AcademicPres

Available online at www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic 1842-4309

Not Bot Horti Agrobo, 2012, 40(1):229-233

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

Determination of Apomictic Fruit Set Ratio in Several Romanian Walnut (*Juglans regia* L.) Cultivars

Sina COSMULESCU[1*], Mihai BOTU[1,2], Gheorghe ACHIM[1,2]

[1]*University of Craiova, Department of Horticulture and Food Science, Agriculture and Horticulture Faculty, 13 A.I. Cuza Street, 200585, Craiova, Romania; sinacosmulescu@hotmail.com (*corresponding author)*

[2]*University of Craiova, SCDP Valcea, 464 Calea lui Traian Str., 240273, Ramnicu Valcea, Romania; btmihai@hotmail.com, gheorgheachim555@yahoo.com*

Abstract

Apomixis, a natural process that allows clonal propagation by seed, is an important feature of walnut (*Juglans regia* L.). This study was carried out to determine the ability of apomictic fruit setting in twelve Romanian walnut cultivars. 'Sibisel 44', 'Geoagiu 65', 'Germisara', 'Muscelean', 'Sarmis', 'Valcor', 'Valmit', 'Valrex', 'Jupanesti', 'Velnita', 'Orastie' and 'Argesean' cultivars were used in the experiment. Female flowers were isolated with pergament paper bags and the apomictic fruit set was determined 8 weeks after anthesis. The apomixis degree was determined as the number of fruit calculated in relation to the number of isolated flowers. The percentage of apomictic fruit set without pollination in cultivars analyzed is low, ranging from 7.86% ('Orastie' cultivar) up to 12.46% ('Jupanesti' cultivar). The results indicated that apomictic fruit set is insufficient for economical seed and crop production in these Romanian walnut cultivars.

Keywords: apomictic rate, bloom date, cultivars, dichogamy, *Juglans regia*

Introduction

Apomixis is a natural trait of reproduction in plants where the embryo is formed without the union of male and female gametes. Plants produce fruits with genetically identical seeds to their female parent (Ulukan, 2009). Apomixis provides a method of clonally propagating plants through seed (Koltunow *et al*., 2001; Spillane *et al*., 2001) and is of great interest owing to its potential application in crop improvement (Albertini *et al*., 2009).

Walnut (*Juglans regia* L.) is a heterozygous plant. Research conducted in walnut fruit formation, have shown that some of the genotypes can form fruits without fecundation, through parthenocarpy or apomixis. Opportunities for fruit formation without pollination in walnut, under normal conditions, have raised a keen interest among researchers in biology of flowering and seed formation (Badalov, 1983; Germain *et al*., 1973; Hanna and Bashaw, 1987; Laiko, 1990; Valdiviesso, 1990). In terms of economic analysis, the phenomenon of parthenocarpy is undesirable because some of the fruits resulted in this way are small, dry, with no commercial value. Fruit formation by apomixis, has, however, theoretical and practical importance; the embryo is homozygous and it accurately transmits the characters of mother plant. Thus, walnut breeders can use apomixis in traditional breeding programs for cultivar improvement (Guo-Liang *et al*., 2007; Hanna, 1995).

Many researchers have reported apomixis in walnut (Germain *et al*., 1973; Laiko, 1990; Şan and Dumanoğlu, 2006; Valdiviesso, 1990), but it was found that the formation of fruit is weak, almost zero in some cases, so there is no apomictic crop to be expected. Sometimes apomixis percentage is higher, between 23.5% and 81.2%, in some of the genotypes (Laiko, 1990; Solar *et al*., 1995), but apomictic nuts cannot be always obtained. A higher percentage of fruit formation through apomixis (the average apomixis rate in walnut was 38.25%, while the highest value was 78.6%) is described by Guo-Liang *et al*. (2007) under conditions in China.

Guoliang *et al*. (2010) reported a new cultivar of walnut tree ('Qinquan 1'), which was selected from walnut seedling resources in the Northern China and characterized by apomixis; the average apomixis rate was 48.53%, and the highest rate could reach up to 75.7% (Wu *et al*., 2010). Under conditions in Turkey, the percentage of apomictic fruits has varied between 0.5% and 1.6% (Şan and Dumanoğlu, 2006). Asadian and Pieber (2005) reported a maximum apomixis percentage of 58% in 'Milotai 4/R' variety, in the experiments carried out in Austria, and Valdiviesso (1990) showed that the growth of apomictic fruits in 'Rego' cv. was 7% in Portugal.

In Romania there were found no cultivars or types to possess valuable features of apomixis (Cosmulescu, 2003). Experiments made on walnut types in Oltenia showed that they are characterized by a low ability to form fruits without pollination. Among all isolated flowers, the percentage of fruits obtained was between 0-4.3% (Cosmulescu, 2003).

Having in view that apomixis-an asexual method of reproduction through the seed- provides unique opportunities for developing superior cultivars in the future (Hanna and Bashaw, 1987), the objective of this study was to determine the genetic potential for apomixis in Romanian walnut cultivars. Identification of apomictic cultivars is an important issue for solving the genetic variation.

Materials and methods

The experiments were conducted at the experimental walnut orchard (Fig. 1) belonging to the University of Craiova and located at SCDP Valcea Research Station, Romania (45°08' N and 24°22' E). The study period was over 3 years (2008-2011). During this period, the average annual temperature in the area was 11.3°C (1.1°C more than the multi-annual average). The absolute minimum temperatures and the lack of spring frosts have not caused damages to catkins and female walnut flowers. The annual average of rainfall was 771.5 mm (59.5 mm more than the multi-annual average). Twelve Romanian walnut cultivars (18 to 20 year-old trees), with different types of flowering, different pistillate bloom dates (Cosmulescu *et al*., 2010a) and good quality fruits (Cosmulescu *et al*., 2009, 2010b) were tested (Tab. 1).

Tab. 1. Cultivars, type of flowering and bearing of walnut cultivars

No.	Cultivar	Type of dichogamy	The pistillate bloom dates*
1	'Sarmis'	protogynous	early/ semi-early
2	'Sibisel 44'	protogynous	early/ semi-early
3	'Valcor'	protogynous	early/ semi-early
4	'Valmit'	protogynous	early
5	'Valrex'	protogynous	early/ semi-early
6	'Jupanesti'	protandrous	semi-early / semi-late
7	'Argesean'	protogynous	early/ semi-early
8	'Geoagiu 65'	protogynous	early/ semi-early
9	'Germisara'	protogynous	early/ semi-early
10	'Muscelean'	protandrous	early
11	'Orastie'	protogynous	early/ semi-early
12	'Velnita'	protogynous	early/ semi-early

*early (April 20-30), semi-early (May 1-5), semi-late (May 6-10),late (after May 10)

A number of flowers were randomly chosen and then isolated with pergamin paper pocket. Before bagging, any male flowers were removed. Bags were removed after 10 to 15 days, when stigmata were fully dry, and the formation of apomictic seeds was determined 8 weeks after anthesis. The apomixis degree was determined as the number of fruits calculated in relation to the number of isolated

Fig. 1. Research field images

Tab. 2. Descriptive statistics for apomictic rate in twelve Romanian walnut cultivars analyzed

Cultivars /descriptive statistics	'Sarmis'	'Sibisel 44'	'Valcor'	'Valmit'	'Valrex'	'Jupanesti'	'Argesan'	'Geoagiu 65'	'Germisara'	'Muscelean'	'Orastie'	'Velnita'
Mean	9.83	10.16	11.13	10.43	9.43	12.46	8	9.53	9.33	8.36	7.86	9.66
Standard Error	1.87	1.07	2.58	2.10	1.50	2.32	0.79	1.30	1.71	0.78	0.84	1.86
Standard Deviation	3.25	1.85	4.48	3.65	2.60	4.02	1.37	2.25	2.96	1.36	1.45	3.23
Sample Variance	10.58	3.45	20.12	13.32	6.80	16.16	1.89	5.08	8.81	1.86	2.12	10.45
Range	6.5	3.4	8.3	6.8	4.9	8	2.7	4.5	5.8	2.7	2.7	5.6
Minimum	6.5	8.9	6	7.8	7.5	8.7	6.5	7.2	6.8	6.9	6.2	7.8
Maximum	13	12.3	14.3	14.6	12.4	16.7	9.2	11.7	12.6	9.6	8.9	13.4

Tab. 3. Analysis of apomixis rate diferences between pairs of walnut cultivars with multiple range *t* test

	'Valcor'	'Valmit'	'Sibisel 44'	'Sarmis'	'Velnita'	'Geoagiu 65'	'Valrex'	'Germisara'	'Muscelean'	'Argesan'	'Orastie'
'Jupanesti'	1.33	2.03	2.30*	2.63*	2.80*	2.93*	3.03*	3.13**	4.10**	4.47***	4.60***
'Valcor'		0.70	0.97	1.30	1.47	1.60	1.70	1.80	2.77*	3.13**	3.27**
'Valmit'			0.27	0.60	0.77	0.90	1.00	1.10	2.07	2.43*	2.57*
'Sibisel 44'				0.33	0.50	0.63	0.73	0.83	1.80	2.17	2.30*
'Sarmis'					0.17	0.30	0.40	0.50	1.47	1.83	1.97
'Velnita'						0.13	0.23	0.33	1.30	1.67	1.80
'Geoagiu 65'							0.10	0.20	1.17	1.53	1.67
'Valrex'								0.10	1.07	1.43	1.57
'Germisara'									0.97	1.33	1.47
'Muscelean'										0.37	0.50
'Argesean'											0.13
LSD 5%=2.28 LSD 1%=3.11 LSD 0.1%=4.17											

flowers. Experiments were repeated over 3 years. Similar methodologies were used by other authors (Guo-Liang *et al*., 2007; Şan and Dumanoğlu, 2006).

The data were analyzed by using Microsoft Excel®, Descriptive Statistics. Mean comparisons were performed by using multiple range *t* test to examine if differences between cultivars were significant at $p<0.05$.

Results and discussion

The twelve cultivars examined have diverse apomictic capacity (Tab. 2). Their average annual apomictic rate ranged from 7.86% ('Orastie' cultivar) up to 12.46% ('Jupanesti' cultivar). An apomixis rate higher than 10% (average rate over the 3 years) was recorded in only four cultivars ('Jupanesti', 'Valcor', 'Valmit', 'Sibisel 44'). Variance analysis showed that the apomictic rate has significantly differed between the cultivars and years. Apomictic ability of 'Jupanesti' was the highest (12.46%), but the difference of their average value (8.7%/16.7%) was greater than three years. 'Valcor' cultivar had the highest difference of apomictic rate over the three years (6%/14.3%), which were affected by variation (20.12). A low variation of apomixis rate was recorded in cultivars 'Orastie' (2.12%), 'Argesean' (1.89%) and 'Muscelean' (1.86%).

In comparing with other results obtained in different cultivars and other ecological conditions, the apomixis rate found in cultivars analyzed is low. Solar *et al*. (1995) reported that apomixis rates were very high in some walnut genotypes; the average degree of apomixis ranging from 23.5% ('G-26') to 31.5% ('Elit'). According to Soylu and Ertürk (2001), the apomictic fruit set in the 1974/7 walnut type was 36%. Four of the walnut cultivars examined by Guo-Liang *et al*. (2007) had apomictic tendencies and their average apomictic rate was 38.25%; the highest observed rate was 78.6%.

The results obtained in the cultivars analyzed in this study indicated that apomictic fruit set is insufficient for economic seed and crop production in the Romanian walnut cultivars. The result is in accordance with the previous research. Soylu and Ertürk (2001) reported that apomictic fruit set ratio in 'Yalova 2' walnut cultivar was 4%; Valdiviesso (1990) stated for 'Rego' cv the rate of 7% growth of apomictic fruits; and Gao *et al*. (1999) found out that apomixis rates of four apomixis varieties were between 1.5% and 13.0%.

In the present study, the differences among the cultivars are statistically significant. Analysis of apomixis rate diferences between pairs of walnut cultivars with multiple range *t* test (Tab. 3) showed the existence of important differences among cultivars. The cultivar 'Jupanesti' was shown to be notably different in apomixis rate from 'Orastie' and 'Argesean' cultivars (differences from them were 4.60 and 4.47%, respectively); while it was not much differing from 'Valmit' and 'Valcor' (differences were 2.03 and 1.33%, respectively). Differences recorded in apomixis rate in cultivars studied are likely caused by environmental conditions; a fact that was upheld by other researchers too (Guo-Liang *et al*., 2007). Regarding the correlation between type of dichogamy, the pistillate bloom dates and apomixis rate, the literature upholds that apomixis is more common in protandrous cultivars than in the protogynous ones; and genotypes with late flowering are forming parthenocarpic fruits more often than those with early flowering. Probably the apomixis percentage of 12.46% obtained in 'Jupanesti' cultivar would have an explanation in the flowering time (semi-early / semi-late) and protandrous type of dichogamy. The same explanation could be used for other cultivars too, which are forming fruits with no late pollination (in nursery), because flowering and fecundation occur over a period when pollen source is missing. The research conclusion was in accordance with that of Solar *et al*. (1995), who considers that the average degree of apomixis is high in late leafing cultivars.

Conclusions

The results of flower's bagging isolation showed that the twelve tested Romanian walnut cultivars have differed in terms of apomictic ability, and the average apomictic rate was between 7.86% and 12.46%. Apomictic ability was significantly different both over the years and among the cultivars. These data indicate that apomictic fruit set is insufficient to assure economical seed and crop production in walnut genotypes.

Acknowledgements

This work was supported by the Romanian CNCSIS-UEFISCSU, project number PNII- IDEI code 430 /2008.

References

Albertini E, Barcaccia G, Mazzucato A, Sharbel TF, Falcinelli M (2009). Apomixis in the era of biotechnology, p. 405-436. In: Pua EC, Davey M (Eds.). Plant Developmental Biology -Biotechnological Perspectives: Volume 1, Berlin, Springer Verlag.

Asadian G, Pieber K (2005). Morphological variations in walnut varieties of the Mediterranean regions. Internat J Agricult Biol 7:71-73.

Badalov PP (1983). The use of methods of hybridization and apomixis for obtaining the perspective forms of walnut. Forest Prod 12:24-25 (in Russian).

Cosmulescu SN (2003). Biological peculiarities in common walnut, p. 30-50. In: Cociu V (Ed.). Culturile Nucifere. Editura Ceres, Bucureşti.

Cosmulescu S, Baciu A, Achim G, Botu M, Trandafir I (2009). Mineral composition of fruits in different walnut (*Juglans regia* L.) cultivars. Not Bot Horti Agrobo 37(2):156-160.

Cosmulescu S, Baciu A, Botu M, Achim GH (2010a).

Environmental factors' influence on walnut flowering. Acta Hort 861:83-88.

Cosmulescu S, Trandafir I, Achim G, Botu M, Baciu A, Gruia M (2010b). Phenolics of green husk in mature walnut fruits. Not Bot Horti Agrobo 38(1):53-56.

Gao S, Liu C, Liu D, Li L, Ye N, Chen Y (1999). Apomixis Characteristics of *Juglans regia* L. J Northwest For Univ 14(1):45-47.

Germain E, Jalinat J, Marchou M (1973). Biologie florale du noyer. Bull. Technol. Inform. Min Agr Development Rurale FR. N282:661-673.

Guoliang W, He L, Qunlong L, Yong W, Pengfei Z (2010). 'Qinquan 1', a new apomixis walnut cultivar. Fruits 65:39-42

Guo-Liang W, Yan-hui C, Peng-fei Z, Yang Jun-qiang Y, Yu-qin S (2007). Apomixis and new selections of walnut. Acta Hort 760:541-548.

Hanna WW (1995). Use of apomixis in cultivar development. Adv Agron 54:333-350.

Hanna WW, Bashaw EC (1987). Apomixis: its identification and use in plant breeding. Crop Sci 27(6):1136-1139.

Koltunow AM, Scott NS, Chaudhury AM (2001). The use of apomixis in cloning horticultural plants: current applications and molecular prospects. Acta Hort 560:333-343.

Laiko RE (1990). Apomixis of walnut. Acta Hort 284:233-236.

Solar A, Smole J, Simonic S (1995). The ability of apomictic fruit setting in five walnut cultivars (*Juglans regia* L.). Zbornik Biotehniske Fakultete Univerze v Ljubljani, Kmetijstvo 65:103-110.

Soylu A, Ertürk Ü (2001). Research on apomictic seed formation in some walnut cultivars. Proceedings of Turkey First National Symposium on Walnut, Tokat (Turkey), 133-137 p.

Spillane C, Steimer A, Grossniklaus U (2001). Apomixis in agriculture: the quest for clonal seeds. Sexual Plant Reproduct 14(4):179-187.

Şan B, Dumanoğlu H (2006). Determination of the apomictic fruit set ratio in some Turkish walnut (*Juglans regia* L.) genotypes. Turk J Agric For 30:189-193.

Ulukan H (2009). A new alternative for plant breedings, biodiversity and environmental sustainability: apomictics. J Biol Sci 9(8):788-795.

Valdiviesso T (1990). Apomixis in Portuguese walnut varieties. Acta Hort 284:279-283.

Wu GL, Hou LQ, Zhang ZH, Tian JB (2010). New chinese cultivar resources of J*uglans* with special characteristics. Acta Hort 861:155-162.