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Monoecious *P. atlantica* trees in the Yunt Mountains of Manisa province of Turkey

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SUMMARY – *Pistacia* species are dioecious plants. Here we report on a few exceptional monoecious plants of *P. atlantica* found in the Yunt Mountains of Manisa province of Turkey. Distribution of staminate and pistillate inflorescences on the trees was observed during two years. One of them, PA18, was fully monoecious. Within three trees, PA6, PA13 and PA14, had several branches with only staminate flowers in different parts of the trees while the rest of the branches bore pistillate inflorescences. Five trees (PA2, PA8, PA9, PA17 and PA19) had staminate inflorescences on several branches that bore also pistillate inflorescences. We tested the viability of pollen from the monoecious plant with TTC (Triphenyl Tetrazolium Chloride), and the germination of the pollen *in vitro*. Pollen viability ranged from 73.6 to 95.1%. Pollen germination percentages were between 33.8 and 48.3% by the test-tube assay, and 51-64% by the agar-plate assay. Pollen from the fully monoecious tree was used to pollinate *P. vera* cultivars Ohadi and Vahidi. The hybrids will be tested for sex type, and may be used in the future as a starting point for breeding monoecious cultivated pistachio.

Key words: *P. atlantica*, monoecious, pollen germination, sex type.

RESUME – "Arbres monoïques de *P. atlantica* dans les montagnes de Yunt de la province de Manisa en Turquie". Les espèces de *Pistacia* sont des plantes dioïques. Dans cet article sont reportées quelques plantes exceptionnellement monoïques de *P. atlantica* rencontrées dans les montagnes de Yunt de la province de Manisa en Turquie. La distribution des inflorescences avec étamines et pistils sur les arbres a été observée pendant deux ans. L'un d'eux, PA18, était pleinement monoïque. Dans trois arbres, PA6, PA13 et PA14, il y avait plusieurs branches avec uniquement des fleurs à étamines dans différentes parties des arbres tandis que le reste des branches portait des inflorescences à pistils. Cinq arbres (PA2, PA8, PA9, PA17 et PA19) avaient des inflorescences à étamines sur plusieurs branches qui portaient également des inflorescences à pistils. Nous avons testé la viabilité du pollen provenant de plantes monoïques avec du TTC (chlorure de triphényl tétrazolium), ainsi que la germination du pollen *in vitro*. La viabilité du pollen allait de 73,6 à 95,1%. Les pourcentages de germination du pollen étaient entre 33,8 et 48,3% par test au tube d'essai, et de 51-64% par test sur boîte d'agar. Le pollen provenant d'arbres pleinement monoïques était utilisé pour polliniser Ohadi et Vahidi, cultivars de *P. vera*. Les hybrides seront testés pour le type sexuel, et pourraient être utilisés dans le futur comme point de départ pour la sélection de pistachiers à cultiver de façon monoïque.

Mots-clés : *P. atlantica*, monoïque, germination du pollen, type sexuel.

Introduction

All *Pistacia* species are dioecious, i.e., the pistillate and staminate flowers are formed on different trees (Zohary, 1952). Both the staminate and pistillate inflorescences are panicles with up to several hundred individual flowers (Crane and Iwakiri, 1981). Both types of flowers are apetalous, and wind is the pollinating agent. In Turkey, there are approximately 66 millions wild *Pistacia* trees, mainly, *P. terebinthus*, *P. khinjuk* and *P. atlantica* (Kuru and Ozsabuncuoglu, 1990). Because of natural occurrence of male *Pistacia* trees in nearby forests, some growers do not realize 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 will not produce nuts.

Two cases of exceptional sex types were reported in the literature: firstly, Ozbek and Ayfer (1958) found 2 hermaphrodite trees (male and female organs borne in the same flowers) in the vicinity of Antep province of Turkey. They reported that these trees were either seedlings of *P. vera*, or hybrids between *P. vera* and *P. terebinthus*. The second report is by Crane (1974). He found three

exceptional trees: (i) a branch bearing staminate flowers on an female tree of *P. atlantica*; (ii) a hybrid, *P. vera* x *P. atlantica*, bearing approximately equal numbers of pistillate and staminate inflorescences, usually on separate branches; and (iii) a similar hybrid, predominantly staminate, but with several branches bearing pistillate inflorescences.

There are no further studies using such trees to breed hermaphrodite or monoecious *P. vera* cultivars. Here we report on exceptional monoecious plants of *P. atlantica* found in the Yunt Mountains of Manisa province in Turkey and on their possible use in breeding to develop monoecious *P. vera* cultivars.

Materials and methods

A group of nine *P. atlantica* trees bearing staminate and pistillate inflorescences were found in the Korukoy village of Manisa province in the course of seed sampling for rootstock selection. Distribution of staminate and pistillate inflorescences on these trees was observed during two years. Staminate branches of four trees (PA6, PA13, PA14, PA18) were cut on 22/4/1997 and brought to Cukurova University, Adana, to perform pollen tests and cross-pollination. Branches were put in jars containing water in the laboratory on 23/4/1997. In the following 2 days pollen was collected and stored at -20°C until pollination. For storage, pollen was put into film box containing CaCl_2 particles under the cover. Some of the pollen was used for viability and germination tests before storage. Pollen viability was tested according to (Oberle and Watson, 1953; Norton, 1966) with some modifications. 1% concentration of TTC (Triphenyl Tetrazolium Chloride) was used. TTC solution was made up in distilled water. Pollen germination *in vitro* was done using the test tube assay according to Caglar and Kaska (1995) and agar-plate method according to King and Hesse (1938) with some modifications, using 12.5% sucrose plus 50 ppm boric acid for test tube assay and 12.5% sucrose plus 0.5% agar for agar-plate assay. Five replicates (100 to 200 pollen grains per replicate) were evaluated under stereo microscope (x 100) for each test. For TTC test pollen was classified as red (viable), pink (semi-viable) and white (dead). Pink pollen was evaluated as _ viable and _ dead. The pollen of fully monoecious tree, PA18, had very good viability and germination-ability, and was therefore used to pollinate *P. vera* cultivars Ohadi and Vahidi. The nuts that developed were harvested and germinated.

Results and discussion

Morphology of monoecious trees

In the course of seed sampling for rootstock selection we have found a group of nine *P. atlantica* trees bearing staminate and pistillate inflorescences in the Korukoy village of Manisa province. One of them, PA18, was fully monoecious (staminate and pistillate inflorescences were borne as a mixture on all the branches). Staminate and pistillate inflorescences of this tree are shown in Fig. 1. Three trees PA6, PA13 and PA14, had several branches with only staminate flowers in different parts of the trees while the rest of the branches bore pistillate inflorescences. Five trees (PA2, PA8, PA9, PA17 and PA19) had staminate inflorescences on several branches that borne also pistillate inflorescences, while the rest of the branches bore pistillate inflorescences. Sex patterns of the trees appeared similar in the two consecutive years, however, the fully monoecious tree in which staminate inflorescences were more numerous than pistillate ones in first year, while the reverse situation occurred in the second year.

Pollen viability and germination

Pollen viability data and germination rates of monoecious *P. atlantica* trees are shown in Table 1. PA13 and PA18 had the highest pollen viability, 95.1%, whereas PA6 (73.6%) and PA14 (78.6%) exhibited lower values. Pollen germination percentages were between 33.8% (PA14) and 48.3% (PA18) while in the agar-plate method germination percentages were between 51.3% and 64.3%. Such values are similar to those of normal male trees of *P. atlantica* (Kafkas, unpublished data).



Fig. 1. Distribution of pistillate and staminate inflorescences in fully monoecious tree, PA18.

Table 1. Pollen viability and germination percentages of monoecious trees

Tree code	TTC viability test – viable (%)	Germination tests (% germination)	
		Test-tube	Agar-plate
PA6	73.6	33.8	61.9
PA13	95.1	44.7	52.6
PA14	78.6	34.3	51.3
PA18	94.6	48.3	64.3

Hybridization with *P. vera* cultivars

We now have 7 seedlings from the *P. vera* cv. Ohadi x *P. atlantica* monoecious cross, and 3 seedlings from *P. vera* cv. Vahidi x *P. atlantica* monoecious cross. The hybrids are being tested for their sex type, and will be used in the future as a starting point for breeding monoecious cultivated pistachio. More crosses with *P. vera* cultivars will be made in the next season. A larger experiment will be started next spring including pollination and progeny tests in order to understand monoecious sex inheritance. If we could develop a pistachio variety that has both male and female inflorescences in a single tree, together with high yield of quality nuts, we would eliminate the need for male trees in the orchard and increase the yield by 10%.

Possible origin of the monoecious trait

It is interesting to speculate about the origin of the monoecious trait in this population. Firstly, all the monoecious trees were found in one location within an area of approximately 3000-4000 m². The monoecious trait may have arisen as a somatic mutation in one tree, followed by sexual reproduction. The group of trees was arranged in an approximate circle, with the fully monoecious tree (PA18) in the middle. The tree coded PA2 is the oldest tree among them, looking more than 500 years old, whereas the others may be at most 100 years old. It is possible that the younger trees are the progeny of PA2. In that case, the fully monoecious tree would be descendent of PA2 as one would expect. On the other hand, it is harder to explain why the other younger trees appear chimaeric, i.e., they have scattered male branches/inflorescences. An alternative explanation would be that all the chimaeric phenotypes arose as multiple independent somatic mutations; this is however rather unlikely, since the trait is so rare in natural *Pistacia* populations. Still another alternative explanation would be that the trees are genetically related and not chimaeric, but the monoecious trait is somatically unstable or confers a non-uniform phenotype. For example, it may have arisen by a transposable element, or, it may be expressed only in some tree parts, as result of an interaction with an unknown biotic/abiotic environmental factor.

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