Marker-assisted cultivar breeding in pistachio

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Abstract

Pistachio production in Turkey has been increasing year by year. However, the main cultivars such as 'Uzun' and 'Kırmızı' have strong tendency to alternate bearing with small size nuts and low splitting rates. Therefore, we aimed to develop new pistachio cultivars that have low tendency to alternate bearing, large nuts with high splitting percentages. Pistachio has a dioecious character and a very long juvenility period that limits its breeding. Recently, novel sex-linked markers were demonstrated to distinguish sex in pistachio at seedling stage in pistachio. Therefore, we aimed to use those markers for marker-assisted breeding (MAB) in pistachio in the new cultivar breeding program. A total of about 15,000 F_1 progenies were obtained from 45 combinations together with about 500 backcrosses and about 500 pseudo F_2 progenies). About 7,500 male progenies were discarded by marker assisted selection. The breeding orchard was established with female progenies together with adequate number of pollinators in December, 2017. MAB provided almost two times more female progenies in the breeding orchard that will serve more chance to breed new female pistachio cultivars along with keeping the land and resources for 20-25 years.

Keywords: pistachio, breeding, cultivar, MAS

INTRODUCTION

The genus *Pistacia* L. belongs to *Anacardiaceae* family and it has at least 11 species. Among these species, pistachio (*Pistacia vera* L.) has edible nuts and commercial importance. The other species grow in the wild and their seeds are used mainly as rootstock seed source and rarely for fresh consumption, oil extraction or soap production. All *Pistacia* species are dioecious and wind-pollinated. *P. vera* is believed to be the most ancestral species and the other species are probably its derivatives (Zohary, 1952). There are two centers of diversity of pistachio: one comprises the Mediterranean region of Europe, Northern Africa and the Middle East countries. The second comprises the Eastern part of Zagros Mountains from Crimea to the Caspian Sea. Pistachio cultivation extended westward from its center of origin to Italy, Spain, and other Mediterranean regions of Southern Europe, North Africa, and the Middle East, as well as eastward to China, and more recently to USA and Australia (Maggs, 1973; Hormaza et al., 1994, 1998).

Currently, Iran, USA, Turkey and Syria are the main pistachio producers in the world, contributing over 90% of the world production (Faostat, 2017). Most of the pistachio cultivars derived from natural selections. 'Fandoghi' and 'Kalehghouchi' were the major cultivars in Iran in the past, and currently they have newreleased cultivars: 'Akbari' and 'Ahmad Aghai'. Pistachio production in USA is mainly in California, and 'Kerman' is the main female cultivar in the production. Meanwhile, new cultivars were released from cross-breeding programs in California such as 'Golden Hills' and 'Lost Hills'. 'Uzun', 'Kırmızı' and 'Siirt' are main cultivars in pistachio production of Turkey. 'Uzun' and 'Kırmızı' cultivars are produced mainly for industrial purpuse such as baklava, and they cover approximately 70-80% of the production. Both cultivars show strong tendency to alternate bearing where one year is on and the following year is off. Furthermore, their nuts are small with low splitting rates, while their taste and aroma are perfect.

The pistachio tree has a very long generation time; juvenility period is about 5-15

years. This is one of the limiting factors for cultivar breeding programs, making it expensive and time consuming. Another limiting factor is the dioecious character of pistachio where paternal parents are selected with unknown nut characters, since males do not bear nuts. In a typical cross, male plants represent 50% of the progenies, resulting in a considerable waste of breeding land and resources. Therefore, marker-assisted selection (MAS) would be a very powerful tool, for facilitating cultivar development in breeding programs for pistachio. Kafkas et al. (2015) and Khodaeiaminjan et al. (2017) developed 17 sex-linked SNP markers for *P. vera* and validated them by testing in a segregating population and in a large germplasm collection. The authors also demonstrated their potential use by HRM analysis for MAS in a pistachio cultivar breeding program.

Therefore, in this study, we aimed to start a new pistachio cultivar breeding program by using female-specific sex-linked markers developed by Kafkas et al. (2015) for marker assisted selection (MAS).

MATERIAL AND METHODS

The new cultivar breeding program has mainly three different crosses: (1) using three female cultivars and 11 male cultivars or genotypes, (2) backcrosses, and (3) crosses between male and female F_1 progenies derived from same parents that are from an earlier breeding program (Uzun et al., 2011). In the first crosses, 'Uzun', 'Siirt' and 'Kalehghouchi' cultivars were females and 'Peters', 'Kaşka', 'Male-7', 'Male-18', 'Male-23', 'Male-1/76', 'Male-11/148', 'Male-12/119', 'Male-16/32', 'Male-21-125', and 'Male-25/90' were males. Six backcrosses and six crosses between sister F_1 progenies were also performed. The male genotypes and F_1 parents are selections from previous breeding programs (Uzun et al., 2011).

Crosses were performed in the spring of 2016. Female inflorescences were bagged with a paper bag before the stigmas became receptive. To collect pollen for artificial pollination, staminate branches of male trees were cut, and pollens were collected from each paternal parent in the laboratory. The collected pollen was stored at +4°C in the desiccator or at -20°C for longer period. Wheat flour was used as a mixing agent with pollen. All the crosses were performed in Gaziantep Pistachio Research Institute germplasm collection. Artificial pollination was repeated two times for each cross.

The nuts were harvested during harvest season of 2016. The seeds were sown following their stratification at +40°C during 45 days. One leaflet from each progeny was sampled after germination for DNA extraction. DNA extractions were done based on CTAB protocol (Doyle and Doyle, 1987). Two SNP markers (SNP-PIS-167992 and SNP-PIS-174431) linked to sex in pistachio developed by Kafkas et al. (2015) were used for gender determination of the progenies. HRM analysis was performed using a Light Cycler 96 Real-Time PCR instrument (Rosch, Mannheim, Germany). The HRM amplification reactions were carried out in a total volume of 20 µL containing 15 ng DNA, 75 mM Tris-HCl (pH 8.3), 20 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 0.1% Tween 20, 200 µM each of dATP, dTTP, dGTP, and dCTP, 0.25 µM each of reverse and forward primers, 1.0 unit Hotstart Taq DNA polymerase, and 1.5 µM Syto 9 dye (Life Technologies, Carlsbad, CA). The cycling program was: pre-denaturalization for 600 s at 95°C; 45 cycles of 95°C denaturalization for 10 s, 60°C annealing for 15 s, and 72°C extension for 15 s. Amplification cycles were immediately followed by HRM steps of 95°C for 60 s, cooling to 40°C for 60 s, then raising the temperature to 65°C and then 97°C for 15 s. The annealing temperature was decreased in subsequent cycles by 0.5°C per cycle after the first 60°C annealing step, down to 55°C.

RESULTS AND DISCUSSION

'Uzun' is an early flowering cultivar, and it is impossible to pollinate 'Uzun' cultivar with late flowering males in a typical flowering season. The weather became suddenly very hot in the spring of 2016, and there was possibility to pollinate female cultivars with all early and late flowering males such as 'Peters' and 'Kaska' in Gaziantep ecological conditions. The stratified seeds were directly sown to 4-L plastic pots. We obtained totally about 15,000 progenies: 9,500 progenies from 'Siirt' cultivar, 4,000 progenies from 'Uzun' cultivar and 500

progenies from 'Kalehghouchi' cultivar, pollinated with 11 different males together with about 500 backcross progenies and about 500 progenies derived from croosses between sisters F_1 progenies (pseudo F_2).

Sexes of 15,000 progenies were determined by DNA fingerprinting and female ones were labelled together with adequate number of pollinators to transferring them to breeding orchard. About half of the progenies were female. Therefore, approximately half of the progenies (males) were eliminated by MAS. The breeding orchard was established with female progenies together with an adequate number of pollinators in December, 2017 with a distance of 3×1 m in the Gaziantep Pistachio Research Institute.

CONCLUSIONS

This is the first cultivar breeding program using DNA-based marker-assisted selection in pistachio. About 7,500 male progenies were discarded by marker assisted selection. This provides two times more progenies in the breeding orchard that will serve more chance to breed new pistachio cultivars. In other words, we kept the land and resources for 20-25 years. All the segregating populations in this study will not only serve to develop new cultivars, they will also help to develop novel markers linked to economically important characters in pistachio in the near future.

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