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Fruit set and embryo rescue in crosses using parthenocarpic 'Mopanshi' persimmon

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Abstract

The 'Mopanshi' persimmon is a seedless, astringent parthenocarpic cultivar that does not produce male flowers. After pollination using four non-astringent cultivars ('Zenjimaru', 'Nishimurawase', 'Okugosho' and 'Hanagosho'), seeds were produced to different degrees. 'Mopanshi' fruits pollinated with 'Zenjimaru' produced far more seeds than those pollinated with the other three cultivars. The ratio of abnormal seeds obtained from the fruits pollinated with 'Hanagosho' was higher than that obtained from the fruits pollinated with the other three cultivars. Most embryos degenerated in the early to late stages of seed development. Immature embryos were cultured in a modified MS medium (half of NO_3 in MS medium + 0.4 μM BA + 0.1 μM IBA) with the greatest success (52–80%) from embryos taken from fruits 60–80 days after pollination. Seedlings failed to initiate radicles so they were transferred to dark culture conditions for 8 days or to a rooting media that contained 3% sucrose and 1% Chinese ink. The seedlings on the medium darkened with ink rooted at greater than 90.83% compared to 75.83% for dark cultured seedlings. This study demonstrated that 'Mopanshi' persimmon could be used as a female parent in crosses, but embryos needed to be moved to tissue culture conditions to continue to develop and form plantlets.

Keywords: *Diospyros kaki*; Pollination; Embryo culture; Rooting

1. Introduction

The persimmon (*Diospyros kaki* L. 'Mopanshi') originated in the Yangtze River valley. It has become the main cultivar in north China due to its superior quality, high production, frost hardiness and drought resistance. It is also the most prevalent astringent cultivar in China. This cultivar does not have male flowers and the fruits of it are large: the average weight is 250 g and a large fruit can be up to 450 g. It is an outstanding female parent source in the general cross-breeding of persimmon. During recent years, we have introduced some excellent non-astringent cultivars from Japan. But in north of China, these cultivars have got some problems such as they cannot become completely non-astringent during the ripening stage, their low temperature adaptabilities are poor and so on. So it is our aim through breeding to solve the problems of cultivar simplification and ripening periods concentricity. Hybridizing non-astringent cultivars with 'Mopanshi' persimmon as female parent could yield new cultivars of non-astringent or astringent persimmon with early ripening, large size fruit of superior quality and good resistance to frost and drought. Because of its parthenocarpic capacity, this cultivar can only be used as a female parent, but according to the discovery in the recent years' cross-breeding practice, the high percentage of abnormal seeds during the growth of its hybrid seriously hampered the development of the research in cross-breeding.

The technique of embryo rescue could rescue abortive embryos to obtain hybrid seedlings (Bridgen, 1994; Iwamoto, 2001). The objectives of current study were to investigate embryo development in 'Mopanshi' persimmon pollinated by different non-astringent cultivars. The timing and conditions for embryo rescue in abortive seeds were also investigated.

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2. Materials and method

2.1. Pollen source and pollination method

Fifteen 'Mopanshi' persimmon trees at a highly productive age planted at China Agricultural University were used. Pollen of 'Zenjimaru' and 'Nishimurawase' was from the Research Institute of Pomology, Northwest Agriculture Technology University, and pollen of 'Okugosho' and 'Hanagosho' was from the China Agricultural University.

The flowering period of 'Mopanshi' was from May 14 to May 21 in 2003. Style abscission began on May 21. Because the fluorescence of the pollinated variety was 7–10 days earlier than 'Mopanshi', pollen was stored in plastic bags in 4 °C. On May 15–17, petals were removed prior to opening and styles pollinated. Paper bags were placed over the pollinated flowers for one week.

2.2. Fruit and seed set and embryo development

Fruits were harvested the second week after pollination and continued every 14 days for 22 weeks. After eliminating abnormal fruits, 50 fruits of normal growth were chosen from each sample to investigate the fruit growth and stage of embryo development.

2.3. In vitro embryo culture

Fruits were washed and then sterilized for 30 s with 70% ethanol, followed by sterilization with 1% NaClO including 0.1% Tween-20 for 15 min. They were then rinsed four times with sterilized water. Under the condition of asepsis, the young embryos were then immersed in a modified MS medium (half of NO_3 in MS medium + 0.4 μM BA + 0.1 μM IBA). The pH value of the medium was regulated to 5.8, and 25 μM PVP was put into the medium to avoid the browning reaction. Cultures were held at 25 °C with light provided 16 h per day by cool white fluorescent lamps at 36 μmol m^{-2} s^{-1}. Germination and shoot production was evaluated 30 days after in vitro embryo culture.

2.4. Initiation of root growth

The embryos from seeds produced in fruit 80 days after pollination were cultured in vitro for 40 days. The selected 60 Erlenmeyer flasks of young shoots were evenly divided into two groups. The callus from the shoot base was first washed and the dipped into a 1.0 mM IBA for 10 s. Shoots were cultured in the dark for 8 days or in a black medium containing 1% Chinese ink containing charcoal. The medium was modified MS (1/2 NO_3 MS + 3% sucrose) and all other conditions were the same as previously described. Rooting was evaluated after 30 days and there were 10 embryos per treatment.

2.5. Acclimatization

Thirty-five days after rooting, seedlings were acclimatized by gradually reducing humidity in the flask for 3 days. Seedlings were transplanted to pots and moved to a shaded greenhouse with temperature and humidity set at 20 ± 2 °C, and 80%, respectively. Plant survival was evaluated after 1 month.

3. Results

3.1. Effect of pollination on fruit growth and seed set of 'Mopanshi' persimmon

There were no differences in growth between non-pollinated controls and pollinated 'Mopanshi' fruits. The average weight of fruits pollinated with 'Zenjimaru', 'Nishimurawase', 'Okugosho' and 'Hanagosho' and harvested on October 10 were 230.0, 236.3, 228.4 and 231.1 g, respectively, compared to 232.1 g in non-pollinated control fruit (Fig. 1).

No seeds formed in the 'Mopanshi' fruits without pollination, but seeds did form in pollinated fruits (Table 1). The number of seeds per fruit was different for each kind of pollen. The most seeds were produced with 'Zenjimaru' pollen, followed by 'Nishimurawase', 'Okugosho' and 'Hanagosho'. Seeds with abnormal embryos did not develop fully and attain a normal size. Seeds from 'Hanagosho' pollen contained a greater proportion of abnormal embryos compared to the other three kinds of pollen.

The number of seeds and embryos decreased dramatically 100 days after pollination (Table 1). Although embryo degeneration was observed in every period, this phenomenon was more serious in the later period. There are four carpels and eight ovules in 'Mopanshi' fruit.

Seed size differed depending on the pollen source (Table 2). The ratio of seeds under 5 mm was higher for 'Zenjimaru' (8.05%) than the other three cultivars including 'Nishimurawase' (6.43%), 'Okugosho' (6.52%) and 'Hanagosho' (4.07%). The seeds pollinated with 'Nishimurawase' were a little bigger than those pollinated with 'Zenjimaru'. But the two were generally smaller than those pollinated with 'Okugosho' and 'Hanagosho' where hardly any differences existed, and the ratio of seeds beyond 13 mm were 5.43 and 6.77%, respectively.

3.2. In vitro embryo viability

The shape of embryo in the seeds 1–5 mm long was not discernable. Globular embryos could often be seen in the seeds 5–8 mm long, and all three types of embryo could be seen in the seeds more than 8 mm long. The torpedo type could not be seen in seeds shorter than 5 mm, but was found in 30% of seeds 10–13 mm long.

The viability of embryos on 30 days since the embryo culture is shown in Table 1, and for most of the embryo, they almost got mature when it is 60–80 days (torpedo period), and it is easy for them to live in vitro. Before 80 days, the browning reaction happened to the exterior of hybrid seed did not impact the growth of the embryo. The viability of embryos 60–80 days after pollination was higher than at later periods. This result was consistent with that provided by Japan, their persimmon study shows that the optimum period for embryo culture is

Table 1
Effect of pollen parent on number of seeds and embryos, type of embryos and viability of embryo culture in 'Mopanshi' persimmon

Days after pollination	Pollen parent	Number of seeds (%)	Number of embryos	Type of embryos (%)			Surviving individual of embryo culture[a]	Viability of embryo culture (%)
				G	H	T		
40 day, 6 week	Zenjimaru	360 (90)	40	32 (80)	8 (20)	0	6	15.0
	Nishimurawase	306 (75)	29	22 (76)	7 (24)	0	5	17.2
	Okugosho	282 (71)	27	22 (82)	5 (18)	0	3	11.1
	Hanagosho	212 (53)	15	12 (82)	3 (18)	0	2	13.3
60 day, 9 week	Zenjimaru	356 (89)	88	69 (78)	9 (10)	10 (12)	68	77.3
	Nishimurawase	307 (77)	55	45 (81)	6 (11)	4 (8)	37	67.3
	Okugosho	279 (70)	43	35 (82)	5 (10)	3 (8)	31	72.1
	Hanagosho	200 (50)	31	25 (82)	3 (9)	3 (9)	16	51.6
80 day, 12 week	Zenjimaru	348 (87)	76	57 (75)	12 (16)	7 (9)	60	78.9
	Nishimurawase	311 (78)	50	38 (76)	6 (11)	6 (13)	35	70.0
	Okugosho	276 (69)	40	32 (80)	4 (10)	4 (10)	27	67.5
	Hanagosho	172 (43)	27	22 (80)	3 (11)	2 (9)	14	51.9
100 day, 15 week	Zenjimaru	260 (65)	50	35 (70)	8 (15)	7 (15)	32	64.0
	Nishimurawase	180 (45)	38	27 (71)	4 (10)	7 (19)	20	52.6
	Okugosho	171 (43)	31	22 (71)	4 (13)	5 (16)	17	54.8
	Hanagosho	140 (35)	19	14 (72)	2 (11)	3 (17)	8	42.1
150 day harvesting time	Zenjimaru	182 (46)	40	20 (50)	9 (23)	11 (27)	22	55.0
	Nishimurawase	125 (31)	26	12 (46)	7 (25)	7 (29)	10	38.5
	Okugosho	120 (30)	20	10 (50)	6 (30)	4 (20)	6	30.0
	Hanagosho	95 (24)	10	4 (40)	2 (20)	4 (40)	2	20.0

G is globular type, H is heart type, T is torpedo type; values in parentheses are the percentage. Fruits to be tested: every 50. Mean separation in columns by Duncan's multiple range test, 5% level. Culture conditions: (1/2 NO_3)MS + 0.4 μM BA + 0.1μM IBA; 25 °C, 16 h per day, 36 μmol m^{-2} s^{-1}.
[a] Alive number after 30 days of embryo culture.

60–90 days (Sugiura et al., 2000). After 80 days, the browning reaction of the abortive hybrid was worsening, and the seed coats of some embryos had exposed in the fruit. The hybrid endosperm began to harden after 80 days development; it was easy to hurt the cotyledon when getting the embryo at that time, and it can hardly gain the intact embryo when it was 90 days. The ratio of the torpedo embryo increased when time gone. The reason that the viability of embryos 80 days after pollination decreased is just that the viability of torpedo embryo decreased.

3.3. Comparison of dark culture versus a black medium on rooting of shoots in embryo culture

Shoots developed after 40 days of in vitro culture, but seedlings failed to elongate or produce a radicle. Seedlings for embryos using the dark culturing method exposed to brief etiolation or placed on black rooting medium produced roots in over 70 and 90%, respectively (Table 3). Seedlings on black medium produced approximately three times more roots compared to those by dark culture. Shoot elongation was greater in the dark treated seedlings compared to black media alone. Furthermore, dark cultured seedlings showed leaf drop that was not observed using the black medium method. Cultivating 35 days after rootage, the seedlings began to get domesticated, then transplant into the nutrition pot, later put them into the greenhouse, the survival percentage can be up to 87% after 1 month.

4. Discussion

'Mopanshi' persimmon shows strong parthenocarpy and seeds are rarely produced. However, 'Mopanshi' could produce seeds after pollination with non-astringent persimmon pollen, but most of the seeds degenerated in the early or late growth stage. The degree of zygote embryo abortion in 'Mopanshi' was affected by the pollen parent. This appears to

Table 2
Development of seeds in 'Mopanshi' persimmon after 80 days of pollination

Pollen parent	Number of tested fruits	Number of embryos	The number of seeds in different length				
			1–5 mm (%)	5–8 mm (%)	8–10 mm (%)	10–13 mm (%)	13 mm (%)
Zenjimaru	50	348a	28a (8.05)	125a (35.92)	167a (47.99)	21a (6.03)	7a (2.01)
Nishimurawase	50	311b	20b (6.43)	100b (32.15)	158a (50.80)	23a (7.40)	10ab (3.22)
Okugosho	50	276c	18b (6.52)	71c (25.72)	135b (48.91)	37b (13.41)	15b (5.43)
Hanagosho	50	172d	7c (4.07)	39d (22.67)	90c (52.33)	23a (13.37)	13ab (6.77)
Control	50	0	0	0	0	0	0

Note: The letters a–d show the difference at significant ($p \leq 0.05$) level.

Table 3
Effect of different culture conditions on developing of shoots in embryo culture

Method of rooting culture	Pollen parent	Beginning of rooting culture		30 days after rooting culture			
		Shoots height (mm)	Leaves number	Shoots height (mm)	Leaves number	Rooting ratio (%)	Roots number per shoot
Dark culture	Zenjimaru	26.1a	6.5a	40.2a	8.3a	73.3a	1.47a
	Nishimurawase	24.8a	6.4a	40.3a	8.2a	76.7a	1.60a
	Okugosho	25.7a	6.1a	39.2a	8.4a	73.3a	1.86a
	Hanagosho	25.9a	5.4a	38.7a	8.5a	80.0a	1.63a
	Average	25.63	6.10	39.60	8.35	75.83	1.64
Black medium	Zenjimaru	25.3a	6.2a	27.1b	8.1a	90.0b	5.23b
	Nishimurawase	25.6a	6.5a	27.0b	8.0a	93.3b	4.93b
	Okugosho	26.0a	6.2a	26.3b	7.8a	93.3b	5.50b
	Hanagosho	25.7a	5.8a	26.7b	7.9a	86.7b	4.83b
	Average	25.65	6.18	26.78	7.95	90.83	5.12

Dark culture: shoots were planted into modified MS medium (1/2 NO_3 MS + 3% sucrose), firstly put them into dark culturing box (the temperature is 25 °C). Eight days later, treat them by 16 h per day illumination with intensity of 36 μmol m^{-2} s^{-1}. Black medium: shoots were planted into above medium of modified MS medium + 1% Chinese ink. The conditions for culture were 25 °C, illumination of 16 h per day, intensity of illuminance of 36 μmol m^{-2} s^{-1}. Shoots to be tested: each 30. Mean separation in columns by Duncan's multiple range test, 5% level.

be correlated with the pollen parent's ability to set seed (personal observation). There are numerous reasons for embryo degeneration (Kitajima et al., 1993; Hasegawa and Nagata, 1993; Hasegawa et al., 1997; Akira et al., 2000). Among Japanese persimmons, the main cause for seed abortion was related to poor embryo sac development related to chromosome number and ploidy levels (Fukui et al., 1991, 1993; Zhuang et al., 1990a,b). The chromosome number of tested cultivars was $2n = 6x = 90$ (Tang and Luo, 2000; Zhuang et al., 1990a,b, 1992) which should start normal mitosis after hybridization. In 'Mopanshi', abortive seeds first show browning in the endosperm. In addition, more than half of aborted seeds show abnormal growth at the hilum. This phenomenon needs to be investigated further to see its significance in seed abortion.

Embryo rescue was effective in obtaining hybrid seedlings. Sugiura et al. (2000) also recovered hybrids in Japanese persimmon by embryo rescue. In the current study, embryos failed to germinate normally and only produced shoots. The use of a dark medium with IBA initiated root growth.

According to the research results, we can get some conclusions, as follows:

(1) Hybridizing Japanese cultivars of non-astringent persimmons with 'Mopanshi' persimmon as female parent get hybrid seedling through embryo rescue.
(2) The optimum time for the embryo rescue is 60–80 days after pollination.
(3) Black medium is rather effective to rooting culture for 'Mopanshi'.

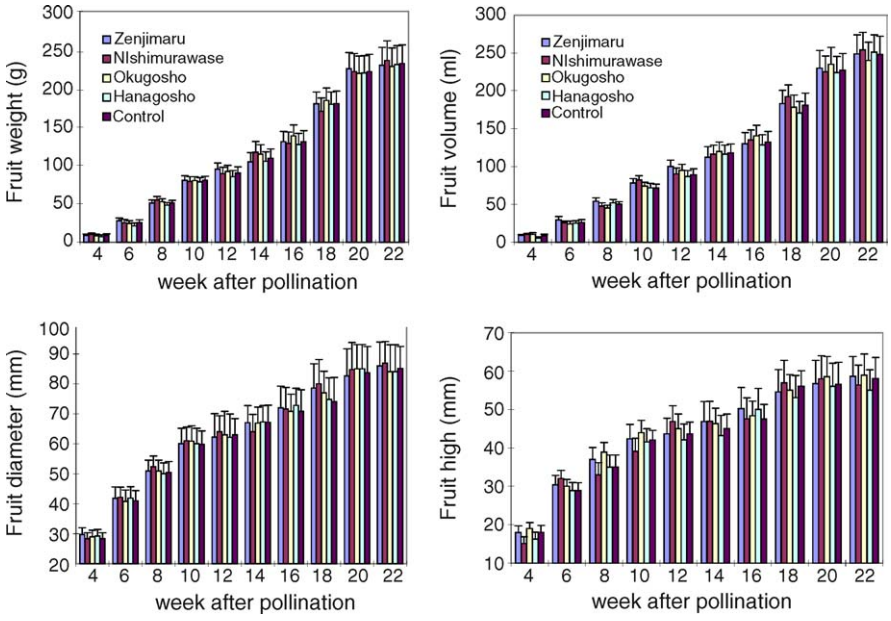

Fig. 1. Effect of pollen parent Zenjimaru, Nishimurawase, Okugosho and Hanagosyo on fruit size, diameter, weight, height and volume of Mopanshi persimmon.

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