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TRANSSEXUAL FORMS OF PISTACHIO (*PISTACIA TEREBINTHUS* L.*)* FROM BULGARIA – BIOTECHNOLOGICAL APPROACHES FOR PRESERVATION, MULTIPLICATION AND INCLUSION IN SELECTION PROGRAMS

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Abstract

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The present article includes a detailed survey of the application of the biotechnological approaches to the multiplication and study of the Pistacia (pistachio) – nut fruit crop not widespread in Bulgaria, which economic significance in Mediterranean region and around the world grows continuously. The main reason for the development of the culture is the high nourishing value of the fruits, the existence of wild species and the potential for inter- and interspecies hybridization and mainly the exceptional drought resistance of the kind which allows its cultivation in soils that are under no irrigation, small-productive, stony and sliding (pliant to erosion) soils.

The *Pistacia* genus (*Anacardiaceae*) includes 12 deciduous species, all of which are trees. These species are dioecious, anemophylic and have their male and female flowers on separate trees. Among them, only *P. vera* is domesticated. For the industrial plantations of pistachio there should be plant a male tree for every 8-11 female trees, thus 10 % from the pistachio plantation is non-productive.

In the summer of 2002, Dr. D. Avanzato found a rare transsexual form of *P. terebinthus* in the Rhodopi Mountain. Later existence of a whole isolated population of these trees is determined. The discovered trees are the first case of a defined transsexual form among *P. terebinthus.*

The study of the area where this transsexual form of *Pistacia terebinthus* was found and the common application of *in vivo* and *in vitro* methods for propagation and molecular markers will allow its preservation and use as a rootstock and as a donor for monoeciousness in the pistachio hybridization programs.

Key words: pistachio, monoecious forms, *in vitro,* micro propagation
Abbreviations: BAP - 6-benzylaminopurine; IBA - indole-3-butyric acid; NAA - a-naphthaleneacetic acid; IAA - indole-3-acetic acid

The economic importance of *Pistacia* (pistachio) in the Mediterranean region and around the world is continuously growing. For the last 20 years the world production of nuts from pistachio has increased 5.4 times, only in Iran that increase is 8.7 times, in the USA – 7.8 times. The main reason for that develop-

ment is the high nourishing value of the fruits, the existence of wild species, the potential for in inter- and interspecies hybridization and mainly the astonishing drought resistance of the species that allows its cultivation in soils that are under no irrigation, small-productive, stony and sliding (pliant to erosion) soils (Kaska, 2002).

The pistachio grows mainly in regions between 30 and 45 degree of latitude in the northern and southern hemisphere and some other microclimatic zones in the different continents where there are suitable ecologic conditions. It is from the *Pistacia* genus (*Anacardiaceae*) that includes more than 12 types of deciduous trees (Zohary, 1952). All of them are dioecious, anemophylic and have their male and female flowers on separate trees. Among them only *P. vera* is domesticated, while *P. integerrima*, *P. atlantica*, *P. terebinthus,* etc. have economic application as rootstocks for the cultivars from *P. vera.*

Pistacia terebinthus has significant importance and is widely spread in the whole Mediterranean region – Italy, Greece, Turkey, Tunisia, Syria, etc., as a wild species and a rootstock in the plantations. The same species is a tree with thick crown and height up to 8 m. It has long, reddish and smooth shoots which change to an ash colour at maturity. The plants grow well in all types of soil, including rocky areas because of their drought resistance. *P. terebinthus* crosses easily and spontaneously with *P. vera* and the hybrids have economic significance as pollen donors for pistachio crops (Avanzato and Quatra, 2004). The female racemes of *P. terebinthus* are relatively short, thick clusters with huge number of small flowers. The male racemes (rachises) during the florescence achieve up to 80 mm. The florescence begins from the middle of the rachis and goes to the top and the base. It takes place in the second half of April.

As we already mentioned, all plants from the Pistacia genus are dioecious, i.e. the male and female racemes are formed in different trees (Zohary, 1952). For industrial plantations of pistachio it is necessary a male tree to be plant for every 8-11 female ones (Maranto and Crane, 1982), hence 10 % of the pistachio plantation is not productive. The eventual creation of cultivars where the male and female racemes are in one tree and it has a high crop of quality fruits would make unnecessary the need male trees to be grown in the plantations and thus it could increase the crop with about 10 %. To achieve that goal it is necessary to find a donor of genes for monoeciousness in the pistachio. Until recently there were only three reports of such trees in the literature.

Ozbek and Ayfer (1958) have found two hermaphrodites, probably hybrid trees of *P. vera* or hybrids of *P. vera* and *P. terebinthus* in Turkey, where the male and female organs are in one and the same blossom.

The second report is that of Crane (1974), where he describes three trees, hybrids between *P. vera* and *P. atlantica*, where the male and female flowers are on separate shoots.

Kafkas et al. (2000) have reported of found wild population of *P. atlantica* that consisted of several monoecious trees in Yunt Mountains in Turkey. The authors used the found forms in experiments of inter- and interspecies hybridization for creation of monoecious cultivars of pistachio and for clarification of the mechanism for determination of the sex in the species from *Pistacia* genus (Kafkas, 2002).

In the summer of 2002 during a visit to the Rhodopes Dr. D. Avanzato found a unique transsexual form of *P. terebinthus* (Avanzato, 2003; Avanzato and Quarta, 2004), and in subsequent common expeditions with Dr. Argir Zhivondov the existence of an isolated population of such trees was discovered. The transsexual forms have the classical habitus of *P. terebinthus*. The first found tree is about 50 years old, and the rest ones are significantly younger. The only determined difference is in the structure of the racemes (Buffa et al., 2007). The plants have both separate male and female racemes, situated on one-year wood, and male and female racemes in one and the same raceme. The found plants are first case of defined transsexuality of *P. terebinthus*. This fact has unusual scientific and practical importance.

From theoretic point of view it is a rare case of defined monoeciousness among a typically dioecious species. From the fact that such form is found in a

small isolated population of *P. terebinthus* can be assumed that monoeciousness can be inherited in the generation of dioecious female plants. The comparison of monoecious and dioecious plants and their hybrids by molecular markers could contribute to the clarification of the mechanism for inheritance of that feature.

From other point of view the discovery of these unique forms has an important economic significance, too. These forms give an opportunity for obtaining interspecies hybrids with cultivars of *P. vera* and transfer of that feature.

The achievement of such ambitious task is possible only if there is a successful elaboration and application of biotechnological and molecular-genetic methods in the multiplication and selection of the species.

The use of tissue cultures for multiplication of the species from Pistacia genus becomes increasingly significant in the last 20 years (Martinelli and Loretti, 1988; Bargchi et al., 1989; Gonzales and Frutos, 1990; Mehlenbacher, 2003).

There are reports of successful experiments of micropropagation of *P. terebinthus* and *P. vera* where the oxidation of the media is controlled by addition of ascorbic acid, and the most successful introduction of culture is achieved at one-month-old seedlings (Gannoun et al., 1995).

The main limiting factor in the introduction and stabilization of in vitro culture for many trees, as well as the pistachio, is the browning of the explants as a result of the increased content of phenols. Tabiyeh et al. (2006) report of decrease in the oxidation and improvement of the growth by dipping the end of the explants in 0.1 mM glutathione before their setting in nutrient media.

Sheibani and Villiers (1995) ascertain that the optimum media for propagation of *P. vera, P. terebinthus* and *P. mutica* is MS (Murashige and Skoog, 1962) with 5 mg/1 BAP, and for rooting – MS with 5 mg/l IBA.

Other authors (Ghorbani et al., 2002) recommend for *in vitro* rooting of *P. vera* DKW media with addition of 2 mg/l IBA and 0.01 mg/l NAA.

In *in vitro* propagation of wild species from genus Pistacia *Behboodi* (2002) examines the influence of different growth regulators. The best results are achieved in the use of BAP and NAA. The results for *P. vera* cv. Mateur are analogous – BAP (2.0 mg/l) stimulates adventitious regeneration of shoots (Chatibi et al., 1998a). Ghoraishi (2006) also mentions that BAP in concentration 20 μM is the most efficient in the micropropagation of *P. mutica*. The author mentions that treating with 100 μM IBA for 5 days significantly improves the rooting of the microplants.

Special attention is drawn to the optimization of the salt content of the nutrient media for getting over the browning of the shoot tip, the existence of red pigmentation on the leaves and improvement of the vitality of the plants (Chatibi et al., 1998b).

Ultrastructure analysis is used for characterization of the shoot-tip necrosis at *in vitro* cultivation of *P. vera* (Abousalini and Mantell, 1995). Damages of the plasmalemma, desintegration of the membranes and organelas and cell autolysis are observed.

In order the propagation to be accelerated *in vitro* techniques based on the somatic embryogenesis are elaborated (Onay, 2000). Development of somatic embryos from leaf explants (Onay, 2000), immature embryos and female flowers (Onay et al., 2004) of *P. vera* is achieved. Regeneration from embryoids and embryogenic masses (Onay et al., 1996) is reported. The influence of the species and the concentration of growth regulators, included in the nutrient media over the formation and growth of the somatic embryos and the germination of the plants is examined (Onay et al., 2004).

Different types of molecular markers are used to study genetic diversity in the genus of Pistacia – isozymes, RELP, RPD, etc. (Mehlenbacher, 2003, Vendramin at al., 2007). Their application for examining the philogenetic and taxonomic connections among the species is very important.

In respect to the selection, markers for defining the sex of the young plants long before they achieve reproductive age are in elaboration (Hormaza et al., 1994; Kafkas et al., 2001).

In conclusion the study of the area of the rare trans-

sexual form of *Pistacia terebinthus* and the common application of in vivo and in vitro methods for propagation and molecular markers will allow its preservation and use as a rootstock and donor for monoeciousness in the selection programs of the pistachio.

The potential for transfer of the monoeciousness feature to cultivars of *P. vera* can allow growing of pistachio plantations without the necessary planting of non-productive male trees for pollination and thus at least 10 % increase of the crop can be achieved.

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