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Recent Advances in the Study of Apomixis in *Juglandaceae*

Guo-liang Wu[1,2*] • Yu-qin Song[2] • Jaime A. Teixeira da Silva[3] • Qun-long Liu[2] • Lan Ji[2] • Jun-qiang Yang[4] • Peng-fei Zhang[5] • Jin-he Bai[6]

[1] College of Forestry and Horticultural Science, Henan Agricultural University, Zhengzhou, 450002 Henan, China
[2] College of Horticultural Science, Shanxi Agricultural University, Taigu, 030801, Shanxi, China
[3] Department of Horticultural Science, Faculty of Agriculture, Kagawa University, Miki-cho, Ikenobe, Kagawa-ken, 761-0795, Japan
[4] Institute of Horticultural Science, Shanxi Academy of Agricultural Science, Taiyuan, 030001, Shanxi, China
[5] Agricultural High Technological School of Xinyang, Xinyang, 464000, Henan, China
[6] Mid-Columbia Agricultural Research and Extension Center, Oregon State University, Experiment Station Drive, Hood River, 3005, OR, USA

***Corresponding author*: * walnut-wu@126.com**

ABSTRACT

Walnut, *Juglans regia* L. is the most economically important member of the *Juglandaceae* family and is the most valuable nut crop in the world. Recently, it has increased greatly in acreage and in production. Although a number of research programs about plant apomixis have been carried out around the world, few were representd by the *Juglandaceae*. This paper presents a brief review of the current status of knowledge in *Juglandaceae* plant apomixis research, especially in walnut, including chemicals induction, bagged isolation, observation of embryology, analysis of isoenzymes, physiological and biochemical characteristics of apomictic seedlings. We further introduce a strain which may be an obligate apomictic walnut, and discuss the problems associated with and the developmental prospects and progress in this field.

Keywords: bagged isolation, embryology, isoenzyme, new strain, walnut (*Juglans regia* L.)

Abbreviations: BA, 6-benzylandenine**; 2,4-D**, 2,4-dichlorophenoxyaceticacid; **IBA**, indole-3-butyric acid; **Kn**, kinetin**; LH**, lactalbumin hydrolysate; **POD**, peroxydase; **DKW**, Driver and Kuniyuki for walnut culture medium

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INTRODUCTION

Some *Juglandaceae* plants are important cultivated forms and have economic value in the world. They belong to the *Juglans* and *Carya* genera. The major forms in China include *Juglans regia* L., *J. sigillata* Dode., *J. cathayensis* Dode., *J. hopeiensis* Hu, *J. mandshurica* Max., *J. nigra* L., *Carya cathayensis* Dode. and *C. ililnoensis* (Wangenh.) K. Kohn. In China, walnut (*Juglans regia* L.) has the top growth area (reached 1300 thousand hm^2 in 2000 and output reached 300.98 thousand t), and there exist more than 4000 cultivars in China (Xi *et al*. 2005). Walnut originated from south-eastern Europe and western Asia and apomictic traits were found many years ago (Loiko 1990; Asadian *et al*. 2005). In fact, apomixis produces seeds through asexual processes. The genetic make-up of the seeds is identical to that of the mother plant. If the mother plant is well adapted to a particular environment or purpose, so will be the offspring (Jefferson 1994; Spillane *et al*. 2001a). Accordingly, to make use of apomictic walnuts to breed seed-

Received: 7 January, 2007. Accepted: 28 January, 2007.

Invited Mini-Review

lings will provide a new way to extend improved cultivars for walnut production (Koltunow 2003).

The selection of varieties with obligate apomixis reduces the effect of bad climatic conditions on yield, and is especially important in the breeding of new varieties in cold regions (Loiko 1990; Valdiviesso 1990) where lower temperature and strong wind would limit the pollination badly.

This paper reviews studies on apomixis in walnut and other related species in order to provide some insight into walnut research and production.

THE CONCEPT AND SIGNIFICANCE OF PLANT APOMIXIS

Concept

The term apomixis was formerly used as a synonym of vegetative propagation. At present, it is preferentially restrictted to the sense of agamospermy, i.e. asexual reproduction by seeds (Jefferson 1994; Sun *et al*. 1996). In apomictic reproduction, the embryo develops autonomously from an unreduced cell having the same set of maternal chromosomes and giving rise to plants that are clones of the mother plant (Sun *et al*. 1996; Carneiro *et al*. 2006).

Classification

Apomixis is a wide-spread phenomenon in the plant kingdom present in more than 36 families of Angiosperms (Hanna and Bashaw 1987; Pichot *et al*. 2001). According to the degree of apomixes, the process can be classified into two types: obligate and facultative. In obligate apomixis, there is no fusion of male and female gametes and the egg cell develops autonomously, by parthenogenesis, generating an embryo that keeps the same set of maternal chromosomes, and leading to the formation of embryos genetically identical to the mother plant.

In facultative apomixis, the plant can reproduce both sexually and asexually, leading to progeny plants with new features. These are desired traits during plant breeding to generate new genotypes better adapted to biotic and abiotic environmental factors.

There is no clear-cut separation between the two types. With a few exceptions it has been observed that apomictic species are polyploid, while the sexual varieties of the same species are diploid (Dong *et al*. 1996; Zhou *et al*. 1998). It is known that most apomicts are facultative apomixis (Sun *et al*. 1996; Gao *et al*. 2000; Carneiro *et al*. 2006).

According to the origin of apomictic generants, apomixis can be differentiated into three types: diplospory, apospory and adventitious embryogenesis. In diplospory, there are two forms (mitotic and meiotic) during gametophytic development, the megaspore-mother cell does not enter meiosis or is arrested at an early stage during meiosis and only undergoes mitotic divisions without a reduction of the genome. In both forms, an embryo sac is formed consisting of an unreduced, diploid egg cell (Leblanc *et al*. 1995). In apospory, aposporous initial cells form from nucellar cells and differentiate after three mitotic divisions into embryo sacs containing a diploid egg cell that directly develop into an embryo without fertilization. This type is common in the Poaceae. Adventitious embryogenesis initiates from the somatic tissues of the ovule, nucellar or integumental cells. These cells developed directly into embryos and compete with the sexual embryo that is formed after fertilization. This type is lacking in the Poaceae but is common in other families (Koltunow 1993; Leblanc *et al*. 1995; Ma *et al*. 2001).

Recent progress

It was always thought that apomixis occurred in all Angiosperms, although only few reports occurred in woody plants, mainly in *Malus* spp. (Dong *et al*. 1996), walnut (Schanderl 1964), *Xanthoxylum bungeanum* (Liu Y-H *et al*. 1987), orange (*Citrus*) (Cai *et al*. 1997) bramble (*Rubus alceifolius*) (Amsellem *et al*. 2001) and so on. Recent studies showed that a new species in the *Clusiaceae*, *Hypericum carpatium* demonstrated apomixis, and that it may be a hybrid of *H. maculatum* × *H. perforatum*, with features from the paternal *H. perforatum* (Martonfi 2001). Newly discovered *Cotoneaster* spp. (Rosaceae), such as *Cotoneaster brickellii*, *C. latifolius*, *C. purpurescens*, *C. vandelaarii*, *C. thimphuensis*, *C. hualiensis*, *C. ignescens*, *C. lancasteri*, *C. kingdonii*, *C. shannanensin* and *C. yui*, had apomictic feature (Fryer and Hylmo 2001; Bartish *et al*. 2001). All plants in the *Rosa* section *Caninae* were polyploid and apomicts (Werlemark 2000; Schranz *et al*. 2005).

It was previously thought that apomixis did not occur in the gymnosperms. However one study detected apomixis for the first time in Mediteranean cypress (*Cupresus dupreziana*) (Pichot *et al*. 2001). In this study paternal apomixis resulted from androgenesis. These results in their totality reveal the universality of apomixis in the plant kingdom (Ma S-M *et al*. 2004).

In ground-breaking studies, Chen and Guan (2006) describe how apomixis-related genes can be cloned into higher plants, using guinea grass (*Panicum maximum*) and bahia grass (*Paspalum notatum*) as model plants.

Methods to determine apomixis

At present there are many methods to study and identify plant apomixis, with few comprehensive reviews (Ma *et al*. 2002; Carneiro *et al*. 2006). Ways to study the apomictic characteristics of walnut include induction with chemicals (Xi *et al*. 1996), investigation of the setting ratio by bagging isolation (Loiko 1990; Valdiviesso 1990; Gao S-T *et al*. 1999; Wang G-A *et al*. 2003; Ghavamaldin *et al*. 2005; Bekir *et al*. 2006a, 2006b), observation of the embryo origin and developmental stage by embryology (Chen Y-F *et al*. 2000; Yang *et al*. 2006; Wu G-L *et al*. 2007), analysis of biochemical characteristics by isoenzymes, observation of morphological features of nuts, and investigation of the germination and morphological features of the apomictic seedlings (Wang G-A *et al*. 2003; Yang J-Q *et al*. 2006; Wu *et al*. 2007).

To determine the rate of apomixis, a number of walnut female flowers were isolated and after three weeks bags were removed and flowers counted and recounted after 14 days again. During harvest the number of fruits was also counted. Cultivars having fruits in the bags were recognized as cultivars with apomixis. The degree of apomixis was determined as the number of fruit calculated to the number of isolated flowers.

The degree and capacity of apomixis was also determined as the number of fruit calculated to the number of open-pollination flowers (Zhang *et al*. 2000). Different standards in studying walnut apomixis by different researchers can result in difficulties coordinating research. Consequently, since most researchers defined apomixis as the number of fruits relative to the number of isolated flowers in walnut, we also adopted this definition in this review.

RECENT ADVANCES IN THE STUDY ON APOMIXIS IN *JUGLANDACEA*

The study of apomixis in *Juglandaceae* plants involved intra- and interspecific *Juglans* spp., such as *J. regia* L., *J. nigra* L., *J. mandshurica* Maxim., *J. cinerea* L., *J. sieboldiana* Maxim. and two interspecific hybrids (Badalvo 1983), however, most of them were about walnut (*J. regia* L.).

Chemical induction

Chemically-induced apomixis is rare, and the only report was from Hebei Agricultural University of China, in 1962-1963 using 2,4-D and other chemical. The fruit set ratio treated with 2,4-D at 10-30 mg.l^{-1} was 3.2%-18.5% (Xi R-T *et al*. 1992).

Isolation studies

Presently, there are two ways to isolate walnut female flowers: one is by using bags made of a sleeve of paper to implement spatial isolation; the other is by artificial fertilizing the chapitel or mentor with several kinds of plant pollen and then blocking it.

Study of bagged isolation

Reviewing the literature related to walnut apomixis, it can be seen that the main method of researching walnut apomixis has been to isolate the female flowers with bags, so that parthenocarpy can be artificially induced.

In general, *Juglandaceae* plants (with few exceptions) display a certain frequency of apomixis despite the different cultivars, different years and different regions. A comparison of plants from the same region and of the same cultivar but from different years showed great differences in their apomixis features.

From **Table 1** to **3** the data illustrates an irregular presentation of apomixis within the same region and over different years: some cultivars did not demonstrate apomixis over two successive years, like 'Liaohe No. 3' (Yangling, Shannxi, China); some cultivars changed a little in their capacity to develop apomixis, like 'Taishan No. 2' (Taian, Shangdong, China), 'Zhonglin No. 1' and 'Liaohe No. 1', but others changed irregularly. This phenomenon is mainly affected by environmental factors, especially air temperature in the different years, and all these plants are facultative apomicts. It is noticeable that cv. 'Zuoquanmian' had the highest percentage of apomixis in three years, especially in 2003 when other cultivars did not demonstrate apomixis, and its apomictic behaviour was steady. This cultivar is considered to be obligate apomict, which is in agreement with former conclusions (Loiko 1990).

The method that uses spatial isolation with bags after emasculation prevents pollen from reaching the female flowers. They are bagged approximately a week before pistillate blooming in walnut (Loiko 1990; Valdiviesso 1990; Gao S-T *et al*. 1999; Zhang *et al*. 2000; Wang G-A *et al*. 2003; Ghavamaldin *et al*. 2005). The bags used for isolation were composed of a sleeve of paper which had been waterproofed with tar, and they were tied at one end with a thin cloth which prevented pollen contamination, but allowing enough ventilation and light to enter (Valdiviesso 1990).

Mentor pollen isolation study

The results of a two-year study showed that it was possible to increase the apomictic fruit setting ratio from 0-8.92% (CK, or control) to 33.33%-55.0% by using inactive walnut pollen in fertilization; simultaneously, it was found that inactive walnut pollen can reduce the fall rate (Liu D-L *et al*. 1999). However, when pistillate flowers of walnut in bags were pollinated with Chinese pine (*Pinus tabulateformis*) pollen, there was almost no mentor effect in the rate of apomixis (Liu D-L *et al*. 1999).

The results of a study conducted in Turkey showed that the pistils of 5 genotypes (Yalova-1, Bilecik, 07-KOR-1, Tokat-1 and Kaman-1) that had apomictic fruit set could be pollinated with freshly collected pollen of apple cv. 'Golden Delicious '(*Malus domestic* Borkh.), and the percenttage of apomictic fruit set ranged from 0.5% to 1.9%. As a result of pollination with apple pollen, open pollination set was 37% (CK), while the highest apomictic fruit set was 6.4% ('Tokat-1') (San *et al*. 2006b).

Embryologic studies

Paraffin sections are an effective way to systematically observe the dissectional construction of the plant ovule. It allows one to observe the position of the gametes, megasporegenesis and formative process of the embyo sac, and to distinguish the phenomenon of meiosis and morphological characters of the ovule and other cells. These characteristics were the key to identifying apomixis in plants (Li *et al*. 1992; Ma S-M *et al*. 2002).

In earlier work, Schanderl suggested that the embryo obtained from apomixis was not formed in the place where the embryonic sac was usually found but rather situated near the micropyle, always in the area between the micropyle and the nucellar cavity. Sometimes it is formed very close to the micropyle or a bit far apart from it, but never more than 13 tiers of cells, from a nucellar somatic cell (Schanderl 1964).

The result of Chen Y-F (2000) showed that there was an

Table 1 The nut setting in walnut without pollination (Yangling, Shannxi, China) (from Gao S-T *et al*. 1999, with permission).

Cultivars	1996			1997		
	Female flowers	№ setting	%	Female flowers	№ setting	%
Xinlin No. 3	54	3	5.6	135	2	1.5
Liaohe No. 1	23	1	4.3	23	3	13.0
Liaohe No. 2	73	5	6.8	50	0	0
Xifu No. 1	32	0	0	40	5	12.5
Xiangling	7	0	0	20	0	0

Table 2 The nut setting in walnut without pollination (Taian, Shandong, China) (from Zhang *et al*. 2000, with permission).

Cultivars	1998			1999		
	Female flowers	№ setting	%	Female flowers	№ setting	%
Xingling	230	36	11.3	150	17	11.3
Fenghui	230	21	9.1	180	17	9.4
Luguang	200	18	9.0	210	28	13.3
Yuanfeng	160	21	13.1	230	33	14.3
Liaohe No. 1	110	15	13.6	180	28	15.6
Lufeng	150	20	13.3	190	18	9.5
Zhonglin No. 1	250	24	9.2	230	31	9.5
Taishan No. 1	350	42	12.0	150	18	12.0
Taishan No. 2	270	38	14.1	250	32	12.8

Table 3 The nut setting in walnut without pollination (Taigu, Shanxi, China) (from Wu *et al*. 2007, with permission).

Cultivars	2003			2004			2005		
	Female flowers	№ setting	%	Female flowers	№ setting	%	Female flowers	№ setting	%
Zhonglin No. 1	53	8	15.1	33	0	0	35	7	20.0
Liaohe No. 1	39	2	5.1	42	0	0	50	6	13.3
Taigu Mian	123	8	6.5	142	0	0	83	5	6.0
Zuoquan Mian	131	67	51.1	103	8	7.8	134	85	63.4

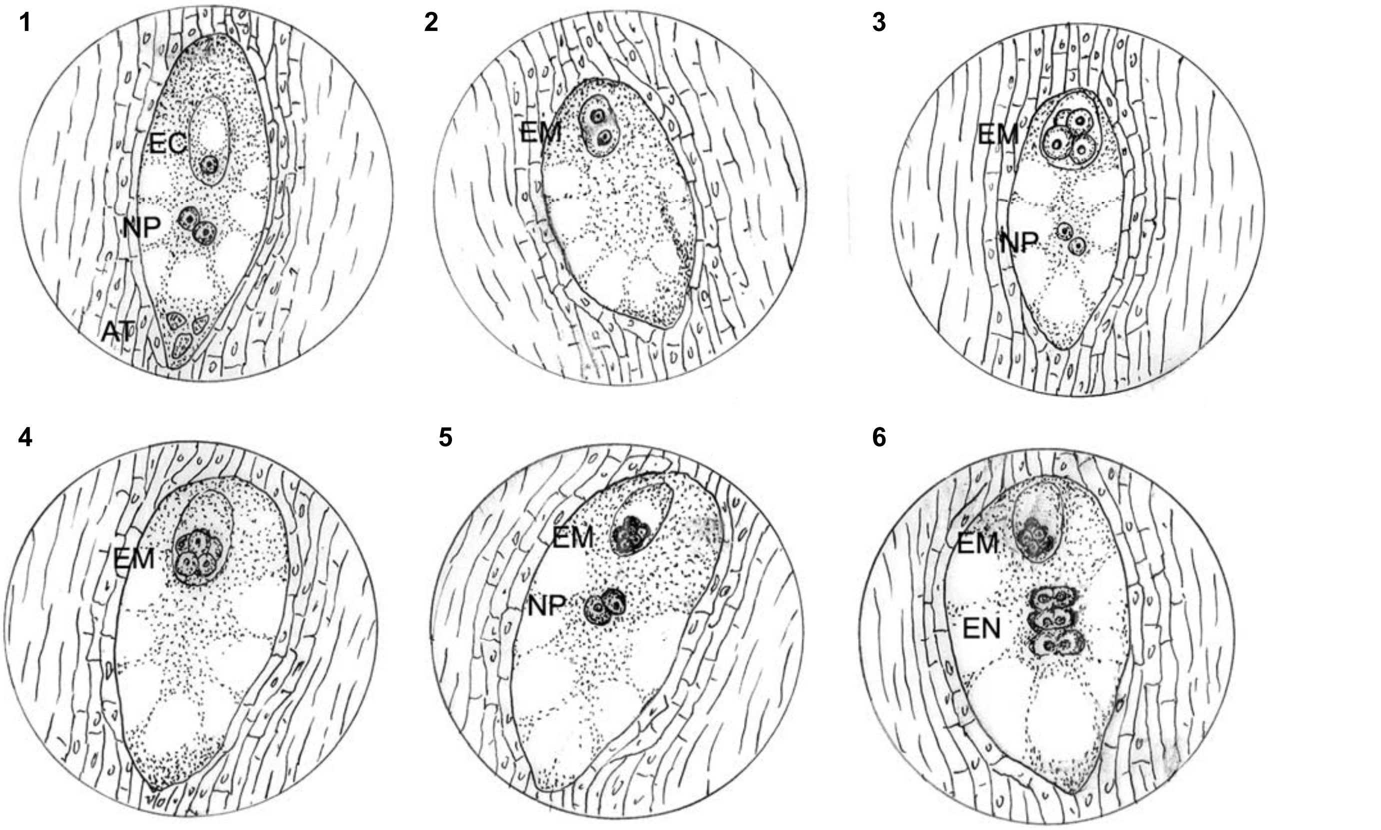

Fig. 1 Apomictic embryology of walnut showing the non-pollination embryo sac and embryo development. 1, egg cell, two polar nucleus (PN) and three antipodals (AT), ×800; 2, two-cell embryo (EM), ×550; 3, four-cell embryo (EM), ×550; 4, eight-cell embryo (EM), ×550; 5, the polar nucleus where fusion occurs, ×600; 6, three endosperm cells that split (EN), ×1000. (adapted from Chen Y-F *et al*. 2000 and Wu G-L *et al*. 2007, with permission)

intact and typical *Polygonum*-type of megaspore in three walnut cultivars (Xilin No.3, Liaohe No.1 and Xifu No.1), and the embryo derived from apomixis expectedly came from egg cells and remained parthenogenic. The egg cell of the embryo sac in apomixis began to cleave after a short period of dormancy (about two weeks) from a two-cell, four-cell, eight-cell up to a globular embryo, developed more and became a multicellular embryo (**Fig. 1**; Chen Y-F *et al*. 2000). Our recent results confirm this process of apomixis in walnut (Wu *et al*. 2007).

Studying the features of apomictic walnut seeds

Germination of seeds

Reaction of young embryos under tissue culture of walnut seeds

The percentage of germination of apomictic seeds was generally low, the amount was very poor and apomictic seeds could not always be obtained. However, somatic embryogenesis from immature cotyledons of a few apomictic seeds could provide the possibility of mass clonal propagation of cultivars in walnut species that are difficult to use in *in vitro* techniques such as shoot tip culture.

In a study of somatic embryogenesis, explants from immature cotyledons of seeds thought to be of apomictic origin of 4 genotypes were cultured 8 weeks after anthesis on an initial medium consisting of DKW (Driver and Kuniyuki,1984) medium for 3 weeks: DKW + 1 mg l^{-1} BA + Kn 2 mg l^{-1} + 0.01 mg l^{-1} IBA + 250 mg l^{-1} LH; then subculture on DKW + 0 (i.e. hormone-free) for four weeks; the percentage of cotyledons that formed embryos ranged from 3.6% to 25%, and secondary embryogenesis from somatic embryo explants progressed well (San *et al*. 2006a).

Germination of seeds

The germination of apomictic seeds is usually lower than seeds from open-pollination, averaging 74.1%, far less than from open-pollination (96.8%.; see **Table 5**). This phenomenon showed that embryo development was poor.

The cotyledons of apomictic walnut seeds developed poorly, frequently lacking a quarter or half the cotyledon. From **Tables 4** and **5** it can be seen that the germination of apomictic walnut seeds depends on the year, the average from 23.0%, 54.4% to 43.7% (see **Table 4**), but it was lower than CK (96.8%) (Loiko 1990; Wang G-A *et al*. 2003; Yang 2006).

There was a high rate of empty nuts (with a hardy shell and seed capsule but no cotyledon) in walnut nuts derived from apomixis following harvest, the average of two years reaching 7.1% while only 0.5% of nuts were derived from natural pollination (**Table 5**). The emergence rate (the ratio of walnut seed number to the walnut seedling number in the apomixes) of walnut arising from apomixis was relatively low with an average value of 74.1% in two years in Taigu and Shanxi (locations of tests: regions in north China) while 96.8% of walnuts resulted from natural pollination, outclassing apomixis.

A lot of walnut seeds developed incompletely with a quarter to half of the cotyledon missing following examination of apomictic walnuts. The average emergence rate in 'Xinjiang' early-fruit walnut with apomictic characteristics was 40.36%, much lower (see **Table 5**, 96.8%) than common walnut (Wang G-A 2003; Yang 2006).

The spherical embryo appeared at a later stage in apomictic walnut than from natural pollination and the apomictic embryos developed slowly, which may explain why nuts are empty or have a shriveled kernel when the walnut matures. Besides slow embryo development, the apomictic embryo also developed the endosperm poorly and the delay in its development also resulted in the incomplete development of matured nuts.

The main likely reasons for the degeneration and halted development of apomictic seeds were likely: i) there was no natural apomictic megaspore formed in the ovule; ii) the apomictic embryo degenerated in mid-development for

Table 4 The germination of walnut nuts having apomixis (Wensu, Xinjiang, China) (from Wang G-A *et al*. 2003, with permission).

Cultivars	1999			2000			2001		
	№ seeded	№ of seedlings	%	№ seeded	№ of seedlings	%	№ seeded	№ of seedlings	%
Zha343	15	2	11.7	8	6	75.0	6	4	66.7
Xinzaofeng	14	11	78.6	8	5	62.5	9	7	77.8
Xinxin2	5	2	40.0	14	2	14.3	14	4	28.6
Xinwen81	32	2	6.3	21	13	61.9	12	8	66.7
Xinwen179	46	6	13.0	36	15	41.7	32	3	9.4
Xinwen185	21	3	14.3	10	8	80.0	37	11	29.7
Xinfeng	19	1	5.3	24	12	50.0	29	6	20.7
Xincunfeng	34	5	14.7	4	2	50.0	34	17	50.0
Total	186	32	-	121	63	-	173	60	-
Average			23.0			54.4			43.7

Table 5 Germination rate of nuts from different walnuts (from Yang 2006, with permission).

Source	Percentage of empty nuts before sowing (%)			The emergence rate of seedlings (%)		
	2004	2005	Total	2004	2005	Total
Apomixis seedlings	6.7 (6/89)	7.4 (2/27)	7.1	72.3 (60/83)	76.0 (19/25)	74.1
Progeny from open-pollination	1.0 (1/100)	0 (0/30)	0.5	97.0 (96/99)	96.7 (29/30)	96.8

Table 6 Plant height of different biennial walnut seedlings (from Yang 2006, with permission).

Source of seedling	n	$\bar{x}$	σ	CV	u
Progeny from apomixis	60	51.4	12.4	0.2	3.38
Progeny from open-pollination	72	62.4	24.0	0.4	(u: 0.01=2.58）

Table 7 Isoenzyme zymogram of POD from apomictic seedlings (from Wu *et al*. 2007, with permission).

Source of seedling	Zymogram appearance percentage (%)		Regularity (%)
	Rf=0.38	Rf=0.29	
Progeny from apomixis	88	84	86
Progeny from open-pollination	77	33	55

being uncompetitive in absorbing and translocating nutrition.

Growth characteristics of apomictic seedlings

Seedlings derived from apomixis were significantly (P = 0.01) shorter than those from natural pollination (**Table 6**). This indicated a great trend in reduction of walnut seedling height from apomictic than from natural pollination. The coefficient of variation in plant height was 0.2 in the former and 0.4 in the latter, and expected result that would explain the lower degree of separation in apomictic seedlings than those derived from sexual reproduction.

The apomixis seedlings performed uniformly, which may be due to the fact that apomictic offspring did not involve the combination of male and female gametophytes and that the segregation of characters in heterozygosis did not exist in seed development. In the control, there was a great difference among individuals with the segregation of character in their seedling progenies with high heterozygosity of the seeds originating from crosses.

Isoenzymes to study walnut apomictic seedlings

Isoenzymes were used to study plant apomixis since this method is quick, cheap and generates considerable polymorphism, and has already been used widely in orchard fruit apomictic studies. Five-month old orange seedlings were studied by isoenzyme analysis of phosphoglucoisomerase and phosphoglucomutase in the lamina. The isoenzyme zymogram indicated a clear distinction between zygotic and adventitious embryos that originated from the nucellar embryo cell (Elisiario *et al*. 1999). According to Liu H-Z (1989), apomictic plants of *Malus sikkimeniis* had a relatively highly uniform peroxidase isoenzyme zymogram besides showing developmental uniformity and parthenocarpy. Results of this study showed that the percentage banding pattern of *M. sikkimeniis* apomictic seedlings was above 80%, but 100% in *Malus xiaojinensis* and 50% in *Malus baccata* L. as controls, which showed that germplasm homozygosity of offspring originating from apomixis was higher than that originating from sexual reproduction. Enzymes are the product of gene expression, and thus isoenzyme zymogram uniformity showed direct proof of germplasm homozygosity. Plant isozymes have been a good biochemical index to study plant development and cell differentiation due to their tissue and phase specificity (Kuhns *et al*. 1978; Cheng S-Z *et al*. 1987; Dong S-Z *et al*. 1989).

We studied peroxidase isoenzyme using the lamina of apomicts and common walnut seedlings. The results showed two clear banding patterns in the test (**Table 7**; **Fig. 2**; Wu G-L *et al*. 2007). The performance frequency of the peroxidase isoenzyme zymogram (POD) among individual plants was used to calculate the banding pattern performance percentage while its mean value represented the uniformity. In this test, 'Liaohe No. 1' seedlings from a natural pollination as the control had an isoenzyme zymogram uniformity of 55% but 86% in apomictic seedlings.

The results showed that isoenzyme zymogram uniformity of POD in apomictic seedlings was higher than the control. This indicated that the heredity background uni-

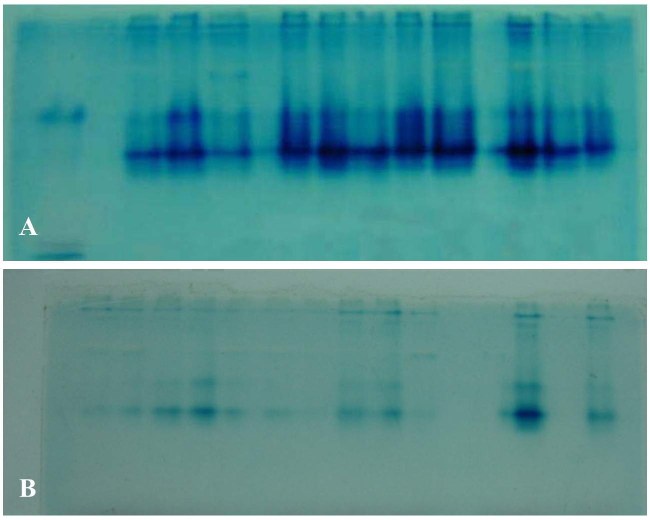

Fig. 2 Isoenzyme zymogram of POD of apomixes seeding in walnut. (**A**) apomictic seedlings; (**B**) naturally pollinated seedlings (from Wu G-L *et al*. 2007, with permission).

Table 8 Main economic characters of walnut strains (from Wu *et al*. 2007, with permission).

Strains	Apomictic capacity ratio (%)	Single fruit weight (g)	Shell thickness (mm)	Seed of ratio (%)	Single seed weight (g)	Seed colour	Yield per m^2 (kg)
Zuoquan Mian	55.8 A	10.3 A	1.18	55.2	7.8	White-yellow	0.23 A
Taigu Mian (CK)	12.5 B	10.4 A	1.20	55.3	7.9	White-yellow	0.15 B

formity of apomictic seedlings was higher than seedlings derived from control seeds, and that its characters developed uniformly with a little degree of separation (Wu *et al.* 2007).

Study on the selection of new strains with apomictic traits in walnut

The selection of varieties with obligate apomixis decreases the effect of unfavourable climatic conditions on yield (Loiko 1990).

Different forms of walnut with apomixis can be used in hybridization, so this has great significance in introducing walnut to regions of severe climatic conditions. There are few studies about variety selection in apomictic walnut, but no reports on the selection of new cultivars (Badalov 1983). On the basis of researching apomixis walnut, we managed to make a new cultivar selection and found a single cultivar which combined economic characteristics, such as single fruit weight and yield per m^2, and also had apomictic ability (**Table 8**; Wu *et al.* 2007). Selected forms having regular apomixis are referred to as obligate apomicts (Loiko 1990).

CONCLUSIONS

Walnut, *Juglans regia* L., is the most valuable nut crop in the world and the most economically important member of the genus *Juglans*. Recently, it has increased greatly in acreage and in production around the world. Although a number of plant apomixis research programs have been carried out around the world, few are dedicated to walnut. Allogamic biological features which are restricted by environmental conditions in walnut also affect output badly. Over the years it has been one of the aims to seek to breed new cultivars by using apomixis.

Though there have been many papers and reviews about apomixis (Bartish *et al.* 2001; Grossniklaus *et al.* 2001; van Baarlen *et al.* 2002; Wang Z-W *et al.* 2004; Carneiro *et al.* 2006), basically they were limited to herbaceous, monocotyledonous plants (Sun *et al.* 1996; Gao J-W *et al.* 2000; Akiyama *et al.* 2004; Albertini *et al.* 2004). Among woody fruit trees most studies were related to *Malus* spp. (Dong S-Z *et al.* 1987, 1989; Liu H-Z *et al.* 1989; Zhou Z-Q *et al.* 1995; Dong W-X *et al.* 1996; Zhou *et al.* 1998) while apomixes in other fruit trees were scarcely reported (Liu Y-H *et al.* 1987; Cai *et al.* 1997, 2002; Nybom 2004; Carneiro *et al.* 2006). Over 60 years of studying walnut apomixis we now know that there are two types of apomixes, obligate and facultative in walnut, following the observation and analysis of morphological features. Using these characteristics to breed new varieties may enhance the adaptability of this important nut crop and obtain steady output.

The walnut tree is a perennial woody plant, large and with many phenols in its tissues, which causes some problems in research related to increasing the growth cycle, difficulties in sampling and in experimenting. At present in depth research is limited. The mechanisms underlying regeneration and the genetic regulation of apomixis in walnut are far from being clarified.

Along with the advances in biotechnology, an interest in apomixis has increased, and the study of apomixis has deepened to the molecular level in most crop plants (Jefferson 1994; Grossniklaus *et al.* 2001; Bartish *et al.* 2001; Ma S-M *et al.* 2002; Eckardt 2003; Akiyama *et al.* 2004; Albertini *et al.* 2004; Chen and Guan 2006). The study of apomixis ultimately seeks a regular pattern or mechanism to control the expression of genes responsible for apomixis. This would allow the transmission of the male progenitor characteristics to the progeny (Spillane *et al.* 2001b; Albertini *et al.* 2004; Carneiro *et al.* 2006).

Many of the results obtained so far would promote research of the mechanisms of apomixis in walnut, many of which will contribute to programs aimed at the generation of improved and asexually seed-propagated walnut (Spillane *et al.* 2001b; Albertini *et al.* 2004). Using novel breeding strategies, combined with novel genetic, molecular and cytological methods it is now possible to attempt to understand the genetic regulation of sexual and asexual ways of reproduction for utilizing apomixis in agriculture and walnut improvement in the near future.

ACKNOWLEDGEMENTS

This work was supported by the Research Foundation of Science and Technology of Shanxi Province, Project No. 021036; the China National Natural Science Foundation, Project No. 30571290, the Shanxi Province Natural Science Foundation, Project No. 2006011081.The authors thank Dr GX Chen, the professor and the library curator of Shanxi Agricultural University ; Dr DY Zhu, the professor and the vice-president of Henan Agricultural University; Ms. Li-fang Cheng, the associate professor of Shanxi Agricultural University for their kind help.

REFERENCES

* In Chinese

Akiyama Y, Conner JA, Goel S, Morishige DT, Mullet JE, Hanna WW, Ozias-Akins P (2004) High-resolution physical mapping in *Pennisetum squamulatum* reveals extensive chromosomal heteromorphism of the genomic region associated with apomixis. *Plant Physiology* **134**, 1733-1741

Albertini E, Marconi G, Barcaccia G, Raggi L, Falcinelli M (2004) Isolation of candidate genes for apomixis in *Poa pratensis* L. *Plant Molecular Biology* **56**, 879-894

Amsellem L, Noyer JL, Hossaert-Mckey M (2001) Evidence for a switch in the reproductive biology of *Rubus alceifolius* (*Rosaceae*) towards apomixes, between its native range and its area of introduction. *American Journal of Botany* **88**, 2243-2251

Asadian G, Pieber K (2005) Morphological variations in walnut varieties of the Mediterranean regions. *International Journal of Agricultural and Biology* **7**, 71-73

Badalov P (1983) The use of methods of hybridization and apomixis for obtaining the perspective forms of walnut. *Forest Production* **12**, 24-25 (in Russian)

Bartish IV, Hylmo B, Nybom H (2001) RAPD analysis of interspecific relationships in presumably apomictic *Cotoneaster* species. *Euphytica* **120**, 273-280

Cai X, Sun D-L, Xing S-P (2002) Ultrastructure of nucellar embryo and cytochemical localization of ATPase activity in nucellar cell of *Xanthoxylum bungeanum. Acta Botanica Boreal-Occident Sinica* **22**, 791-795*

Cai X, Mu X-J, Zhu Z-Q, Hua Z-M, Huang Y-H, Sun Q-X (1997) Polyembrony and multiple seedlings in apomictic plants. *Acta Botanica Sinica* **39**, 590-595*

Carneiro VTC, Dusi DMA, Ortiz JPA (2006) Apomixis: occurrence, applications and improvements. In: Teixeira da Silva JA (Ed) *Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues* (1st Edn, Vol I), Global Science Books, London, pp 564-571

Chen L-Z, Guan L-M (2006) Advances in cloning and expression of apomixis-specific genes in flowering plants. In: Teixeira da Silva JA (Ed) *Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues* (1st Edn, Vol I), Global Science Books, London, pp 572-577

Chen Y-F, Lang S-L, Gao S-T, Gao Y (2000) A cytoembryological study on apomixis in walnut (*Juglans regia* L.). *Acta University Agricultural Boreali – Occidentalis* **28**, 16-20*

Cheng S-Z, Yang W-H (1987) Taxonomic studies of ten species of the genus *Juglans* based on isozymic zymograms. *Acta Horticulturae Sinica* **14**, 90-96*

Dong S-Z, Yu H (1987) The study of peroxydase isoenzyme in the species of *Malus sieboldii*. *China Fruits* **3**, 34-36*

Dong S-Z, Yu H (1989) The analysis of peroxidase isoenzyme in *Malus hupe-*

nensis. *Journal of Fruit Science* **6**, 103-105*

Dong W-X, Jing S-X, Xuan J-H (1996) Research and utilization of the apomictic characteristic in *Malus* – a literature review. *Acta Horticulturae Sinica* **23**, 343-348*

Driver JA, Kuniyuki AH (1984) *In vitro* propagation of Paradox walnut rootstock. *HortScience* **19**, 507-509

Eckardt NA (2003) Patterns of gene expression in apomixis. *The Plant Cell* **15**, 1499-1501

Elisário PJ, Santos GG, Guerreiro AR, Ollitrault P, Luro F, Leitão JM (1999) Isozyme analysis revealed that the Portuguese mandarin "Carvalhais" originated as a single clone. *Scientia Horticulturae* **82**, 145-152

Fryer J, Hylmo B (2001) Captivating cotoneasters. *New Plantsman* **8**, 227-238

Gao J-W, Li Z-D, Sun Q-X, Liu J-Z (2000) Advances of apomixis in plants. *Advances of Biotechnology* **20**, 43-47*

Gao S-T, Liu C-B, Li L, Ye N-L, Chen Y-F (1999) Apomixis characteristic of *Juglans regia* L. *Journal of Northwest Forestry University* **14**, 45-47*

Grossniklaus U, Nogler GA, van Dijk PJ (2001) How to avoid sex? The genetic control of gametophytic apomixis. *The Plant Cell* **13**, 1491-1498

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

Jefferson RA (1994) Apomixis: A social revolution for agriculture. *The Center for the Application of Molecular Biology to international Agriculture (CAM BIA), Biotechnology and Development Monitor* **19**, 14-16

Koltunow AM, Grossniklaus U (2003) Apomixis: A developmental perspective. *Annual Review of Plant Biology* **54**, 547-574

Koltunow AM (1993) Apomixis: Embryo sacs and embryos formed without meiosis or fertilization in ovules. *The Plant Cell* **5**, 1425-1437

Kuhns LJ, Fretz TA (1978) Distinguishing rose cultivars by polyacrylamide gel electrophoresis. II. Isoenzyme variation among cultivars. *Journal of the American Society Horticultural Science* **103**, 509-516

Leblanc O, Peel MD, Carman JG, Savidan Y (1995) Megasporogenesis and mega-gametogenesis in several *Tripsacum* species (*Poaceae*). *American Journal of Botany* **82**, 57-63

Li P, Chen F, Zhou G-M (1992) Apomixis application in breeding and cyto-embryological study method. *Journal of Sichuan University* **29**, 288-293*

Liu D-L, Gao S-T, Chen Y-F (1999) A study of apomixis characteristics of *Juglans regia* L. by distant and deactive mentor pollen. *Journal of the Northwest Forestry University* **14**, 35-37*

Liu H-Z, Pu F-S, Ren Q-M, Liu L-J (1989) Some characters of apomictic *Malus*. *Acta Horticulturae Sinica* **16**, 1-4*

Liu Y-H, Wang F-X, Qian N-F (1987) Apomixis in *Zanthoxylum bungeanum* and *Z. simulans*. *Acta Genetica Sinica* **14**, 107-113*

Loiko RE (1990) Apomixis of walnut. *Acta Horticulturae* **284**, 233-236

Ma G-H, Zh N-X, Huang X-L (2001) Apomixis in the Gramineae. *Journal of Tropical and Subtropical Botany* **9**, 83-92*

Ma S-M, Wang Y-F, Ye X-L, Zhao N-X, Liang C-Y (2002a) Genetic mechanism and molecular basis of apomixis in plants. *Hereditas* (Beijing) **24**, 197-199*

Ma S-M, Wang Y-F, Ye X-L, Liang C-Y (2004) Recent advances in the study on apomixis in plant. *Journal of Tropical and Subtropical Botany* **12**, 477-481*

Ma S-M, Wang Y-F, Ye X-L, Zhao N-X, Liang C-Y (2002b) The methods for identification of apomixis in plant. *Acta Botanica Boreal-Occident Sinica* **22**, 985-993*

Martonfi P (2001) New species of the genus *Hypericum* sect. *Hypericum* (*Guttiferae*) from Slovakia. *Folia Geobotanica* **36**, 371-384

Nybom H (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology* **13**, 1143-1155

Pichot C, Elmaataoui M, Raddi SP (2001) Surrogate mother for endangered *Cupressus*. *Nature* **412**, 39

San B, Humanglu H (2006a) Determination of the apomictic fruit set ratio in some Turkish walnut (*Juglans regia* L.) genotypes. *Turkish Journal of Agricultural Forestry* **30**, 189-193

San B, Humanglu H (2006b) Somatic embryogenesis from immature cotyledons of apomictic and non-apomictic seed in walnut (*Juglans regia* L.). *Turkish Journal of Agricultural Forestry* **30**, 111-117

Schanderl H (1964) Untersuchungen uber die blutenbiologie und embryonenbildung von *Juglans regia* L. *Biologischez Zentrablatt* **83**, 71-102

Schranz ME, Dobes C, Koch MA, Mitchell-olds T (2005) Sexual reproduction, hybridization, apomixis, and polyploidization in the genus *Boechera* (Brassicaceae). *American Journal of Botany* **92**, 1797-1810

Spillane C, Steamer A, Grossniklaus U (2001a) Apomixis in agriculture: the quest for clonal seeds. *Sexual Plant Reproduction* **14**, 179-187

Spillane C, Vielle-Calzada J, Grossniklaus U (2001b) APO2001: a sexy apomixer in Como. *The Plant Cell* **13**, 1480-1491

Sun J-S, LiuY-S, Xin H-W (1996) Apomixis in angiosperms. *Chinese Bulletin of Botany* **13**, 1-8

Valdiviesso T (1990) Apomixis in Portuguese walnut varieties. *Acta Horticulturae* **284**, 279-283

van Baarlen P, de Jong HJ, van Dijk PJ (2002) Comparative cyto-embryological investigations of sexual and apomictic dandelions (*Taraxacum*) and their apomictic hybrids. *Sexual Plant Reproduction* **15**, 31-38

Wang G-A, Maimaiti A, Cun W-G, Ba T (2003) Application of apomixis in the varieties genetic purification of walnut. *Xinjiang Agricultural Sciences* **40**, 90-93*

Wang Z-W, Li R-T, Guo D-D (2004) Progress of apomixis in plant. *China Biotechnology* **24**, 35-37*

Werlemark G (2000) Evidence of apomixis in hemisexual dogroses, *Rosa* section *Caninae*. *Sexual Plant Reproduction* **12**, 353-359

Wu G-L, Zhang P-F, Yang J-Q, Song Y-Q (2007) The study of apomixis traits and new strains selected in walnut. *Acta Horticulturae*, in press

Xi R-T, Zhang Y-P (1996) China fruit records (walnut). Forestry Press, Beijing, pp 93-197*

Xi R-T, Zhang Y-P (1992) *China Walnuts*, Forestry Press, Beijing, pp 182-183*

Xi R-T, Liu M-J (2005) China dry fruits. Forestry Press, Beijing, pp 78-112*

Yang J-Q (2006) Study on physiological and biochemical characteristics of apomictic walnut seedlings. MSc Thesis, Shanxi Agricultural University, China*

Zhang M-Y, Xu Y, Ma F-X (2000) Apomixis ability of *Juglans regia* L. *Journal of Fruit Science* **17**, 314-316*

Zhou Z-Q, An H-M, Yang F-Y, Wu L-R (1998) Study on the interspecific and intraspecific differences of apomixes in *Malus* Mill. *Journal of the Southwest Agricultural University* **20**, 114-116*

Zhou Z-Q, Li Y-N (1995) Recent advances in the study on apomixis in *Malus*. *Acta Horticulturae Sinica* **22**, 341-347*