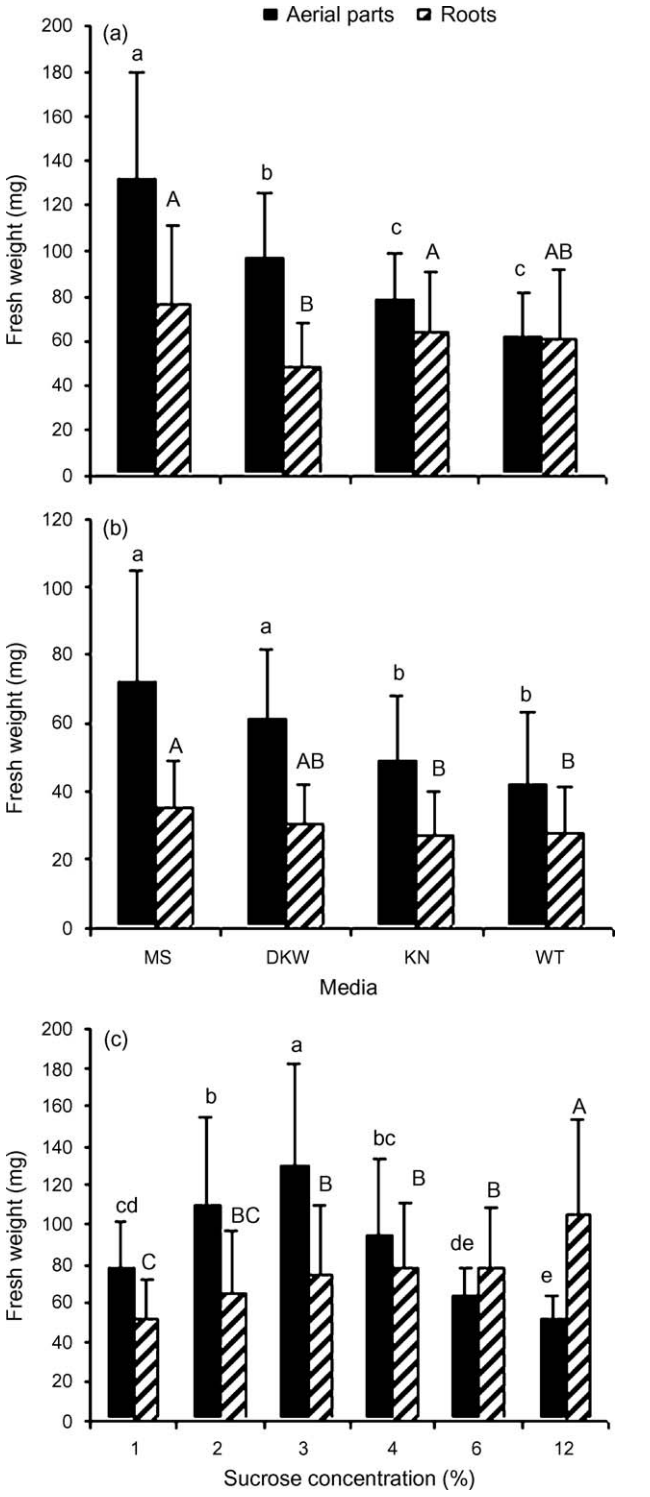

Fig. 2. Effect of the culture medium, agar concentration (a: 0.4%; b: 0.8%) and sucrose concentration (c) on the fresh weight of aerial parts and roots of seedlings after 1 month of culture. Bars with the same letter are not significantly different at $P < 0.05$.

Table 1
Effect of the culture medium and its agar concentration on the mean length of aerial parts and roots and the number of newly formed leaves per seedling after 30 days of culture. Values represent the mean ± SE of 30 explants per treatment. Values followed by the same letters within columns are not significantly different at $P < 0.05$.

	Length (cm)		Mean number of leaves per seedling
	Roots	Aerial parts	
Gelled medium (0.8%)			
MS	3.4 ± 1.8 bc	1.8 ± 0.5 a	7.6 ± 2.8 a
DKW	2.7 ± 1.3 c	1.8 ± 0.6 a	7.5 ± 2.3 a
KN	3.7 ± 1.9 ab	1.4 ± 0.5 b	6.1 ± 2.6 b
WT	4.3 ± 1.6 a	1.1 ± 0.4 b	5.0 ± 2.1 b
Gelled medium (0.4%)			
MS	4.0 ± 1.9 a	2.5 ± 0.8 a	9.3 ± 1.8 a
DKW	4.0 ± 1.8 a	2.6 ± 0.7 a	8.4 ± 1.6 ab
KN	4.8 ± 2.1 a	1.7 ± 0.5 b	7.5 ± 2.4 bc
WT	4.6 ± 1.9 a	1.3 ± 0.4 c	7.0 ± 1.6 c

containing at least one axillary bud. Different BA concentrations (4.4; 8.9; 17.8 and 26.6 μM) with or without IBA (0.5 μM) or GA_3 (2.9 μM) were studied. The basal nutrient medium that gave the best results during the first isolated embryo *in vitro* development phase was used. After 4 weeks of culture, the mean number of axillary shoots produced from primary explants and their qualitative features (colour, presence of callus...) were assessed.

Rooting of shoots was attempted through a two-step process. The shoots selected for rooting (1–2 cm long), were cultured on half-strength MS supplemented with 12.3 μM IBA. After 1 week, the shoots were transferred onto the same medium lacking hormone for root elongation. After 4 weeks of culture, the rooting percentage was recorded.

2.3. Statistical analysis

All results were analysed with a complete randomised design using Statgraphics Plus software. The results were tested using an analysis of variance (ANOVA) and for each significant difference, means were differentiated using Duncan's multiple-range test at $P < 0.05$. The results are expressed as mean ± standard error (SE).

3. Results

3.1. In vitro germination of isolated embryos

3.1.1. Effect of mineral solutions and agar concentration

No significant differences were noted in the efficiency of germination of embryo axes cultured in the different mineral solutions studied, which ranged from 85.4 to 95.8% on WT and DKW media, respectively (Fig. 1).

The quantitative and qualitative effect of the culture medium and its agar concentration was, however, noted at the end of the experiments. The mean fresh weight of seedlings cultured in gelled medium, at an agar concentration of 0.4%, was greater than in a solid medium at an agar concentration of 0.8% in all the nutrient solutions tested (Fig. 2a and b).

The results summarised in Table 1 also highlight the efficiency of the nutrient solutions on the development of isolated *P. vera* L.

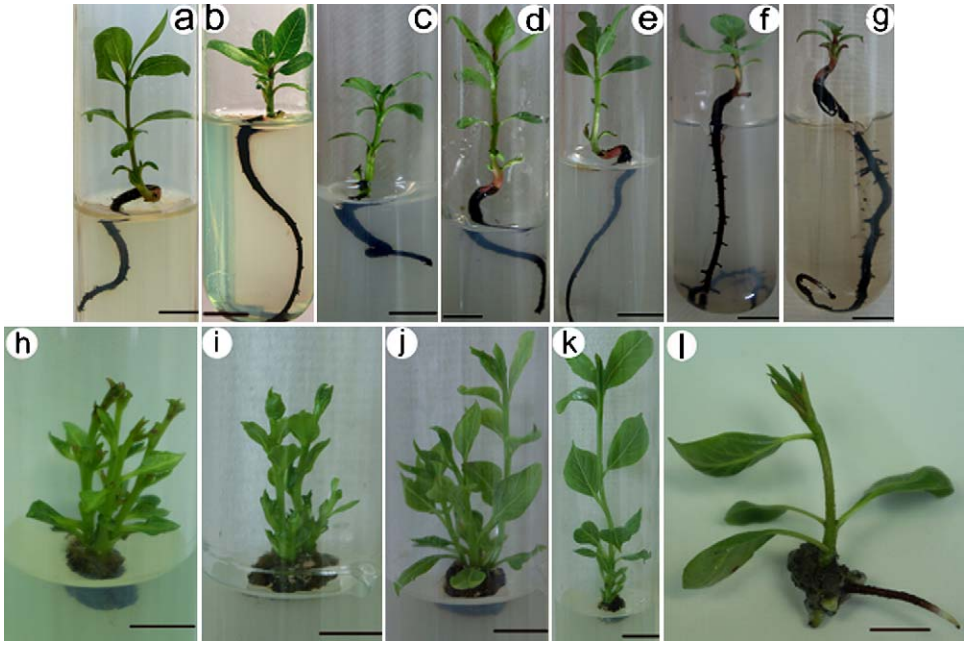

Fig. 3. *In vitro* culture of isolated embryos and proliferation of axillary buds from *Pistacia vera* L. *In vitro* embryo development on MS (a) and KN (b) solidified at 0.4% agar. (c–g) Effect of the sucrose concentration on the *in vitro* development of embryos isolated after 30 days of culture on the basic MS medium including 0.4% agar supplemented with sucrose at concentrations of 1% (c), 2% (d), 4% (e), 6% (f), 12% (g) and the control medium with 3% (cf. a). (h–k) *In vitro* proliferation of axillary buds on basic MS medium enriched with 8.9 μM (h), 17.8 μM BA (i), 17.8 μM BA + 0.5 μM IBA (j), 17.8 μM BA + 0.5 μM IBA + 2.9 μM GA_3 (k). Rooting of shoot after 30 days of culture on 1/2 MS + 12.3 μM IBA (l) (bar = 1.0 cm).

Table 2
Effect of the sucrose concentration on the mean length of aerial parts and roots and the number of newly formed leaves per seedling after 30 days of culture on the basic MS medium (hormone-free), solidified at 0.4% agar. Values represent the mean ± SE of 25 explants per treatment. Values followed by the same letters within columns are not significantly different at $P < 0.05$.

Sucrose (%)	Length (cm)		Mean number of leaves per seedling
	Roots	Aerial parts	
1	3.2 ± 2 c	1.8 ± 0.6 c	6 ± 2.3 c
2	3.5 ± 2 c	2.6 ± 0.9 a	7.2 ± 1.9 b
3	4.0 ± 2.1 bc	2.5 ± 0.8 ab	9.4 ± 2 a
4	5.9 ± 2.7 a	2.1 ± 0.8 bc	5.7 ± 2.1c
6	5.3 ± 2.5 ab	1.4 ± 0.3 d	5.4 ± 1.6 c
12	4.8 ± 3.4 ab	1.0 ± 0.2 e	1.9 ± 1.8 d

embryos. In nutrient-rich solid media (DKW and MS) with an agar concentration of 0.4%, better development of aerial parts was noted with high foliar organogenesis (Fig. 3a). The nutrient-poor media (WT and KN) promoted root development (Fig. 3b).

3.1.2. Effect of the sucrose concentration

The effects of the sucrose concentrations on the length of the aerial and root parts, as well as the foliar organogenesis of isolated embryos are presented in Table 2.

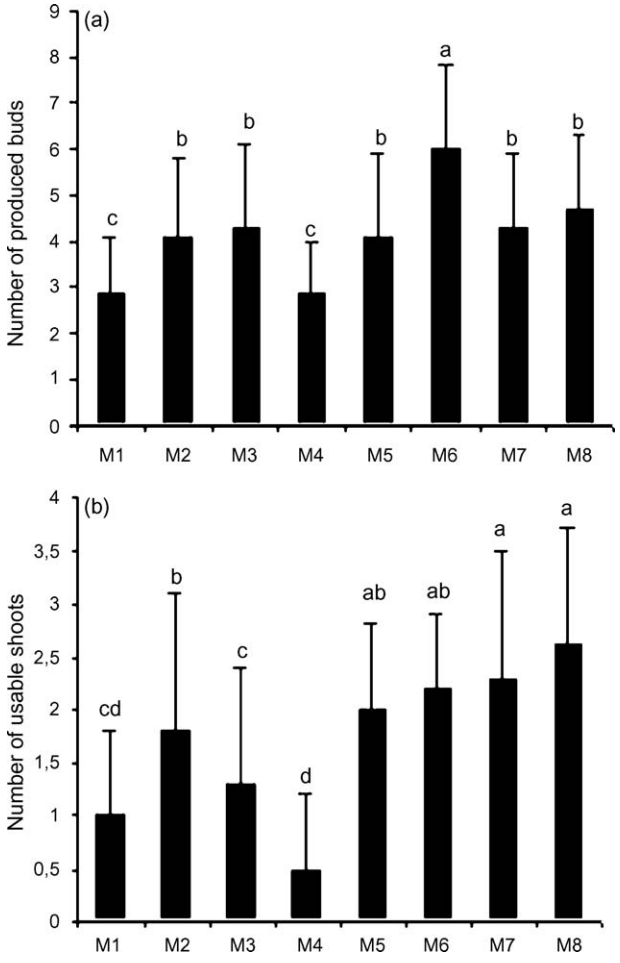

Fig. 4. Effect of the BA concentration combined or not with IBA (0.5 μM) or GA_3 (2.9 μM) on the mean number of produced buds (a) and extended axillary shoots (b) after 4 weeks of culture on Murashige and Skoog propagation medium (M1: 4.4 μM BA; M2: 8.9 μM BA; M3: 17.8 μM BA; M4: 26.6 μM BA; M5: 8.9 μM BA + IBA; M6: 17.8 μM BA + IBA; M7: 8.9 μM BA + IBA + GA_3; M8: 17.8 μM BA + IBA + GA_3). Bars with the same letter are not significantly different at $P < 0.05$.

Sucrose concentrations of 2–4% were more conducive to embryo axis development, thus enhancing the growth rate and organogenesis. At higher concentrations (6 and 12%), there was a decrease in stem elongation and leaf production. A low sucrose content (1%) slowed down embryo growth (Fig. 3c–g).

Growth inhibition of aerial parts was associated with fresh root matter production. The root fresh weight was significantly higher when the sucrose concentrations increased (Fig. 2c).

3.2. Effect of growth regulators on in vitro axillary shoot proliferation

The impact of different BA concentrations, with or without IBA or GA_3, was analysed in MS propagation medium. After 4 weeks of culture, BA stimulated bud formation at 8.9 and 17.8 μM (Fig. 3h–i). Low and high BA concentrations significantly reduced the mean number of burst axillary shoots, while having a negative impact on their subsequent development (Fig. 4a).

Joint application of BA and IBA had a positive effect on shoot proliferation. The mean number of vegetative axes was significantly higher in the presence of 17.8 μM BA combined with 0.5 μM IBA (Figs. 3j and 4a). The addition of GA_3 (2.9 μM) to the medium reduced the number of produced buds per plantlet but enabled us to obtain, on average, 2.3–2.6 extended microcuttings, some of which were over 2 cm long (Figs. 3k and 4b).

3.3. Rooting of shoots

After 4 weeks of a two steps culture, the formation of roots was observed in 23.3% of shoots. All rooted shoots had only one root and exhibited important callus development at their base (Fig. 3l).

4. Discussion and conclusion

In this study, we investigated *in vitro* embryo germination and proliferation of *P. vera* L. The results obtained showed that the enriched media DKW and MS induced homogeneous seedling development. However, nutrient-poor Knop and White media seemed to be more favourable for root system development. These results were similar to the findings of Tombolato and Monet (1984) who pointed out that enriched media are beneficial for fresh matter synthesis with respect to peach embryos. Abousalim et al. (1992) showed, however, that low ionic strength culture media promoted growth of *P. vera* L. plantlets micropropagated from seeds (embryos with their cotyledons). In our study, we used embryo axes (i.e. embryos without cotyledons) to assess the effects of the culture medium and the effects of the nutrient medium were noted on two essential external parts of embryos, i.e. radicle and gemmule.

The physical state of the medium has an effect on explants in culture, and reducing the agar concentration was beneficial to pistachio embryo responses. The total fresh weight of the embryos was found to be always higher in media where the agar content was reduced by half. Casanova et al. (2008) underlined the importance of the agar concentration with respect to *in vitro* organogenesis of *Dianthus caryophyllus* L. The best results were obtained with low agar concentrations, probably because at high agar concentrations extraction and absorption of nutrients from the culture medium is hampered.

Sucrose, when used at different concentrations, significantly oriented embryo axis development in *P. vera* L., with 2–4% being the most favourable for organogenesis of embryos from this species, while also ensuring good morphological features. Conversely, higher concentrations (6 and 12%) clearly slowed down plantlet growth. These results are similar to the findings of Thomas (1980) in *Pinus sylvestris* and Jay-Allemand and Cornu (1986) in *Juglans regia* L. These authors pointed out that the sucrose

concentration has an influence on the growth balance and mitosis location. They also specified that high sucrose concentrations, which induce high osmotic pressure, could reduce water and nutrient transport from roots to aerial parts.

The organogenesis potential of explants depends on several factors, especially the hormonal stimulus provided by the nutrient medium. High cytokinin concentrations generally promote proliferation of many buds (Tanuwidjaja et al., 1998). We also showed that the use of 8.9–17.8 μM BA concentrations significantly increased the number of axillary shoots produced per explant. Low and high concentrations had a negative impact on bud production and subsequent development. Our results are in agreement with those obtained by Barghchi and Alderson (1983) and Abousalim et al. (1991) in pistachio. Similar results were obtained in other woody species such as *Pinus pinaster* Sol (Rancillac, 1981), *Dalbergia sissoo* (Gulati and Jaiwal, 1996), *Annona muricata* L. (Lemos and Blake, 1996), *Irvingia gabonensis* (Omokolo Ndoumou et al., 2003), *Ricinodendron heudelotii* (Fotso et al., 2004) and *Annona glabra* L. (Deccetti et al., 2005).

In our experiments, it was necessary to use 0.5 μM IBA with 17.8 μM BA to maximise axillary shoot proliferation. Combining an auxin, especially IBA, with BA during the bud proliferation phase has been shown to be beneficial in several fruit species, including: *Juglans regia* L. (Driver and Kuniyuki, 1984; Sánchez-Zamora et al., 2006), *Annona muricata* L. (Lemos and Blake, 1996); *Ricinus communis* L. (Sujatha and Reddy, 1998); and *Jatropha curcas* L. (Sujatha et al., 2005). However, Chalupa (1984) showed that adding low auxin concentrations (IBA or ANA) did not have a significant effect on bud burst in *Quercus robur*. Moreover, Zobayed et al. (2002) noted that for *Annona* sp., adding an auxin to the propagation medium reduced the budding rate as compared to using BA alone.

The presence of GA_3 in the proliferation medium did not enhance bud yield in pistachio, as also observed in other woody species: *Ceratonis siliqua* L. (Sebastian and McComb, 1986), and *Annona muricata* L. (Lemos and Blake, 1996). The inhibitory effect of GA_3 on caulogenesis in many plant species has been reported by several authors (El Kbiach et al., 2002; Deccetti et al., 2005). According to George (1996), the effect of GA_3 on leafy shoot proliferation varies according to the interaction between other growth regulators. For a long time, auxin and GA_3 were thought to act through mainly independent mechanisms. Recently, interactions between these hormones have been clearly demonstrated (Hedden, 2004; Weiss and Ori, 2007). Auxin has a stimulatory effect on metabolites and can alter the GA_3 content, which is increased by synthesis stimulation. Thus supra-optimal GA_3 concentrations could be reached with auxin application, which would account for the reduction.

Abousalim et al. (1991) found that IBA at 12.3 μM is the optimum concentration for *in vitro* rooting of pistachio. In our experiments, the rooting percentage obtained was only 23.3% and shoots rooted were characterized by an abundant callus formation. A similar problem of callus formation was also mentioned by Ozden-Tokatli et al. (2005). These authors showed that the high callus production at the basal ends of the shoots with the high level of IBA inhibited rooting induction of *P. vera* L.

Trials carried out here on *P. vera* L. enabled us to determine the most suitable conditions for embryo axis development and *in vitro* shoot proliferation from them. Further studies on the rooting of this recalcitrant species are currently under way.

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References

Abousalim, A., El Mahboul, B., Walali, L.D., 1991. La multiplication *in vitro* de *Pistacia vera* L. Rev. Rés. Amélior. Prod. Agr. Milieu Aride 3, 73–79.
Abousalim, A., El Mahboul, B., Walali, L.D., 1992. Germination *in vitro* de graines et croissance de plantules de pistachier (*Pistacia vera* L.). Rev. Rés. Amélior. Prod. Agr. Milieu Aride 4, 17–23.
Barghchi, M., Alderson, P.G., 1983. *In vitro* propagation of *Pistacia vera* L. from seedling tissues. J. Hortic. Sci. 58, 435–445.
Can, C., Özaslan, M., Töremen, H., Sarpkaya, K., Iskender, E., 2006. *In vitro* micrografting of Pistachio, *Pistacia vera* L. var. Siirt, on wild pistachio rootstocks. J. Cell. Mol. Biol. 5, 25–31.
Casanova, E., Moysset, L., Trillas, M.I., 2008. Effects of agar concentration and vessel closure on the organogenesis and hyperhydricity of adventitious carnation shoots. Biol. Plant. 52, 1–8.
Chalupa, V., 1984. *In vitro* propagation of oak (*Quercus robur* L.) and Linden (*Tilia cordata* Mill.). Biol. Plant. 26, 374–377.
Deccetti, S.F.C., Paiva, R., Duarte De Oliveira, P., Aloufa, M.A.I., 2005. La micropropagation d'*Annona glabra* L. à partir de segments nodaux. Fruits 60, 319–325.
El Kbiach, M.L., Lamarti, A., Abdali, A., Badoc, A., 2002. Culture *in vitro* des bourgeons axillaires de Chêne-liège (*Quercus suber* L.). I—Influence des cytokinines sur l'organogenèse et la callogenèse de nœuds de plantules. Bull. Soc. Pharm. Bordeaux 141, 73–88.
Fotso, O., Donfagsiteli, T.N., Mbouna, D., Omokolo, N.D., 2004. Propagation de *Ricinodendron heudelotii* par bouturage *in vitro*. Fruits 59, 351–358.
Gamborg, O.L., Miller, R.A., Ojima, K., 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50, 151–158.
Garcia, J.L., Troncoso, J., Sarmiento, R., Troncoso, A., 2002. Influence of carbon source and concentration on the *in vitro* development of olive zygotic and explants raised from them. Plant Cell. Tissue Org. Cult. 69, 95–100.
George, E.F., 1996. Plant Propagation by Tissue Culture, Part I, The Technology, 2nd ed. Edington, Exigetics, UK, 1574 p.
Gulati, A., Jaiwal, P.K., 1996. Micropropagation of *Dalbergia sissoo* from nodal explants of mature trees. Biol. Plant. 3, 169–175.
Gürel, S., Gülsen, Y., 1998. The effect of different sugar, agar and pH levels on *in vitro* shoot production of Almond (*Amygdalus communis* L.). Tr. J. Bot. 22, 363–373.
Hedden, P., 2004. Gibberellin metabolism and its regulation. J. Plant Growth Regul. 20, 317–318.
Jay-Allemand, C., Cornu, D., 1986. Culture *in vitro* d'embryons isolés de noyer commun (*Juglans regia* L.). Ann. Sci. Forest 43, 189–198.
Lemos, E.E.P., Blake, J., 1996. Micropropagation of juvenile and mature *Annona muricata* L. J. Hortic. Sci. 71, 395–403.
Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tabacco tissue cultures. Physiol. Plant. 15, 473–479.
Omokolo Ndoumou, D., Fotso, O., Mbouna, D., 2003. Propagation d'*Irvingia gabonensis* par microbouturage *in vitro*. Fruits 59, 31–38.
Onay, A., 2000. Micropropagation of Pistachio from mature trees. Plant Cell Tissue Org. Cult. 60, 159–162.
Ozden-Tokatli, Y., Ozudogru, E.A., Akcin, A., 2005. *In vitro* response of pistachio nodal explants to silver nitrate. Sci. Hort. 106, 415–426.
Rancillac, M., 1981. Perspectives d'application des cultures d'organes *in vitro* à la multiplication végétative du pin maritime, *Pinus pinaster*. Sol. Ann. Sci. Forest. 38, 55–70.
Sánchez-Zamora, A., Cos-Terrer, J., Frutos-Tomas, D., Garcia-Lopez, R., 2006. Embryo germination and proliferation *in vitro* of *Juglans regia* L. Sci. Hort. 108, 317–321.
Sebastian, K.T., McComb, J.A., 1986. A micropropagation system for carob (*Ceratonia siliqua* L.). Sci. Hort. 28, 127–131.
Sujatha, M., Reddy, T.P., 1998. Differentiel cytokinin effects on the stimulation of *in vitro* shoot proliferation from meristematic explants of castor (*Ricinus communis* L.). Plant Cell Rep. 17, 561–566.
Sujatha, M., Makkar, H.P.S., Becker, K., 2005. Shoot bud proliferation from axillary nodes and leaf sections of non-toxic *Jatropha curcas* L. Plant Growth Regul. 47, 83–90.
Tanuwidjaja, C., Webb, D.T., Sagawa, Y., 1998. Micropropagation of Akia (Wikstroemia uva-ursi A. Gray). Plant Cell Tissue Org. Cult. 53, 85–90.
Thomas, M.J., 1980. Applications physiologiques de la culture d'embryons isolés. In: Connaissance de la biologie des arbres par la culture in vitro, 2ème réunion de la section française de l'AIPTC sur l'arbre in vitro, 14-15 nov., Paris.
Tombolato, A., Monet, R., 1984. Utilisation d'axes embryonnaires de pêcher pour apprécier l'effet d'un milieu nutritif minéral sur le développement des tiges ou des racines en culture *in vitro*. Agronomie 4, 927–931.
Weiss, D., Ori, N., 2007. Mechanisms of cross talk between gibberellin and other hormones. Plant Physiol. 144, 1240–1246.
Yang, Z., Lüdders, P., 1993. *In vitro* propagation of Pistachio (*Pistacia vera* L.). Gartenbauwissenschaft 59, 30–34.
Zobayed, S.M.A., Armstrong, J., Armstrong, W., 2002. Multiple shoot induction and leaf and flower bud abscission of *Annona* cultures as affected by types of ventilation. Plant Cell Tissue Org. Cult. 69, 155–165.