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Studying Artificial Pollination of Four Midseason Male Genotypes of Pistachio (*Pistachia vera* L.) on Three Iranian Cultivars and Selection of Best Pollinizer

Hossein Afshari[1*] • Alireza Talaei[2] • Hossein Hokmabadi[3]**

[1] Horticulture Department, Islamic Azad University, Damghan Branch, P. O. Box: 36716-39998, Iran
[2] Horticulture Department, Faculty of Agriculture, University of Tehran, Iran
[3] Horticulture Department, Iran's Pistachio Research Institute, P.O. Box: 77175-435, Rafsanjan, Iran

Corresponding author: * **afshari2000ir@yahoo.com** ** **hokmabadi@pri.ir**

ABSTRACT

In order to achieve good pollination and fruit set in a pistachio orchard, the ratio of male to female trees has to be considered. In addition, the quality of pollen source and ability of the pollen to germinate and pollinate female flowers are important. In this study the effect of overlapping pollen with tree commercial pistachio cultivars on fruit set and other traits were examined. This study was conducted as a factorial experiment with randomized complete design at the Pistachio Research Institute, Iran in 2005-2006. Among the fruits of the three cultivars which were pollinated with four male genotype pollen (R27, R28, N2, N16) only total fruit weight and blankness were affected by pollen type. The highest fruit weight was related to the fruits of cv. 'Kaleghuchi' (3.4 g) and the lowest was observed in cv. 'Ohadi' (2.2 g). Also, the highest rate of blankness in the first year was observed for pistachio cv. 'Ahmadaghaii' (27%) and R27 pollen resulted in the highest amount of blankness in both years (33% in 2005, 25% in 2006). Incidentally, the highest fruit weight and lowest blankness were established by male genotype pollen of N16. No factor such as year, cultivar, pollen type, or any combination or interaction among them affected fruit set. However, in the first year of the experiment fruit set was 14.9%, higher than the second year (14%).

Keywords: 'Ahmadaghaii', fruit set, 'Kaleghuchi', male genotype, 'Ohadi', pollen

INTRODUCTION

Pistachio is a dioecious plant: its male and female flowers grow on separate trees. The fruits, which belong to the group of dry fruits, have edible kernels. Good pollination is crucial to achieve sound and full fruits (Ak 1998). In many fruit-bearing trees a number of flowers and fruits drop before maturation of the fruits and the dropped ovaries may be non-pollinated, pollinated with incompatible or ruined pollen after formation of the ovule (Shuraki and Sedgley 1994). Fruit dropping may also be affected by pollination time or be related to flower age or environmental factors (temperature) during the flower opening (Crane and Iwakiri 1981). In general, the time of germination for female flowers after pre-germination is a determining factor in fruit set (Shuraki and Sedgley 1994). Choosing suitable pollen (which produce lots of live pollen when commercially produced types are flowering) from seed plants produced through controlled hybridization of male lines or cultivars is one of the significant goals in replication of pistachio scions (Crane and Iwakiri 1980; Martinez, and Herrero 1994; Martinez and Herrero 1994). In order for a good pollination and fruit set to happen in an orchard, the ratio of male and female trees (1:8) should be considered carefully. Besides, the quality of the pollen source and the potential of the pollen in germination and fertilization of female flowers are of utmost significance (Crane and Iwakiri 1980). The effect of pollen parent (xenia) on kernel and fruit traits of several species such as almond, chestnut, and pecan has been observed (Kunar and Dos 1996). For pistachio too, there has been considerable research on the effects of pollen parent on the properties of the fruits (Crane and Iwakiri 1980; Kunar and Dos 1996; Gouta *et al*. 2002). Crane and Iwakiri (1980) have shown that none of the pollen from different sources is effective on the size and fruit blankness rate. Ak (1998) reported that fruits pollinated with domestic pistachio pollen were far better than those obtained with other wild pistachio pollen. Gouta *et al*. (2002) studied the effects of domestic, atlantica, and tereibintus pollen types on two female genotypes of Mateor (domestic) and El Gotear; and concluded that for both types, the fruits set from pollination with atlantica were much better. Yet the weight of the kernels produced by different pollen types showed no difference. Also for Mateor type the percent of blank fruits produced by free pollination was quite low (8%) (Crane and Iwakiri 1980). Riazi *et al*. (1997) studied the effect of xenia and metaxenia on three cultivars of Iranian commercial pistachios ('Ohadi', 'Kaleghucii', 'Momtaz'). They reported that the pollen of mutica and atlantica decreased the growth of kernels and percent of split fruits; while the pollen of 'Momtaz' and 'Soltani', from the domestic category, increased the size and number of split fruits (56% and 52% respectively). Shuraki and Sedgley (1994) demonstrated that there was a significant difference in the grow of pollen tubes and potential of producing better fruits between cultivars, and many fruit features such as weight, diameter, width, length, and split percent are positively influenced by them.

In this research the effects of overlapping pollen on 3 commercial pistachio types in different germination and fruit set times, and some of their features were studied and the best pollinizers were recognized.

MATERIALS AND METHODS

The present study was conducted in 2005 and 2006 on 3 cultivars of commercial pistachio, namely 'Kaleghuchi', 'Ohadi' and 'Ahmadaghaii' by using pollen from four genotypes (R27, R28, N2, and N16) in Iran's Pistachio Research Institute. Eight similar trees (in same age) of each type were chosen for the project. The flower

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buds were tagged at the time of swelling, 6 out of 7 branches containing 3 buds were isolated in special bags immediately. The branches having male flower clusters were separated before blooming completely, sent to the lab, and their branch ends were put inside water containers. Once the anthers opened, male flowers of the four different genotypes were shaken on white cloths in 4 separate rooms, and pollen was sieved through a 0.1 mm mesh. Hands and all tools were disinfected with 70% alcohol before and after each operation so as to prevent infection. Pollen seeds were hand-pollinated with a hand brush at one stage (when 70% of the flowers had opened) by brushing over pollen flowers. Immediately after pollination, the bags were closed then opened 8 days later when the flowers were counted. The average number of flowers in each cluster was considered to be 105 ('Ahmadaghaii' = 109, Ohadi' = 101, 'Kaleghuchi' = 105). The final counting of fruits was done a week before harvest and the percentage fruit set was calculated. Seven named branches were used for this goal: 4 branches were considered for pollination with R27, R28, N2, and N16 genotypes; one branch was reserved for pollination with a combination of the 4 pollen types, one branch was considered for free pollination (positive control) and the last branch remained unpollinated (negative control). At harvest time 10 fruits of each branch were chosen for quantitative and qualitative tests. The weight of the fruit including and excluding the green skin, length, width, diameter of the fruit with hard shell, kernel weight, number of blank fruits, number of split fruits, and number of fruits with early splitting were the measured features. The weight of fruits and kernels were measured by digital balance, the length and width were measured by calipers, and the features of early split and cracked fruits were assessed by eye.

These tests were conducted in a factorial design within completely random blocks, the differences in the means were assessed by ANOVA and the differences in the means (assessed at $P<0.05$ or $P<0.01$) were analyzed using Duncan's Multiple Range Test by SAS and MStatC software.

RESULTS

Fruit set percentage

None of the factors such as year, cultivar, pollen type, or their interaction had any significant effect at $P<0.05$ on the final fruit set. However, in the first year of the experiment the fruit set rate (14.9%) was slightly higher than the second year (14%) (**Table 1**). Also, despite the lack of a significant difference between the fruits produced through pollination by given and control pollen, it was observed that fruits pollinated freely (positive control) had a higher fruit set (15.3%) than the other types (**Figs. 1, 2**).

Fruit weight

Based on the data obtained from the analysis of the variance of factors such as year, cultivar, and pollen type the mutual effects of pollen and cultivars in both years (2005 and 2006) on the weight of pistachio dehulled fruits were significantly different ($P<0.01$). In the second year the fruits had an average weight of 2.69 g, which decreased slightly in the first year of the experiment (2.59 g) (CV = 7.19%). Therefore in the first year of the experiment the highest fruit weight was seen in 'Kaleghuchi' (3.3 g) and the lowest in 'Ohadi' (2.1 g). The same pattern could be observed in the second year, i.e. the fruits from 'Kaleghuchi' type had the highest weight (3.4 g) and those from 'Ohadi' had the lowest weight (2.2 g). Assessing the effect of pollen type on fruit weight in 2005 indicated that the fruits pollinated by R28 and N16 and a combination of pollen had the highest weights (2.8, 2.7, and 2.6 g, respectively) while dehulled fruits pollinated with free pollen (positive control) or with R27 genotype had the lowest weight (2.3 and 2.4 g, respectively). The second year was the same: in both experiment years the highest weight of fruits was observed in 'Kaleghuchi' pollinated with N16 and a combination of other pollen (3.8 g), and 'Ohadi' fruits pollinated with N2 or free (negative control) pollen demonstrated the lowest weight (2.1 g) (**Tables 1-4**).

Table 1 Effect of experiment year on features of fruit set and blankness rates of pistachio.

Year of experiment	Fruit weight with skin	Fruit blankness	Fruit set rate
Year 1	2.69 a	20 a	14.9 a
Year 2	2.59 b	5 b	14.0 a

Values with the same letters within a column are not significantly different according to DMRT ($P\leq0.05$).

Table 2 Effect of pollen type on some pistachio features.

Features	R27	R28	N2	N16	Control	Combined
Fruit weight, skin included	2.5 c	2.7 b	2.53 c	2.85 a	2.45 c	2.8 ab
Fruit weight, skin excluded	29.0 a	16.0 bc	18.0 b	12.0 cd	9.00 d	17.0 bc

Values with the same letters within a row are not significantly different according to DMRT ($P\leq0.05$).

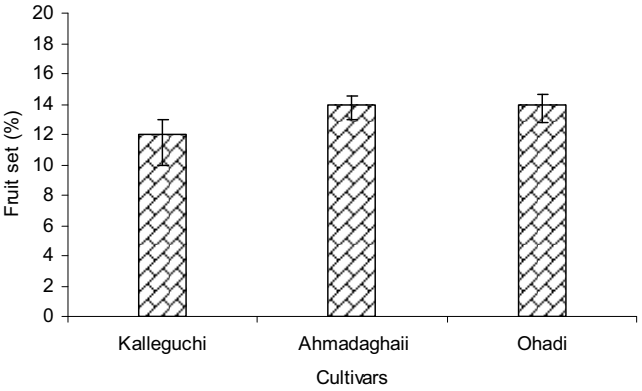

Fig. 1 Effect of fruit set rate in different cultivars. Error bars are ± standard error of the means (n = 6).

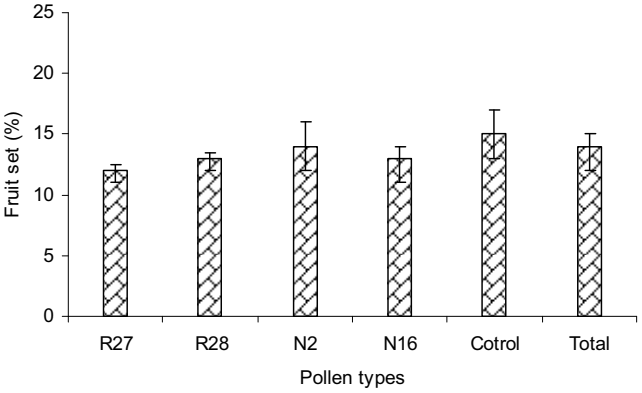

Fig. 2 Effect of pollen type on fruit set rate. Error bars are ± standard error of the means (n = 6).

Hulled fruit weight

The data obtained from ANOVA suggested that factors such as year, cultivar, pollen type, and the interactive effects of factors on traits such as weight of the fruits excluding green skins had no significant statistical difference. Only the effect of cultivar on the weight of hulled fruit had a significant statistical difference at $P<0.01$ (CV = 21.4%). Thus fruits of 'Kaleghuchi' type were heavier (2 g) than 'Ahmadaghaii' and 'Ohadi' types (1.4 and 1.3 g, respectively) (**Tables 2, 4**).

Fruit length

Among various factors and their effects on the length of hulled fruits, only the cultivar factor had a significant difference at the 5% level. This indicates that pollen type and its interaction with other factors had no effect on the length of the fruits. Maximum fruit length was observed in 'Ahmadaghaii' (2.8 cm) type, and then 'Kaleghuchi' (2.1 cm)

Table 3 Mutual effects of pollen type and cultivar.

	Fruit weight with skin			Fruit blankness		
	Cultivar					
Pollen	**'Kaleghuchi'**	**'Ohadi'**	**'Ahmadaghaii'**	**'Kaleghuchi'**	**'Ohadi'**	**'Ahmadaghaii'**
R27	3.0 c	2.3 def	2.4 de	26 b	37 a	22 bc
R28	3.2 b	2.5 d	2.4 de	20 bc	7 de	22 bc
N2	3.2 b	2.1 f	2.4 de	7 de	26 b	19 bc
N16	3.8 a	2.3 def	2.5 d	21 bc	0 e	15 cd
Control	3.0 c	2.1 f	2.5 d	4 de	0 e	20 bc
Combination	3.8 a	2.2 ef	2.4 de	22 bc	0 e	20 bc

Values with the same letters within a column are not significantly different according to DMRT ($P \leq 0.05$).

Table 4 Effect of cultivar on some features of pistachio fruits.

Features	**Cultivars**		
	'Ohadi'	**'Kaleghuchi'**	**'Ahmadaghaii'**
Fruit weigh, skin included	2.10 c	3.30 a	2.40 b
Fruit weigh, skin excluded	1.40 b	2.00 a	1.30 b
Fruit length, shell included	1.90 bc	2.10 b	2.80 a
Fruit length, shell excluded	1.46 a	1.44 a	1.20 a
Fruit width	1.39 a	1.50 a	1.42 a
Fruit kernel	0.90 a	1.00 a	1.30 a
Early-popping	3.00 a	4.00 a	4.00 a
Irregular cracking	20.00 b	24.00 a	19.00 a
Fruit blankness	13.00 b	18.00 a	21.00 a

Values with the same letters within a column are not significantly different according to DMRT ($P \leq 0.05$).

and 'Ohadi' (1.9 cm) types (CV = 19.4%) (**Table 4**).

Fruit diameter

None of the factors and their interactions had any effect on the diameter of hulled fruit (CV = 22.4%), i.e. the effects of different pollen seeds on the diameters of three different cultivars and the diameters of the fruits of 3 studied types were not statistically different. The diameter of studied fruits was 1.46 cm for 'Ohadi', 1.44 cm for 'Kaleghuchi', and 1.20 cm for 'Ahmadaghaii' type (**Table 4**).

Fruit width

As for fruit diameter, there was no significant difference between the effects of various factors and their interactions on the widths of studied fruits as shown in the variance analysis table (CV = 30.54%). Nevertheless, the width of studied fruits was 1.50 cm for 'Kaleghuchi', 1.42 cm for 'Ahmadaghaii', and 1.39 cm for 'Ohadi' types (**Table 4**).

Kernel weight

There was no significant statistical difference among the effects of factors such as year, cultivar, pollen type and their interactions on kernel weight. This means that pollen type had no effect on the increase or decrease of kernel weight, yet the studied fruits apparently showed significant differences in 'Ahmadaghaii' (1.3 g), 'Kaleghuchi' (1 g), and 'Ohadi' (0.9 g) types (**Table 4**).

Early splitting rate

According to the ANOVA table the rate of early splitting among various cultivars was not affected by various pollination factors, and none of the factors or their interactions could influence the early popping rate in a significant way (CV = 21.2%). Indeed early splitting was 4% and 3% in 'Ahmadaghaii' and 'Kaleghuchi' types, respectively (**Table 4**).

Fruit cracking rate

According to the ANOVA table, from among all factors and their interactions on the rate of irregular cracking of the fruits being studied, only cultivar showed a significant difference at the 1% level. Maximum irregular cracking was observed in 'Kaleghuchi' type (24%), while 'Ohadi' (20%) and 'Ahmadaghaii' (19%) ranked next (**Table 4**).

Blankness rate

According to the ANOVA table factors such as year, cultivar, type of pollen and the interactive effects of pistachio and pollen type on the fruit blankness rate had significant differences at the 1% level (CV = 28.4%). In the first year three factors (pollen type, cultivar and their interaction) and in the second year two factors (pollen type and its interactions) showed significant statistical differences at the 1% level. Maximum fruit blankness rates in the first year were observed for 'Ahmadaghaii' (27%), 'Ohadi' (20%) and 'Kaleghuchi' (13%). Maximum blankness in the first year was observed in R27 pollen (33%). From the mutual effect of pistachio and pollen type in the first year on the blankness rate it was observed that maximum blankness resulted from the effect of R27 pollen on 'Kaleghuchi' type (42%) and minimum blankness resulted in control fruits by a combination of pollens and N16 pollen on 'Kaleghuchi' type (5%). Yet in the second year R27 pollen caused maximum blankness (25%), and pistachios pollinated freely (positive control) had minimum blankness rates (4%). Also, in the second year of pollination (2007) the effect of R27 pollen on 'Kaleghuchi' resulted in maximum blankness (35%) and 'Kaleghuchi' types pollinated freely (positive control) and with N16 and a combination of pollen showed minimum blankness (5%) (**Tables 1, 3, 4**).

DISCUSSION

Pistachio nuts are dioecious. Pistachio flowers, which have no petals, are pollinated by insects. Therefore, pollination occurs by the transport of pollen from male to female trees by wind. In order to obtain a yield pollination and fertilization are necessary pre-requisites. Therefore, pistachio orchards must contain male trees and the ratio of male to female should be 1/8. In Iran, flowering time is during the first two weeks in April for most districts. To get maximum nut production, it is necessary to have enough male trees to insure adequate pollination. Viability and pollen tube growth and morphological characterization of pollen grains are recognized as important characteristics of plants. Modifying these specifications leads to genetic improvement of pistachio. Therefore, some simple methods of breeding and selection of pollinzers to improve potential of new pistachio orchards are required (Kamyab 2006). In this experiment the better fruit set of the first year (i.e. 2005) was probably due to the pollen was fresh because of moderate weather condition in this year of the experiment, and the higher fruit set in the control group was probably due to the fact that the flowers of other groups were harmed by isolating bags. The lack of influence of pollen grains on the final fruit set is in agreement with the studies of Riazi *et al*. (1996). They found also that the pollen source had no effect on final fruit set and fruit weight. Pollen of wild pistachio species reduced kernel weight, number of split nuts, and increased the percentage of blank nuts of three commercially grown pistachio cultivars. Also the higher rate of fruit set in the con-

trol group is in agreement with the studies of Kamyab (2006). The effect of pollen type on the increase or decrease of fruit weight when compared to the control group points to the existence of xenia in pistachio. Crane and Iwakiri (1980) explained that xenia was evident in the effect of hybrid and *P. atlantica* pollen in reducing kernel length and dry weight.

The increase in total fruit weight due to pollen taken from domestic pistachios is in agreement with the results reported by Ak (1998). The cause of total fruit weight during the second year (i.e. 2006) may be nutritional factors or use of fresher pollen in comparison to the first year, which resulted in low blankness rates as well. The pollen seeds of various genotypes did not influence the weight of hulled fruits, although the pollen of N16 genotype resulted in heavier fruit (2.17 g) than other genotypes. Riazi *et al*. (1996) and Kamyab (2006) did not observe any effect of pollen seeds on the weight of skinned fruit. The lack of influence on features like length, diameter, and width of the fruits by various pollen seeds is in agreement with the results reported by some researchers (Ak and Kaska 1998; Gouta *et al*. 2002; Kamiab 2006), although these results differ among the three studied types of pistachio.

Gouta *et al*. (2002) and Riazi *et al*. (1996) did not observe any effect of pollen grain of domestic pistachio on the weight of pistachio kernels, which is in agreement with our results, although the kernels of 'Ohadi' type had a minimum weight which was established in previous research (Riazi *et al*. 1996; Gouta *et al*. 2002).

Early splitting and irregular cracks in the green skin of the fruit are mainly influenced by their female parent; factors such as irrigation, nutrition, environment and other factors which affect the female parent have direct effects on them as well (Doster and Michailides 1995). Since pollen type had no effect on pistachio fruit weight, it can be said that pollen grain did not affect such features. Yet there are differences among pistachio types in terms of irregular cracks: 'Kaleghuchi' fruits had more cracks (46.61%). Panahi and Talaie (2002) and Kashaninejad *et al*. (2005) also observed more irregular cracks on 'Kaleghuchi' fruits. The higher blankness rate of the first year as compared to the second year may probably be due to, among other causes, high fruit set and high yield in first year of experiment. Pollen grains of R27 and N2, due to their lack of fertilization by ovules or the destruction of the produced fetus, caused more blank fruits in comparison to other types of pollen. N16 pollen and flowers pollinated freely (positive control) resulted in a maximum rate of kernelled pistachios. Also this group resulted in higher fruit set rates. It was observed in previous experiments (Ak and Kaska 1998; Gouta *et al*. 2002; Kamiab 2006) that N16 pollen had the highest rate of fertilization, fruit growth, and fruit set. Yet the percentage of fruits with higher amount of kernels is in agreement with the findings of several research results (Ak and Kaska 1998; Gouta *et al*. 2002; Kamiab 2006).

Flowering inside bags, gradual germination of the control group and their pollination, and introduction of positive competition among several pollen types on the stigmas of control flowers may be the cause of higher kernel rates in freely-pollinated flowers.

GENERAL CONCLUSIONS

All pollen of different male genotype of pistachio are compatible with the pistachio female flowers and can fertilize the female flower of pistachio. However, it is necessary to select each female and each cultivation area for the best pollinizer. We found in Rafsanjan pistachio area that the best pollinizers for three commercial pistachio cultivars, viz. 'Ahmadaghaii', 'Ohadi' and 'Kaleghuchi', were N16 and N28 genotypes. We also found that characteristics of the hull of pistachio (epicarp and mesocarp) were affected by the female parent and not the male pollen source. Therefore it is recommended to obtain best results in fruit set and yield of pistachio to select the best pollinizer or graft a few different genotypes for better performance.

REFERENCES

Ak BE (2001) Effect of different *Pistacia* species pollen on fruit dimension and weight in Kirmizi variety. Proceedings of the XI GREMPA Seminar, 1-4 Sept., 1999, Sanliurfa, Turkey, Cahiers Options Mediterraneennes (Vol 56), pp 311-314

Ak BE (1998) Effects of different *Pistacia* species pollen on fruit dimension and weight in the siirt cultivar. *Acta Horticulturae* **470**, 294-299

Ak BE, Kaska N (1998) Determination of viability and germination rates of *Pistacia* spp. Pollen kept for artificial pollination. *Acta Horticulturae* **470**, 300-306

Alipour H, Azghandi AV, Esmailpour A (2002) Introducing suitable pollinizers for "Ahmad Aghaii" pistachio (An Iranian commercial cultivar). *Acta Horticulturae* **591**, 371-374

Crane JC, Iwakiri BT (1980) Xenia and metaxenia in pistachio. *HortScience* **15**, 184-185

Crane JC, Iwakiri BT (1981) Morphology and reproduction in pistachio. *Horticulture Reviews* **3**, 376-393

Doster MA, Michailides TJ (1995) The development of early split pistachio nuts and their contamination by molds aflatoxins, and insects. *Acta Horticulturae* **419**, 359-364

Gouta H, Chelli Ak, Chrab M, Aissa IB (2002) Preliminary results on the effects of different pistachio species pollen on fruit characteristics of two commercial cultivars "Mateur" and "El Guetta". *Acta Horticulturae* **591**, 405-409

Kamiab F (2006) Determination the best pollinizer for 4 pistachio commercial cultivars and study the sexuality in pistachio by RAPD method. MSc thesis, Tehran University, 135 pp

Kashaninejad M, Mortazavi A, Safekordi A, Tabil LG (2006) Some physical properties of pistachio (*Pistacia vera* L.) nut and its kernel. *Journal of Food Engineering* **72**, 30-38

Kunar K, Dos B (1996) Studies on xenia and methaxenia in almond. *Scientia Horticulturae* **71**, 545-549

Martinez PE, Herrero M (1994) Male Performance in pistachio (*Pistacia vera*). *Journal of Horticultural Science* **69**, 1117-1122

Panahi B, Talaie A (2002) Survey and characterization of pistachio germplasm in Iran. *Acta Horticulturae* **591**, 263-264

Riazi GH, Rahemi M, Khanizadeh Sh (1996) Effects of selected pistachio pollen on development and quality of pistachio nuts of three commercially grown cultivars. *Journal of Plant Nutrition* **19**, 635-641

Shuraki YD, Sedgley M (1994) Effect of pistil age and pollen parent on pollen tube growth and fruit production of pistachio. *Journal of Horticultural Science* **69**, 1019-1027

Shuraki YD, Sedgley M (1996) Fruit development of *Pistacia vera* (Anacardiaceae) in relation to embryo abortion and abnormalities at maturity. *Australian Journal of Botany* **44**, 35-45