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Determination of the Apomictic Fruit Set Ratio in Some Turkish Walnut (*Juglans regia* L.) Genotypes

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Abstract: This study was carried out to determine the amount of apomictic fruit set in 10 Turkish walnut (*Juglans regia* L.) genotypes in 2001, 2002 and 2003. Yalova-1, Şebin, Bilecik, KR-1, KR-2, Şen-2, 07-KOR-I, Tokat-1, Kaman-1 and Kaman-5 cultivars and selections were used in the experiment. Female flowers were bagged and/or pollinated with pollen of the apple cv. 'Golden Delicious' (*Malus* x *domestica* Borkh.), and the apomictic fruit set was determined 8 weeks after anthesis. The percentage of apomictic fruit set without pollination ranged from 0.5% to 1.6% in 5 walnut genotypes, namely Yalova-1, Bilecik, 07-KOR-I, Tokat-1 and Kaman-1, in the first 2 years. In the third year, the highest apomictic fruit set (6.4%) was obtained from Tokat-1 after pollination with apple pollen. Apple pollen did not induce apomixis in the other 4 genotypes. The results indicated that apomictic fruit set is insufficient for economical seed and crop production in these ten Turkish walnut genotypes.

Key Words: Walnut, *Juglans regia* L., apomixis

Bazı Türk Ceviz (*Juglans regia* L.) Genotiplerinde Apomiktik Meyve Tutum Oranının Belirlenmesi

Özet: Bu çalışma on Türk ceviz (*Juglans regia* L.) genotipinde apomiktik meyve tutumu miktarını belirlemek için 2001, 2002 ve 2003'de gerçekleştirilmiştir. Çalışmada Yalova-1, Şebin, Bilecik, KR-1, KR-2, Şen-2, 07-KOR-1 Tokat-1, Kaman-1 ve Kaman-5 tip ve çeşitleri kullanılmıştır. Dişi çiçekler keseler içerisine alınmış ve/veya 'Golden Delicious' (*Malus* x *domestica* Borkh.) elma çiçek tozlarıyla tozlanmış ve apomiktik meyve tutumu tam çiçeklenmeden 8 hafta sonra belirlenmiştir. Tozlama yapılmaksızın apomiktik meyve tutum oranı Yalova-1, Bilecik, 07-KOR-I, Tokat-1 ve Kaman-1 ceviz genotiplerinde, iki yılda % 0.5-1.6 arasında değişmiştir. Üçüncü yılda en yüksek apomiktik meyve tutum oranı (% 6.4) elma çiçek tozlarıyla tozlamadan sonra Tokat-1 çeşidinden elde edilmiştir. Elma çiçek tozu diğer dört genotipte apomiksisi uyarmamıştır. Sonuçlar, on Türk ceviz genotipinde apomiktik meyve tutumunun ekonomik anlamda tohum ve meyve üretimi için yetersiz olduğunu göstermiştir.

Anahtar Sözcükler: Ceviz, *Juglans regia* L., apomiksis

Introduction

Apomixis, embryo sacs and embryos formed without meiosis or fertilization in ovules, gives rise to fertile seeds the embryos of which are derived directly from maternal cells. Therefore, these embryos have a genetic constitution identical to that of the female parent (Hanna and Bashaw, 1987; Koltunow, 1993; Garcia et al., 1999). Apomixis is a form of vegetative reproduction by the seed in tree crops such as citrus, apple and some tropical fruits (Westwood, 1993). Moreover, it could be interesting to increase crop production in some plants without pollination and fertilization.

Apomictic reproduction has traditionally been separated into 2 mechanisms: adventitious embryony and gametophytic apomixis. Adventitious embryony (nucellar embryony) refers to the formation of embryos from ovule tissues outside of the embryo sac, although fertilization of the polar nuclei to form the endosperm is usually necessary to support the embryo development. In gametophytic apomixis, embryo sacs are produced from unreduced cells. Their egg cells develop parthenogenetically, giving rise to embryos and offsprings bearing the maternal genotype. Two major types of gametophytic apomixis have been described based on the origin of megagametophyte. In apospory, one or more unreduced female gametophytes are formed mitotically from nucellar cells, while in diplospory, megasporocytes develop into mature unreduced female

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gametophytes either directly or after drastically altered meiotic processes producing unreduced megaspores (Garcia et al., 1999; Martinez et al., 2003). Apomixis has been investigated in the Juglandaceae. The mechanism of apomixis in walnut has been reported as adventitious embryony (Valdiviesso, 1990), apospory (Terziiski and Stefanova, 1990) or diplospory (Sartorius and Stosser, 1991). Badalov (1989) also reported that apomictic embryos developed mainly from the egg cell with subsequent doubling of the chromosome number, leading to homozygosity. Despite considerable variability in approach, studies have shown that apomixis rates were very high in some walnut genotypes and ranged from 23.5% to 81.2% (Loiko, 1990; Solar et al., 1995; Soylu and Ertürk 2001), but were generally below 17.3% (Badalov, 1989; Terziiski and Stefanova, 1990; Valdiviesso, 1990; Gao et al., 1999; Zhang et al., 2000; Soylu and Ertürk, 2001). The objective of this study was to determine the percentage of apomictic fruit set in 10 Turkish walnut genotypes.

Materials and Methods

Pistillate flowers of Yalova-1, Şebin; Bilecik, KR-1 (Gültekin-1), KR-2 (Yavuz-1), Şen-2 (24-KE-21), 07-KOR-I, Tokat-1 (Tokat-40, 60 TU-1), Kaman-1 and Kaman-5 walnut (*J. regia* L.) genotypes were used in this study. The experiments were conducted at Atatürk Central Horticultural Research Institute, Yalova, and a grower's orchard in Beynam village, Bala, Ankara, over 3 years.

The pistillate flowers of 10 genotypes were bagged approximately a week before pistillate blooming in 2001 and 2002. In addition, pistils of 5 genotypes that had apomictic fruit sets in 2001 and/or 2002 were pollinated with freshly collected pollen of the apple cv. 'Golden Delicious' (*Malus x domestica* Borkh.) to stimulate an apomictic fruit set in 2003. The pistillate bloom dates of genotypes during the 3 years are shown in Table 1. In the apomixis experiments, the number of pistillate flowers was between 48 and 227 on 3 trees for each genotype

Table 1. The pistillate bloom dates of some Turkish walnut (*J. regia* L.) genotypes in 2001, 2002 and 2003.

Year	Genotype	Beginning of blooming	Full blooming	End of blooming	Location
	Yalova-1	12 April	18 April	24 April	Yalova
	Şebin	12 April	18 April	24 April	Yalova
	Bilecik	18 April	24 April	01 May	Yalova
	KR-1	22 April	26 April	01 May	Yalova
2001	KR-2	18 April	24 April	28 April	Yalova
	Şen-2	02 April	08 April	13 April	Yalova
	07-KOR-I	20 April	28 April	08 May	Yalova
	Tokat-1	29 March	12 April	14 April	Yalova
	Kaman-1	01 May	07 May	15 May	Beynam Village*
	Kaman-5	03 May	06 May	15 May	Beynam Village
	Yalova-1	25 April	30 April	06 May	Yalova
	Şebin	21 April	29 April	04 May	Yalova
	Bilecik	15 April	22 April	28 April	Yalova
	KR-1	27 April	04 May	09 May	Yalova
2002	KR-2	29 April	07 May	14 May	Yalova
	Şen-2	22 April	28 April	04 May	Yalova
	07-KOR-I	27 April	06 May	11 May	Yalova
	Tokat-1	16 April	22 April	30 April	Yalova
	Kaman-1	10 May	15 May	21 May	Beynam Village
	Kaman-5	08 May	14 May	21 May	Beynam Village
	Yalova-1	7 May	10 May	16 May	Yalova
	Bilecik	5 May	8 May	15 May	Yalova
2003	07-KOR-I	7 May	12 May	18 May	Yalova
	Tokat-1	5 May	7 May	15 May	Yalova
	Kaman-1	6 May	9 May	16 May	Yalova

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and year. In addition, 41 to 383 flowers on 3 trees for open pollination in these genotypes were selected as controls during the studies. Trees on walnut seedlings were approximately 25 years old. The pollination bags, which were double layer, were removed 2 weeks after pollination, and fruit set by apomixis and open pollinated flowers were determined 8 weeks after anthesis.

The data were analyzed using analysis of variance (ANOVA) in accordance with an F-test ($P = 0.05$) and means were compared by least significant difference (LSD) ($P \leq 0.05$). Transformed angle values were used for percentage data.

Results and Discussion

As shown in Table 2, the percentage of apomictic fruit set of the walnut genotypes was very poor. In the first year, only 07-KOR-I had one apomictic fruit. The percentage of apomictic fruit set was only 1.6% in this genotype. In the second year, apomictic seeds occurred in Yalova-1, Bilecik, Tokat-1 and Kaman-1. The percentage ranged from 0.5% to 1.9%. These data indicate that apomictic fruit set is insufficient for economic seed and crop production in the walnut genotypes. The result is in agreement with previous reports (Badalov, 1989; Terziiski and Stefanova, 1990; Valdiviesso, 1990; Gao et al., 1999; Zhang et al., 2000; Soylu and Ertürk, 2001). Soylu and Ertürk (2001) reported that apomictic fruit set ratio in Yalova 2 walnut cultivar was 4%. However, Loiko (1990) and Solar et al. (1995) reported that apomixis rates were very high in some walnut genotypes and ranged from 23.5% to 81.2%. Furthermore, according to Soylu and Ertürk (2001), the apomictic fruit set in the 1974/7 walnut type was 36%.

Apomictic fruit set without pollination occurred in 5 walnut genotypes, Yalova-1, Bilecik, 07-KOR-I, Tokat-1

Table 2. Fruit set by apomixis in some Turkish walnut (*J. regia* L.) genotypes.

Year	Genotype	The number of pistillate flower	The number of apomictic fruit	The percentage of apomictic fruit set (%)
2001	Yalova-1	55	-	-
	Şebin	64	-	-
	Bilecik	57	-	-
	KR-1	-	-	-
	KR-2	65	-	
	Şen-2	71	-	-
	07-KOR-I	62	1	1.6
	Tokat-1	48	-	-
	Kaman-1	84	-	-
	Kaman-5	80	-	-
2002	Yalova-1	185	1	0.5
	Şebin	204	-	-
	Bilecik	227	2	0.9
	KR-1	215	-	-
	KR-2	174	-	-
	Şen-2	165	-	-
	07-KOR-I	210	-	-
	Tokat-1	205	4	1.9
	Kaman-1	192	1	0.5
	Kaman-5	180	-	-
2003*	Yalova-1	197	-	-
	Bilecik	200	2	1.0
	07-KOR-I	200	1	0.5
	Tokat-1	171	11	6.4
	Kaman-1	160	2	0.6

* Pistils of 5 genotypes that had apomictic fruit set in 2001 and/or 2002, were pollinated with pollen of the apple cv. 'Golden Delicious' (*Malus x domestica* Borkh.) to stimulate apomictic fruit set in 2003.

and Kaman-1, in 2 years. As a result of pollination with apple pollen, apomictic fruit set (6.4%) was only stimulated in Tokat-1 genotype. Liu et al. (1999) reported that when pistillate flowers of walnut in bags were pollinated with Chinese pine (*Pinus tabulaeformis*) or non-activated walnut pollen, non-activated walnut pollen increased the rate of apomixis, while Chinese pine pollen did not affect the rate.

In 2000, prior to the study, it was stated that the apomictic fruit set ratios in Kaman-1 and Kaman-5 walnut genotypes are very high and these genotypes could be propagated by seeds and fruits set without pollination (personal communication). However, our results showed that Kaman walnut selections did not have a tendency to undergo apomixis. Kaman-1 had only one apomictic fruit in 2002 and 2003, but Kaman-5 did not have any.

In the present study, the percentage of fruit set in open pollinated flowers of 10 Turkish walnut genotypes ranged from 7% to 93.3% in different years (Table 3). There were significant differences among the genotypes in each year. The rate of fruit set ranged from 65% to 93.3% in KR-1, KR-2, Kaman-1 and Kaman-5, while it ranged from 7% to 56.1% in the other cultivars and selections (Table 3). Westwood (1993) reported that the fruit set ratio in walnut may be 50% or more of their flowers. The fruit set ratios of Yalova-1, Şebin, Bilecik, Şen-2, 07-KOR-l and Tokat-1 were lower than 50%, but no problems concerning the productivity of these genotypes were reported.

Conclusion

In the present study, data on Yalova-1, Şebin, Bilecik, KR-1, KR-2, Şen-2, 07-KOR-l, Tokat-1, Kaman-1 and

Table 3. Fruit set by open pollinated flowers of some Turkish walnut (*J. regia* L.) genotypes.

Year	Genotype	The number of pistillate flower	The number of fruit	The percentage of fruit set (%)
	Yalova-1	77	25	32.5 de*
	Şebin	78	29	37.2 d
	Bilecik	78	22	28.2 de
	KR-1	-	-	-
2001	KR-2	45	42	93.3 a
	Şen-2	66	15	22.7 e
	07-KOR-l	106	39	36.8 d
	Tokat-1	41	23	56.1 c
	Kaman-1	133	118	88.7 b
	Kaman-5	68	58	85.3 b
	Yalova-1	302	147	48.6 c*
	Şebin	383	84	21.9 d
	Bilecik	246	119	48.3 c
	KR-1	238	163	68.4 b
2002	KR-2	291	204	70.1 b
	Şen-2	217	49	22.5 d
	07-KOR-l	234	101	42.7 c
	Tokat-1	174	79	45.4 c
	Kaman-1	76	65	85.5 a
	Kaman-5	97	80	82.5 a
	Yalova-1	100	7	7.0 c*
	Bilecik	100	26	26.0 b
2003	07-KOR-l	100	25	25.0 b
	Tokat-1	100	37	37.0 b
	Kaman-1	100	65	65.0 a

*Letters in the same column for each year indicate significant differences (LSD, $P \leq 0.05$).

Kaman-5 showed that apomixis was observed in 5 walnut genotypes, Yalova-1, Bilecik, 07-KOR-l, Tokat-1 and Kaman-1, in very low levels. Further research is needed to form lines from few apomictic seeds for clonal propagation. In vitro techniques such as somatic embryogenesis from immature cotyledons of apomictic seeds could be useful to solve the problem.

At present, immature cotyledons of seeds thought to be of apomictic origin are in culture for somatic embryogenesis. At the same time, DNA based molecular studies to identify the apomictic origin will be performed. This should offer new possibilities for genetic studies and breeding.

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