A project on developing monoecious pistachio (*Pistacia vera* L.) populations and determination of sex mechanism in *Pistacia*

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SUMMARY – Exceptional wild *P. atlantica* populations with several monoecious trees were recently found in the Yunt Mountains of the Manisa province of Turkey. This project aims to develop monoecious *P. vera* cultivars, and to determine sex mechanism and inheritance in *Pistacia* by inter- and intra-specific hybridization using these trees. Two monoecious *P. atlantica* genotypes were used both as male and female parents: One is "fully monoecious" i.e., they bore a mixture of male and female inflorescences. Another genotype has several branches with only staminate flowers, while the rest of the branches bore pistillate inflorescences. One *P. atlantica* genotype and two *P. vera* cultivars ('Ohadi' and 'Siirt') were used as other female parents. One *P. atlantica* and one *P. vera* genotypes were used as male parents as well. Altogether, 20 intra- and inter-specific crosses between 5 female and 4 male genotypes were performed. Four of the crosses will mainly serve to breed monoecious *P. vera* genotypes and the rest of them will help to understand sex inheritance mechanisms in *Pistacia*. The crosses between *P. atlantica* genotypes were done in the spring of 2001 and the hybridizations between *P. vera* and monoecious *P. atlantica* genotypes were performed in the spring of 2002. The plants of 2001 crosses are now one year old and they were morphologically characterized. The seeds from 2002 crosses were germinated and transplanted into plastic bags. The orchard will be established with approximately 3000 plants. About 1500-1600 plants are hybrids between *P. vera* cultivars and monoecious *P. atlantica* genotypes and the rest of them are from the other combinations. The breeding orchard will be set up in the Pistachio Research Institute in Gaziantep province with drip irrigation in the autumn of 2003.

Key words: Monoecious, *Pistacia vera*, breeding, sex mechanism, *P. atlantica*.

RESUME – *"Projet sur le développement de populations monoïques de pistachier (*P. vera *L.) et détermination du mécanisme sexuel chez* Pistacia*". Des populations sauvages exceptionnelles de* P. atlantica *possédant plusieurs arbres monoïques ont été récemment trouvées dans les montagnes de Yunt de la province de Manisa en Turquie. Ce projet vise à développer des cultivars monoïques de* P. vera*, et à déterminer le mécanisme et l'hérédité sexuels chez* Pistacia *par hybridation inter et intra-spécifique en utilisant ces arbres. Deux génotypes monoïques de* P. atlantica *ont été utilisés comme parents mâles et femelles à la fois : l'un était pleinement monoïque, c'est-à-dire qu'il portait un mélange d'inflorescences mâles et femelles. Un autre génotype possédait plusieurs branches avec seulement des fleurs à étamines, tandis que le reste des branches portait des inflorescences à pistil. Un génotype de* P. atlantica *et deux cultivars de* P. vera *('Ohadi' et 'Siirt') ont été utilisés comme autres parents femelles. Un génotype de* P. atlantica *et un génotype de* P. vera *ont été utilisés comme parents mâles également. Au total, 20 croisements intra et inter-spécifiques ont été réalisés entre 5 génotypes femelles et 4 génotypes mâles. Quatre de ces croisements serviront principalement à sélectionner des génotypes monoïques de* P. vera *et le reste d'entre eux servira à comprendre l'hérédité et le mécanisme sexuels chez* Pistacia*. Les croisements entre génotypes de* P. atlantica *ont été faits au printemps 2001 et les hybridations entre les génotypes de* P. vera *et les génotypes monoïques de* P. atlantica *ont été réalisées au printemps 2002. Les plants des croisements 2001 ont maintenant un an d'âge et ils ont été caractérisés morphologiquement. Les semences des croisements 2002 ont germé et ont été transplantées dans des sachets en plastique. Le verger sera établi avec quelque 3000 plants. Environ 1500-1600 plants sont des hybrides entre des cultivars de* P. vera *et des génotypes monoïques de* P. atlantica*, et le reste proviennent d'autres combinaisons. Le verger d'amélioration sera installé à l'Institut de Recherches sur le Pistachier dans la province de Gaziantep avec irrigation localisée à l'automne 2003.*

Mots-clés : *Monoïque,* Pistacia vera*, amélioration, mécanisme sexuel,* P. atlantica.

Introduction

All *Pistacia* (Anacardiaceae) species are dioecious (Zohary, 1952). Male and female flowers are apetalous, and wind is the pollinating agent. Because of the natural occurrence of male *Pistacia* trees

in nearby forests, in Turkey, some growers do not appreciate the importance of male trees in the orchard, and obtain lower yields. For commercial pistachio orchards, approximately one male tree is needed for 8-11 females (Maranto and Crane, 1982). As a result, 10% of a typical pistachio orchard is not productive.

In the literature, only three cases of individual *Pistacia* trees of exceptional sex types were reported. Firstly, Özbek and Ayfer (1958) found two hermaphrodite trees (with male and female organs borne in the same flowers) in the vicinity of the Antep province of Turkey. They estimated that these trees were either seedlings of *Pistacia vera* L., or hybrids between *P. vera* and *P. terebinthus.* The second report (Crane, 1974) described three trees with exceptional phenotypes: (i) a branch bearing staminate flowers on an female tree of *P. atlantica*; (ii) a hybrid between *P. vera* and *P. atlantica* bearing approximately equal numbers of pistillate and staminate inflorescences, mostly on separate branches; and (iii) a similar hybrid, predominantly staminate, but with several branches bearing pistillate inflorescences.

Recently, Kafkas *et al.* (2000) reported an exceptional wild population of *P. atlantica* with a few monoecious trees found in the Yunt Mountains of the Manisa province of Turkey. The distribution of staminate and pistillate inflorescences was observed during two years and varied between trees. All branches of one of the trees were "fully monoecious", i.e. they bore a mixture of male and female inflorescences. Three trees had several branches with only staminate flowers, while the rest of the branches bore pistillate inflorescences. Five other trees had inflorescences of both sexes on several branches, and pistillate inflorescences on the remaining branches. The authors tested pollen germination of monoecious trees and used them for pollination with *P. vera*. The pollen germination rates were similar with normal male trees of *P. atlantica* and the hybridization with *P. vera* resulted normal fruit set and viable seed production. Therefore, large scale of crosses was planned and has been performed with the current project to develop monoecious pistachio cultivars and to understand sex mechanism in *Pistacia*. In a further study, this study will also have populations that will be used to clone the gene(s) that are responsible for sex determination in *Pistacia*. This project firstly presented in the 3rd Pistachio and Almond Symposium in Zaragoza and published in Acta Horticulture (Kafkas, 2002) by giving very preliminary results. In this paper, progresses in the project during last two years are given.

Materials and methods

A group of monoecious trees found by Kafkas *et al.* (2000) in Koruköy village in the Manisa province of Turkey are main materials of this study. Two types of monoecious *P. atlantica* genotypes were used: (i) a fully monoecious tree (Type I), i.e. all branches bore a mixture of male and female inflorescences; and (ii) a tree have several branches with only staminate flowers, while the rest of the branches bore pistillate inflorescences (Type II). The monoecious *P. atlantica* genotypes and two female *P. vera* cultivars (cvs 'Siirt' and 'Ohadi') with a dioecious *P. atlantica* tree grown in the Yunt Mountains are used as female genotypes. The monoecious *P. atlantica* genotypes and dioecious male *P. vera* and *P. atlantica* trees are used as pollinators. In total, twenty different crosses were performed between five female and four male parents.

Before the stigma becomes receptive, female inflorescences were bagged with a paper bag. To collect pollen for artificial pollination, staminate branches of four pollinators in this project were cut and put in water-containing cap in the laboratory to perform pollen tests and cross-pollination. In the following two days, pollen was collected and stored at -20°C until pollination. Before pollination, pollen aliquots were taken for viability and germination tests. Wheat flour was used as a mixing agent with pollen at a ratio of 1:1.

The harvested nuts were stratified for 60 days and germinated. Germinated plantlets were transferred to the 5-liter plastic bags. The plants will be transplanted to the orchard in the autumn of 2003 and drip irrigation will be set up.

Besides cross pollination study, the parents used in this study were described morphologically. The crosses between *P. atlantica* genotypes and four pollinators were also characterized. The distribution of male/female branches or inflorescences in the monoecious trees was followed, year-by-year, by labelling them within the tree as well.

When the plants will be reach the reproductive stage, the progenies will be tested for their sex type, and will be used as breeding parents to try and obtain monoecious cultivated pistachio. The segregation populations will also be used for molecular studies to clone gene(s) that are responsible for sex determination in *Pistacia*.

Results and discussion

The cross pollination study was done during three years (2001, 2002 and 2003). In 2001, the crosses between *P. atlantica* and four pollinators, in 2002 the crosses between *P. vera* and pollinators and missing crosses were performed. In 2003, additional crosses and missing ones were done as well.

In the first year (2001), all the crosses were actually performed in Manisa province. But we had some problems in the combinations between *P. vera* cultivars and four pollinators by not getting any nuts. Therefore we planned to perform that crosses in Gaziantep Pistachio Research Institute located 1200 km far from Manisa province. The pollens were transported to Gaziantep province in dry ice in 2002 and in 2003.

Table 1 shows targeted and current plant numbers. In this project, we aimed to obtain 500-700 plants from the crosses between *P. vera* cultivars and monoecious Type I, 100-200 plants from the crosses between *P. vera* cultivars and monoecious Type II, 50-100 plants from rest of the crosses. Totally it is targeted to have 2000-3400 plants in the project (Table 1). Currently, we almost completed all the combinations and we have in total 2855 plants in the plastic bags. We have only several missing plants in the crosses between *P. vera* cv. 'Ohadi' and *P. atlantica*, *P. vera* and monoecious *P. atlantica* Type II (Table 1). However, we also did additional crosses in this year (2003) to increase the plant numbers especially between *P. vera* and monoecious genotypes.

Table 1. Targeted and current plant numbers in the crosses

No	Female		Male	Targeted plant numbers	Current plant numbers
1	*P. atlantica*	X	M-PA18 (Type I)	50-100	108
2	*P. atlantica*	X	M-PA13 (Type II)	50-100	108
3	*P. atlantica*	X	*P. vera*	50-100	108
4	*P. atlantica*	X	*P. atlantica*	50-100	108
5	M-PA18 (Type I)	X	M-PA18 (Type I)	50-100	106
6	M-PA18 (Type I)	X	M-PA13 (Type II)	50-100	58
7	M-PA18 (Type I)	X	*P. vera*	50-100	50
8	M-PA18 (Type I)	X	*P. atlantica*	50-100	101
9	M-PA13 (Type II)	X	M-PA18 (Type I)	50-100	70
10	M-PA13 (Type II)	X	M-PA13 (Type II)	50-100	52
11	M-PA13 (Type II)	X	*P. vera*	50-100	54
12	M-PA13 (Type II)	X	*P. atlantica*	50-100	98
13	*P. vera* cv. 'Ohadi'	X	M-PA18 (Type I)	500-700	630
14	*P. vera* cv. 'Ohadi'	X	M-PA13 (Type II)	100-200	90
15	*P. vera* cv. 'Ohadi'	X	*P. vera*	50-100	43
16	*P. vera* cv. 'Ohadi'	X	*P. atlantica*	50-100	47
17	*P. vera* cv. 'Siirt'	X	M-PA18 (Type I)	500-700	574
18	*P. vera* cv. 'Siirt'	X	M-PA13 (Type II)	100-200	243
19	*P. vera* cv. 'Siirt'	X	*P. vera*	50-100	102
20	*P. vera* cv. 'Siirt'	X	*P. atlantica*	50-100	105
	Total plant numbers			2000-3400	2866

A pistachio variety that has both male and female inflorescences and hermaphrodite flowers in a single tree, together with high yield of quality nuts, may eliminate the necessity of male trees in the orchard and increase the yield per hectare by about 10%.

Sex determination mechanisms in plants are diverse, and may involve either sex chromosomes or individual sex gene(s). In *Pistacia*, the genetic mechanism of sex determination is still unknown. A better approach to understand how sex determination operates in a dioecious and monoecious species is to study differences at the DNA level. When the plants will be reach the reproductive stage, the progenies will be tested for their sex type, and will be used for molecular studies to clone gene(s) that are responsible for sex determination in *Pistacia*

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