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BREEDING SYSTEM AND PARENTAL EFFECT ON FRUIT CHARACTERS OF *IDESIA POLYCARPA* (FLACOURTIACEAE), A PROMISING PLANT FOR BIODIESEL, IN NORTHWEST CHINA

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Abstract

Idesia polycarpa Maxim. is a promising plant for biodiesel in China. We have reported the flowering phenology, breeding system and parental effect on fruit characters of this species distributed in Qinling-Bashan Mountain (Shaanxi Province) nature reserve. As a dioecious plant, the male and female flowered almost synchronously. The anthesis was from around 10^{th} of May and proceeded until the end of May or the beginning of June at population level. To determine the breeding system of *I. polycarpa*, three pollination treatments were carried out on every three female plants: natural pollination (NP), apomixy (AP) and cross pollination (CP). Reproductive success of apoximy treatment indicated that, as a dioecious plant, *I. polycarpa* could also reproduce by apomixis. However, the mean fruit set under apomixy treatment was markerly lower (3.6–13.33%) than that of two pollination treatments (>65.69%). Fruit quality (fruit length, fruit width, 100 fruit weight, seed production and seed germination) of the cross pollination treatment was greater than the other two treatments, suggested that pollen competition in cross pollination treatment was the most intense in three treatments. To study parental effect on fruit characters, cross pollination was carried out with three female plants and three male plants. The result showed that maternal parents ($P<0.001$) and parental interaction significantly affected all the fruit characters (including 100 fruit weight, pulp/fruit ratio, oil content and seed germination) while paternal parents showed significant effects on 100 fruit weight, pulp/fruit ratio and seed germination. Fruit set was only significantly affected by maternal parents ($P=0.001$). Our findings will facilitate future breeding programs of *I. polycarpa* in parental selection.

Key words: *Idesia polycarpa* Maxim., Fruit set, Breeding system, Fruit character, Parental effect.

Introduction

Idesia polycarpa Maxim. is a dioecious plant of the Flacourtiaceae family. This tree is native to some asian countries such as Korea, Japan and China (Kim *et al.*, 2005). This plant received considerable attention during the last ten years for some interesting characters. Firstly, the fruit of this species are high oil content. The oil contents in pulp and seed are about 43.6% and 26.26% (% dry basis), respectively(Zhu 2010; Yang *et al.*, 2009). The oil contains unsaturated fatty acids and multiplex vitamins, which could effectively prevent cardiovascular or hyperlipidemia disease (Dmytryshyn*et al.*, 2004). Recently, one new compound, idescarpin, isolated from *I. polycarpa* fruit showed a great potential against obesity and diabetes through the anti-adipogenic activity and the up-regulation of PPARγ. (Lee *et al.*, 2013). The feasibility of producing biodiesel from *I. polycarpa* fruit oil was also studied. It showed that the fuel properties of the *I. polycarpa* fruit oil biodiesel were similar to the No. 0 light fuel, which indicated that it could be an acceptable substitute for petrodiesel (Yang *et al.*, 2009). All these suggested that this species has important agricultural and commercial potential, and therefore some indigenous communities have become interested in planting this species as a basis for development of community-based enterprise.

The development of *I. polycarpa* as a promising commercial plantation species will depend on the development of high-quality genetic material. The lack of basic knowledge of breeding system is one constraint on such development. As an entomophilous plant, the natural population density of *I. polycarpa*is very low. Plant is sporadic and scattered in the field. Moreover, the sex ratio of *I. polycarpa* in the location of our study was female-biased (Wang *et al.*, 2014). This seems to make it difficult for pollen grains being shed on stigma. So it is necessary to elucidate the breeding system of *I. polycarpa* and find out if it can produce asexually. This is particularly important since fruits are the object of economic importance (Silva *et al.*, 2011).

Knowledge of breeding system and parental effect on fruit characters will facilitate the breeding program of *I. polycarpa*. Through field observations, monitoring of flowering phenology and controlling pollination experiments, we tried to address the following questions: (1) Do the male and female plants flower synchronously? (2) Can *I. polycarpa* reproduce by apomixis? (3) What is the parental effect on fruit set and fruit characters in *I. polycarpa*? And we hope the basic data would play important role in formulating the future breeding program of *I. polycarpa*.

Material and methods

Study site: The trial was carried out in May 2011. According to Zhu *et al.*(2010), the study was set up in the montane forest of the Qinling-Bashan Mountain, located in the south of Shaanxi Province in China (32°54′54"N; 106°09′54"E; 968–1006 m below sea level). The climate is humid and temperate. The annual mean temperature is 12.9°C, the average temperature in the hottest month is 24–27.5°C and in the coldest month is 0–3°C, the annual mean precipitation is 1103.6 mm (Hu *et al.*, 2010). This area was mainly dominated by sharp-tooth oak forest.

Plant material: The population of *I. polycarpa* currently shows normal and healthy growth. They were monitored for the flowering phenology during the flowering period from early May to early June of 2011. Three adult females and three males were selected from the population with a minimum distance of 10 m and identified respectively for the experimental pollination.

Flowering phenology observation: We recorded the process of anthesis and flower withering of *I. polycarpa*. Field observations were performed weekly before the flowering period. During the anthesis, daily observation was taken on the population. The start, intensity and end of the flowering event were recorded. Flowers were considered to be authetic when anthers were dehiscent and releasing pollen, or the stigma was receptive even if the petals were not fully expanded. The stigma receptivity was tested hourly by means of peroxidase activity using hydrogen peroxide, following Dafni (1992).

Controlled reproductive experiments and cross pollination: Inflorescences of three female plants were randomly selected and bagged to study the breeding system of *I. polycarpa*. The number of flowers of every inflorescence was reducedto 50. The right time of experimental pollination was determined according to the test of stigma receptivity. The following three treatments were carried out on the inflorescences of every female tree: (1) natural pollination (NP). Inflorescences had no manipulation, but were marked and monitored; (2) apomixy (AP). Inflorescences were bagged without pollination; (3) cross pollination (CP). The stigma of the flower from every three female plants was deposited by pollinium of the flower from three male plants, which means that there were 3×3=9 cross combinations. All the treatments and every cross pollination were carried out with 10 inflorescences per tree and every inflorescence with 50 flowers. All bags used in this experiment were made of fine-meshed nylon fabric. Inflorescences were bagged before anthesis and immediately covered again after pollination. All bags were kept on inflorescences until the end of anthesis. Fruit set of the plants in three treatments were recorded at the end of flowering periods when ovaries were distinctly expanded. Fruit were collected at the middle of October when they turned red.

For each treatment and cross combination, fruit were randomly divided into three replications.

100 fruit weight (g): One hundred fruit were selected randomly from each replication. Weight of fruit was determined using an electronic balance to the nearest 0.01 g.

Fruit length and fruit width (mm): Thirty fruit were chosen randomly from the same 100 representative fruit. Polar diameter and equatorial diameter of these fruit were measured with a digital caliper to the nearest 0.01 mm.

Pulp/fruit ratio (%): Pulp of the same selected 100 fruit was weighted to nearest 0.01g. Then average pulp/fruit ratio was calculated as percent (%).

Oil content (%): Oil extraction was carried out with the 3 replications described above following the method of Gohil and Pandya (2008) with minor modification: fruit were dried in hot air oven at 60°C till constant weight and then crushed into powder. The oil was extracted in chemical extraction solvent using petroleum ether (boiling point of 60–90°C) for 4 hours by heating it on a water bath. Petroleum ether was evaporated and oil content was calculated according to the percentage of the powder sample.

Seed germination (%): In early April 2012, seeds were placed in plastic round boxes containing potting mixture of sand, soil and farm yard manure in the ratio of 1:1:1 (by volume) in three replications. Every replication was carried out with 300 seeds. Seed germination was evaluated 20 days after sowing when no seed germination was observed.

Data analysis: Fruit set was calculated as per inflorescence of every treatment and cross. We analyzed differences of fruit set and fruit characters between any two treatments with Tukey-Kramer pairwise comparison. The effect of maternal parents, paternal parents and their interaction on fruit set and fruit characters was analyzed by one-way Analysis of Variance (ANOVA). Correlation analysis was performed on the fruit of natural and controlled pollination treatments to determine the relationships between all fruit traits observed (fruit set and fruit characters)

Results

Flowering phenology of *I. polycarpa*: Male and female flowers were synchronous, that is, the number of female and male opening flowers peaked at the same time (around 20th of May). Both male and female plants began to flower around 10th of May and proceeded until the end of May or the beginning of June at population level. The flowering period usually lasted almost 20 days. The anthers dehisced approximately 5–6 h after the flower opened and the pollen was always available during the anthesis of both floral types. While the stigma had receptivity even if the flower was not fully open. Each inflorescence contained 150–300 flowers, and the male flowered as much as female. Male inflorescence lasted 9 to 12 days while the female lasted 5 to 10 days. At the end of the male anthesis, the stamen withered and then the rest of the flower began to dry. Within 3 to 4 days of the female anthesis, the stigma apparently turned into dark in color, with no receptivity. Then the tepals fell after 5–6 days when the style blacked, and the fruit began to develop.

Fruit set in different treatments: Fruit sets of the three pollination treatments on three females are shown in Figure 1. Fruit formed in all the treatments, although there were 11 inflorescences of apomixy treatment without fruit (6 in ♀1, 1 in ♀ 2 and 4 in ♀3). Based on Tukey-Kramer analysis, three treatments showed significant difference in fruit production ($P<0.0001$). The fruit set of apomixy treatment was the lowest (3.6–13.33%). Natural pollination was greater than cross pollination and the mean fruit set of three female plants were all greater than 90%, and the lowest inflorescence was 78%. The mean fruit set of cross pollination treatment of the three female plants was 75.43%, 81.79% and 65.69%, respectively.

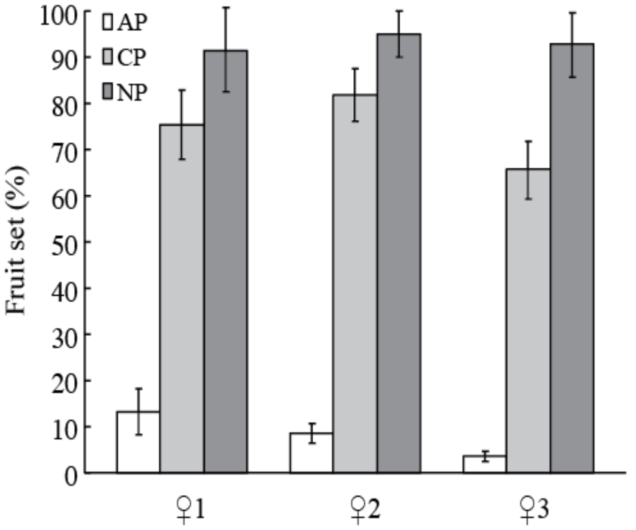

Fig. 1. Mean fruit sets of three pollination treatments in three maternal parents. Error bars represent the standard error. A Tukey-Kramer pairwise comparison indicated that every treatment significantly differed from others (AP: apoximy; CP: controlled pollination; NP: natural pollination).

Fruit characters of different treatments: The mean value of fruit characters data of three treatments, including 9 cross combinations are shown in Table 1. The Tukey-Kramer analysis figured out that fruit characters of three pollination treatments were significantly different except oil content in natural pollination and apoximy treatments (P=0.175). Fruit quality of cross pollination treatment was generally greater than the other two treatments. It was generally bigger, heavier (43.21–66.34g) and had lower pulp/fruit ratio (39.39–52.06%) and higher seed germination rate but had lower oil content (25.66–33.61%) than the other two treatments. On the contrary, fruit of apomixy treatment was much smaller, lighter (19.86–45.92g) and had higher pulp/fruit ratio (50.19–54.48%) and lower seed vigor than the other treatments but as high as natural pollination in oil content (32.05–38.59%).

Parental effect on fruit set and fruit characters: The parental effect on fruit characters was shown in Table 2. Fruit set was significantly affected by maternal parents (P=0.001). Fruit set of different cross combinations varied between different parental pairs, and the mean fruit set of every parental pair ranged from 58.76% to 85.43%. But for all the combinations assessed, paternal effect and the parental interaction were not significant on fruit set. Significant effect of maternal parents (P<0.0001) and parental interaction were found in all the fruit characters (100 fruit weight, pulp/fruit ratio, oil content and seed germination). The effect of paternal parents were significant in 100 fruit weight, pulp/fruit ratio and seed germination and oil content was not affected by paternal parents (P=0.059).

Correlation of fruit characters: Fruit set and the four fruit characters, including fruit length, fruit width, 100 fruit weight and seed germination, exhibited a significant high positive correlation among each other (Table 3). Whereas oil content only had significantly positive correlation with pulp/fruit ratio (r=0.850). Two highest correlations were observed on fruit width and 100 fruit weight (r=0.940), fruit width and fruit length (r=0.905).

Table 1. Mean value for various fruit characters of three pollination treatments (including different parental pairs) in *I. polycarpa* (average ± SD).

Treatment	Fruit length (mm)	Fruit width (mm)	100 fruit weight (g)	Pulp/fruit (%)	Oil content (%)	Seed germination (%)
♀1×♂1	9.52±0.46	9.70±0.58	51.73±1.10	39.39±0.79	25.66±1.48	59.50±1.89
♀1×♂2	9.34±0.59	9.95±0.43	48.70±0.66	41.70±0.56	29.09±1.65	49.67±3.14
♀1×♂3	8.97±0.44	9.10±0.55	43.98±0.71	42.38±0.73	29.31±0.76	60.67±2.00
NP1	9.34±0.56	9.36±0.63	46.43±1.56	49.19±1.63	32.43±1.45	57.67±2.22
AP1	7.16±0.48	6.86±0.64	19.86±1.74	50.19±1.36	33.58±0.85	12.00±3.51
♀2×♂1	9.15±0.55	9.71±0.55	46.65±2.95	45.70±1.15	27.97±0.78	62.00±4.25
♀2×♂2	8.88±0.48	9.30±0.46	43.21±1.67	44.81±1.37	27.74±1.35	65.00±2.25
♀2×♂3	8.84±0.29	9.32±0.41	43.32±2.67	40.49±1.64	26.82±1.18	47.00±2.18
NP2	8.01±0.74	8.31±0.77	35.33±1.77	51.19±1.37	32.98±1.55	58.33±4.98
AP2	7.72±0.68	7.85±0.75	25.60±2.51	52.06±1.49	32.05±0.59	11.50±2.11
♀3×♂1	9.93±0.60	10.87±0.64	66.34±2.09	46.67±1.04	32.93±1.26	33.00±2.40
♀3×♂2	9.59±0.75	10.43±0.83	51.01±1.88	48.34±0.64	33.61±1.23	33.20±1.55
♀3×♂3	9.83±0.79	10.79±0.49	62.80±1.95	48.19±1.16	32.49±0.49	34.67±1.90
NP3	8.46±0.73	9.36±0.75	45.75±1.58	53.67±1.45	37.54±1.59	52.00±1.55
AP3	9.03±0.88	9.58±1.17	45.92±2.31	54.48±1.35	38.59±0.88	15.59±2.31

NP: natural pollination; AP: apoximy

Table 2. ANOVA for fruit set and fruit characters (100 fruit weight, oil content and seed germination) of the controlled pollinated flowers of different parents

Source of variation	df	MS	F	P-value
Fruit set				
Female (F)	2	0.168	8.155	0.001
Male (M)	2	0.021	1.009	0.370
F×M	4	0.047	2.285	0.068
Error	73	0.021		
100 fruit weight				
Female (F)	2	411.63	351.77	<0.0001
Male (M)	2	69.66	59.53	<0.0001
F×M	4	42.97	36.72	<0.0001
Error	18	1.17		
Pulp/fruit				
Female (F)	2	99.25	87.88	<0.0001
Male (M)	2	4.07	3.60	0.048
F×M	4	14.58	12.91	<0.0001
Error	18	1.13		
Oil content				
Female (F)	2	61.44	62.16	<0.0001
Male (M)	2	3.89	3.94	0.059
F×M	4	3.71	3.75	0.046
Error	18	0.988		
Seed germination				
Female (F)	2	1204.87	289.77	<0.0001
Male (M)	2	26.88	6.47	0.020
F×M	4	105.36	25.34	<0.0001
Error	18	4.16		

Discussion

Flowering phenology: In our study site, we found that male and female plants flowered almost synchronously and the anthesis lasted almost 20 days. The stigma showed receptivity before the flower opened while the anther dehisced 5–6 h after the flower opening. The long anthesis of both floral types provides enough opportunity for the pollen to be shed on the stigma. This could reduce the risk of reproductive failure in *I. polycarpa*. However, the flowering phenology of trees varied among populations, locations and climates (Vieira & Silveira 2010; Munguia-Rosas *et al.*, 2011). Further study should be carried out in different populations located in different sites to verify the results.

Breeding system of *I. polycarpa*: Reproductive success of apoximy treatment suggested that, as a diocious plant, *I. polycarpa* could also reproduce by apomixis, although the fruit set was very low (0–18%). Further study should be carried out to confirm the result and figure out the mechanism of apomixis. The fruit set of natural pollination (>90%) was higher than controlled pollination treatment (81.79–65.69%). But in many studies, fruit set of cross-pollination was higher than open-pollinations (Freihat *et al.*, 2008; Srivastav *et al.*, 2014; Zhang *et al.*,2014). A number of mechanisms may lead to this difference, including maternal control of pollen germination, pollen tube growth rate and seed abortion (Marshall & Ellstrand 1988; Swanson *et al.*, 2004; Ruane & Donohue 2007). In this study, the difference in fruit set of two pollination treatments may be caused by the fine-mesh nylon fabric bags used in controlled pollination experiment. The bags will form shading which may potentially affect patterns of resource allocation and fruit set (Fischer *et al.*, 2003; Supaporn *et al.*, 2017).

Fruit quality and pollen competition: In three pollination treatments, fruit of apoximy treatment was the smallest, lightest and had the lowest seed production and seed vigor, while the controlled pollination fruit were the biggest, heaviest and had the highest seed productionand seed vigor compared to the other two treatments. We speculated that it was caused by different amount of pollen onto the stigma. No pollen was used in apoximy treatment. In natural pollination the pollinator could only be touched by a few of pollen. But when we did cross pollination experiment, the whole pollinium was used. The larger pollen load size in controlled pollination treatment enhanced pollen competition which will improve fruit quality and influence the progeny performance (Quesada *et al.*, 1993; Marshall *et al.*, 2000; Zhang *et al.*, 2010). Oil content of the controlled pollination treatment was lower than the other two treatments. That might be because the pulp/fruit ratio of controlled pollination treatment was the lowest and the oil content in pulp was higher than seed (Zhu, 2010).

Parental effect on fruit characters: In our study, maternal parents showed significant effects on fruit set (P=0.001) and all fruit characters (P<0.0001), including 100 fruit weight, pulp/fruit ratio, oil content and seed germination. While paternal parents significantly affected 100 fruit weight, pulp/fruit ratio and seed germination. But the interactions of maternal and paternal parents were found in all the fruit characters. It could infer that maternal genotype of *I. polycarpa* could influence the siring ability of the pollen donor (Muraya *et al.*, 2011). This fact indicated that the selection of female parent in *I. polycarpa* breeding program is of special importance.

Correlation of fruit traits: As the most important fruit character, fruit oil content could only be predicted with pulp/fruit ratio. Fruit yield and seed vigor had significant positive correlation. However, there is a potential effect of environment on the development of fruit traits, such as size, shape, weight and oil content (Shen *et al.*, 2013; Wang *et al.*, 2017). So in different environments, the correlation of fruit characters may not be consistent. A follow-up study on fruit of populations in different environments is a logical next step.

Table 3. Correlation coefficients between fruit characters (size, weight and seed germination) and fruit set.

Trait	Fruit length	Fruit width	100 fruit weight	Seed germination	Fruit set	Oil content
Fruit width	0.905**					
100 fruit weight	0.880**	0.940**				
Seed germination	0.584*	0.787**	0.730**			
Fruit set	0.815**	0.830**	0.798**	0.711**		
Oil content	0.125	0.130	0.155	0.438	0.212	
Pulp/fruit	0.326	0.123	0.090	0.170	0.450	0.850**

* Significant at 5% level, ** significant at 1% level.

Conlusions

The present study suggested that female plant of *I. polycarpa* can reproduce asexually even though apomixis did not happen frequently. In addition, maternal parents showed more significant effects on fruit characters than paternal parents. This suggested that, for better yield and fruit quality, choosing appropriate maternal parent is more important in crossbreeding. As the most important character, fruit oil content could be predicted through fruit pulp/fruit ratio. However, our study was carried out in one population of *I. polycarpa*. Further investigations with different populations are necessary to verify the results and clarify the apomixis mechanism, which will help us to formulate breeding program of *I. polycarpa* in the future.

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