Improvement of Germination Efficiency of Interspecific Hybrids *Pistacia terebinthus* L. × *Pistacia vera* L.

P. Gercheva, A. Zhivondov and L. Nacheva
Fruit Growing Institute
Plovdiv
Bulgaria

D. Avanzato
CRA-Centro di Ricerca per
la Frutticoltura
Rome
Italy

Keywords: pistachio, transsexual form, embryorescue, stratification, perlite

Abstract

An isolated population of a rare transsexual form of *Pistacia terebinthus* L. was discovered in the Rhodopes Mountain in Bulgaria. The trees were studied by means of in vivo and in vitro methods and molecular markers which could allow preservation and use of the transsexual forms as rootstocks and eventually as a donor for monoeciousness in the pistachio hybridization programs. After conventional stratification a very low germination capacity of the seeds was established. The aim of the present research was to improve the germination efficiency of interspecific hybrids *P. terebinthus* × *P. vera*. We carried out in vitro experiments for obtaining plants of seeds from open pollination and purposeful crossings of monoecious forms of *P. terebinthus*. Two methods were tested – in vitro embryo rescue of mature and immature embryos and stratification in perlite after sterilization. The best efficiency of hybrid seed germination was achieved after stratification in perlite. Interspecific hybrids were developed. The survived plants were pricked in plastic pots and grown under black net in the open.

INTRODUCTION

In the summer of 2002 in the Rhodopi Mountain in Bulgaria was found a rare transsexual form of *Pistacia terebinthus* (Avanzato, 2003; Avanzato and Quatra, 2004). Later existence of a whole isolated population is determined. The trees were studied by means of in vivo and in vitro methods and molecular markers which will allow preservation and use of the transsexual forms as rootstocks and eventually as a donor for monoeciousness in the pistachio hybridization programs (Ghercheva et al., 2008; Buffa et al., 2009). The germination capacity of the seeds of *P. terebinthus* after conventional stratification is very low (Caloggero and Parera, 2000; Sfendiyaro and Ozeker, 2001; Ozden-Tokatli et al., 2007).

The aim of the present research was to improve the germination efficiency of interspecific hybrids *P. terebinthus* × *P. vera*.

MATERIALS AND METHODS

Conventional Methods

In mid-September the fruits obtained from open pollination and after sexual hybridization were washed with the aim of removing the mesocarp. The cleared seeds were dried in shade for 20 days, after which they were stored in a dry place until the end of October. At the beginning of November the seeds were mixed with clean sand at a ratio 1:3 (seeds:sand) and put in pots stored in a stratification room. Optimal sand humidity was maintained until the end of March, after which the percentage of germination was reported.

The seeds obtained from open pollination were directly planted in a seedling nursery in the open, imitating natural conditions. The percentage of germination was reported in spring.

In Vitro Embryorescue of Mature Embryos

P. terebinthus embryos (o.p.) were placed in culture in the middle of August, at the beginning of September and at the end of September. The mesocarp was removed from the seeds, after which the embryos were extracted from the hard nut shell using a scalpel.

Sterilization was performed in a laminar box by a standard procedure with 70% ethyl alcohol for 30 s, 5% calcium hypochlorite for 10 min, washing with sterile distilled water 3 times × 10 min. The embryos were cultivated on 7 variants of the nutrient media based on MS (Murashige and Skoog, 1962) with varying concentrations of carbohydrates and plant growth regulators.

In Vitro Embryorescue of Immature Embryos

P. terebinthus fruits (o.p.) were collected by 20-day intervals for a period of two months starting in the middle of June. The explant sterilization was conducted following the standard procedure – washing of the fruits with tap water for an hour, alcohol – 30 s, 5% solution of calcium hypochlorite for 5, 10 or 15 min, washing with sterile water three times for 1, 5 and 10 min.

The thus processed explants were plated on 10 variants of the nutrient media MS (Murashige and Skoog, 1962) with a high concentration of plant growth regulators and sucrose.

In each variant the fruits were placed either intact or after removing of the upper part of the mesocarp with open embryo.

The embryos were cultivated in a growth chamber at a temperature of 22±2°C and 16/8 h photoperiod (40 μmol m^{-2} s^{-1} PPFD).

Stratification in Perlite

Seeds from ripen fruits collected at the end of September with removed mesocarp were sterilized by the above described procedure. Preliminary sterilized glass jars used for in vitro cultivation of plants were half filled with sterile perlite. The mixture was wetted with sterile water it a laminar box. The sterilized pistachio seeds were put in the jars and mixed with the wet perlite. The jars were wrapped in polyethylene folio and stored in a refrigerator at 4°C for 3 months. After stratification in sterile perlite the *P. terebinthus* seeds obtained by open pollination and by crossing with *P. vera* were taken out and seeded in a peat-perlite mixture at a temperature of 22°C and exposed to light of 16/8 h photoperiod (40 μmol m^{-2} s^{-1} PPFD).

RESULTS AND DISCUSSION

Conventional Technology

In order to establish the germination capacity of *P. terebinthus* seeds when using the conventional technologies, thousands of seeds obtained by open pollination were gathered and planted following the methods described. After stratification, as well as after direct planting in soil, a very low seed germination capacity was established. In both cases only single plants were obtained from the hundreds of planted seeds. Thus, alternative forms for obtaining plants were looked for. In order to achieve the experimental aims, we carried out in vitro investigations for obtaining plants by open pollination and purposeful crossings of monoecious forms of *P. terebinthus*.

In Vitro Embryorescue of Mature Embryos

The results from the experiments for in vitro cultivation of mature embryos showed that the major problem was the sterilization of the plant material. Different periods of sterilization from 5 to 15 min were tested, with and without preliminary treatments with ethyl alcohol, but in all the variants fungi infection of extremely vigorous development was established in 50 to 90% of the tubes, which covered the embryos for a day or two. That necessitated during the next years to test sterilizing agents and/or sterilizing systems with the aim of optimizing the process and increasing the efficiency of embryo cultivation.

The experiments carried out showed that the basic factor for in vitro embryo germination was the period of isolating the fruits. The embryos in the fruit seeds isolated in August were well developed, fresh and filling up the whole seed. Placed on the nutrient medium, they quickly developed a stem and a root. The plants obtained in that way were transferred on nutrient media for multiplication and were successfully propagated and cloned under in vitro conditions.

In Vitro Embryorescue of Immature Embryos

The fruits and stones of *P. terebinthus* are small and the extraction of the embryos from the hard nut shell is extremely difficult and time consuming. To avoid this problem we started experiments with immature embryos from green fruits.

After 20-30 days of cultivation most of the embryos enlarged, some of them became light green and formed embryoaxes, but up to now we have not obtained normal plants using this approach (Fig. 1).

Stratification in Perlite

After three-month stratification in sterile perlite more than half of the seeds had cut open to show a developed embryo rootlet. Two weeks later about 70-80% of the seeds germinated. Reaching the height of 3-4 cm the plantlets were pricked in plastic pots and grown under black net in the open (Table 1).

CONCLUSIONS

Alternative forms for obtaining plants (embryo rescue and stratification in perlite) were successfully used to improve the germination efficiency of interspecific hybrids *P. terebinthus* × *P. vera*.

The critical points for in vitro embryo rescue of *P. terebinthus* are the sterilization of the plant material and the season of isolating the fruits.

The best efficiency of hybrid seed germination was achieved after stratification in perlite.

ACKNOWLEDGEMENTS

This research is a part of the project CC16-07/2006, supported by National Science Fund, Ministry of Education and Science, Bulgaria.

Literature Cited

Avanzato, D. 2003. Una forma monoica di *Pistacia terebinthus* L. scoperta in Bulgaria. Frutticoltura 10:51-53.

Avanzato, D. and Quatra**,** R. 2004. Monoecious *Pistacia terebinthus* found in Bulgaria. Crop Wild Relative 2:14-16.

Buffa, R., Caruso, T., Marra, F.P., Vaccaro, A., Avanzato, D., Meli, M. and Zhivondov, A. 2009. Phenological and morphological studies of *Pistacia terebinthus* L. genotypes native of Bulgaria with different asset of tree sexuality. Acta Hort. 825:63-70.

Caloggero, S. and Parera, C. 2000. Improver germination and emergence of *Pistacia* by presowing chemical scarification. Seed Science and Technology 28(2):253-260.

Gercheva, P., Zhivondov, A., Nacheva, L. and Avanzato, D. 2008. Transsexual forms of pistachio (*Pistacia terebinthus* L.) from Bulgaria: biotechnological approaches for preservation, multiplication and inclusion in selection programs. Bulgarian Journal of Agricultural Science 14(5):449-453.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and biossays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Ozden-Tokatli, Y., Ozudogru, E.A., Gumusei, F. and Lambardi, M. 2007. Cryopreservation of *Pistacia* spp. seeds by dehydration and one-step freezing. Cryo Letters 28(2): 83-94.

Sfendiyarolu, M. and Ozeker, E. 2001. The relations between phenolic compounds and seed dormancy in *Pistacia* spp. Proceedings of XI GREMPA Seminar on pistashios and almonds. Turkey.

Tables

Table 1. Number of *P. terebinthus* plants and *P. terebinthus* × *P. vera* hybrids in the nursery in 2008; (t) – transsexual form.

Parent combinations	Plants obtained by:	Number of plants
♀Tree № 8 × ♂ Tree № 2 (transsexual)	Conventional stratification	1
♀Tree № 6 × ♂ *P. vera* 'Greco'	Stratification in perlite	25
♀Tree № 8 × ♂ Tree № 2 (transsexual)	Stratification in perlite	29
P. terebinthus – o.p.	Stratification in perlite	20
P. terebinthus – o.p.	Conventional stratification	33
Total number of plants		108

Figures

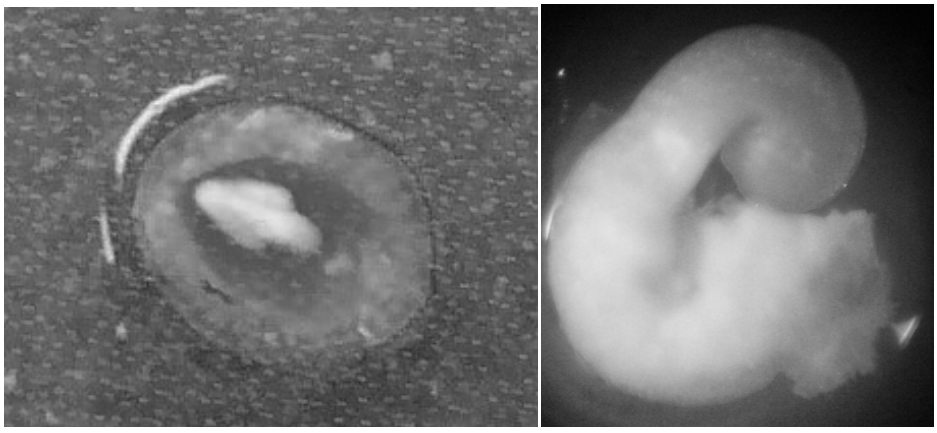

Fig. 1. Immature *Pistacia terebinthus* embryos from green fruits isolated in mid-June.