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Effects of Open- and Self-Pollination on Fruit Set and Seed Quality in *Jatropha curcas* L.

(Kesan Pendebungaan Terbuka dan Penswadebungaan pada Buah dan Kualiti Biji Benih bagi *Jatropha curcas* L.

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ABSTRACT

Investigation on different mating systems is necessary to improve fruit and seed production and seed quality in Jatropha curcas. *In the present study, a total of 150 female flowers belonging to five accessions from Indonesia and Malaysia were screened for traits associated with fruit set and seed quality under open- and self-pollination. The study was conducted at Biodiesel Research Station, Universiti Kebangsaan Malaysia (UKM), Kuala Pilah from April to September 2015. The number of fruits per plant (NFP), number of seeds per plant (NSP) and number of seeds germinated (NSG) showed significant differences among the mating systems. Under open-pollination, the maximum mean values for NFP, NSP, NSG and number of seedlings surviving after three months (NSS) were 10, 29, 27 and 18, respectively. Conversely, self-pollination scored at 9, 26, 22 and 18 for NFP, NSP, NSG and NSS, respectively. Seed weight (SWt) showed significant differences among accessions whereas seed length (SLT) showed significant differences among the mating systems and accessions. UKMJC20 (Indonesia) showed the best performance in terms of NFP under both open- and self-pollination whereas UKMJC21 (Malaysia) showed the best performance in terms of SWt and SLT under both conditions. Two (UKMJC13 and UKMJC21) out of five accessions showed occurrence of apomixis.*

Keywords: Artificial pollination; Jatropha curcas*; mating systems; open-pollination; seed quality*

ABSTRAK

Kajian terhadap kepelbagaian sistem pembiakan adalah penting dalam usaha meningkatkan pengeluaran buah serta biji dan kualiti benih Jatropha curcas. *Dalam kajian ini, sejumlah 150 bunga betina yang terdiri daripada lima penerimaan dari Indonesia dan Malaysia telah dinilai berdasarkan ciri yang berkait dengan penghasilan buah dan kualiti biji benih di bawah pendebungaan terbuka dan pendebungaan sendiri. Kajian ini dilakukan di Pusat Penyelidikan Biodiesel, Universiti Kebangsaan Malaysia (UKM) Kuala Pilah dari April hingga September 2015. Bilangan buah setiap pokok (NFP), bilangan biji setiap pokok (NSP) dan bilangan biji bercambah (NSG) menunjukkan perbezaan bererti antara jenis pendebungaan yang berlainan. Pendebungaan terbuka menunjukkan nilai min maksimum bagi NFP, NSP, NSG dan bilangan anak benih yang berjaya hidup selepas tiga bulan (NSS) masing-masing ialah 10, 29, 27 dan 18. Sebaliknya, pendebungaan sendiri masing-masing menunjukkan nilai 9, 26, 22 dan 18 untuk NFP, NSP, NSG dan NSS. Berat biji benih (SWt) menunjukkan perbezaan yang bererti antara penerimaan sahaja manakala kepanjangan biji benih (SLT) menunjukkan perbezaan yang bererti antara sistem pembiakan dan penerimaan. UKMJC20 (Indonesia) menunjukkan ciri yang terbaik daripada segi NFP dalam pendebungaan terbuka dan pendebungaan sendiri manakala UKMJC21 (Malaysia) menunjukkan ciri terbaik daripada segi SWt dan SLT dalam kedua-dua jenis pendebungaan. Dua (UKMJC13 dan UKMJC21) daripada lima penerimaan menunjukkan kewujudan apomiksis.*

Kata kunci: Jatropha curcas*; kualiti biji benih; pendebungaan bantuan; pendebungaan terbuka; sistem pembiakan*

INTRODUCTION

The monoecious *Jatropha curcas* L. plant bears complete flowers, which are able to exhibit both protandrous and protogynous states during the floral development process (Negussie et al. 2014). The flowers are in racemose inflorescences, with dichasial cyme pattern (Raju & Ezradanam 2002). Male flowers can be easily distinguished from the female flowers; the former has a round head bud with small pedicle, about ten stamens with yellow anthers and five sepals and petals whereas the latter forms a conical head with long sepals, thick long pedicle and three styles and bifid stigmas (Dasumiati et al. 2015). Generally, the inflorescence is composed of a central female flower surrounded by a group of male flowers (Wu et al. 2011). However, in few cases, at the places where female flowers are expected to develop, a substitution by the male flowers is observed (Raju & Ezradanam 2002).

At the reproductive stage, the inflorescence produces flower on a daily basis for an average period of 11 days. During this period, the male flowers open first and the female flowers bloom between the second and sixth day (Chang-wei et al. 2007; Raju & Ezradanam 2002). In other studies, female flowers have been reported to open before its male counterpart (Pranesh et al. 2010; Rincon-Rabanales et al. 2016). The rate of male and female flowers opening was reported the highest at 0700 h (Kaur et al. 2011). The stigma becomes receptive and remains receptive up to the fourth day from the day of bloom (Chang-wei et al. 2007). The female flowers with three-loculus ovary will eventually form a three-seeded fruit (Church 1921), however, in rare cases, some flowers may bear up to four or five loculi in each ovary (Wang & Ding 2012). The development of individual fruit begins immediately after fertilization and the fruiting process becomes apparent at 30 days after the bud formation (Kaur et al. 2011). Fruits then grow to full size over a two-month period. Fruits are the product of random fertilization which includes both the geitonogamy (pollen transfer within the same plant) and xenogamy (pollen transfer between two different plants). The geitonogamous offspring might be selectively eliminated by the plant to accommodate reallocation of resources for the xenogamous fruit (Raju & Ezradanam 2002). In terms of yield component, the fruit and seed set quality of *J. curcas* fluctuates seasonally. According to Che Mat et al. (2016) and Kaur et al. (2011), fruit set, seed weight and kernel weight of *J. curcas* harvested in the first season were significantly lower compared to the second season.

Due to the adhesive nature of *J. curcas* pollen, wind pollination is almost impossible in this species (Chang-wei et al. 2007). A study conducted by Rianti et al. (2010) in Bogor, Indonesia identified *Prenolepis* species, *Apis dorsata*, *Xylocopa confusa* and *Apis cerana* as the biggest contributors for *J. curcas* pollination. In another study by Luo et al. (2012) in Shuangbai and Yuanmou, China, an ant species, *Tapinoma melanocephalum,* was found to play an important role in pollination at both the study sites. European bees (*Apis mellifera*), green flies (*Chrysomya chloropyga*) and carrion flies (*Eristalis tenax*) were identified as pollinators of *J. curcas* in Samaru, Nigeria (Alamu et al. 2013). In Nahan and Arki, India, 16 bee species were found attracted to the *J. curcas* flowers: *Xylocopa fenestrata* (most abundant), *Ceratina gigantica*, *Megachile flaviceps*, *Halictus* sp. and *Andrena* sp. (Mattu & Kumar 2015). Inevitably, most of the insect pollinators for *J. curcas* were from the orders of Hymenoptera, Lepidoptera and Diptera (Kaur et al. 2011; Raju & Ezradanam 2002).

At present, only very few studies have investigated the *J. curcas* mating systems. In a study conducted by Wang and Ding (2012) in Guiyang Guizhou Province, China, no significant differences in the *J. curcas* fruit set rates were observed under the open- (80.51%) and artificial self-pollination (77.38%) systems. Similar results were found in another study by Kaur et al. (2011) in north-western India, where fruit setting rates under open- and self-pollination systems were 79.2% and 72.2%, respectively. Nietche et al. (2014) also found similar result for fruit set in natural and self-pollination in Florida but varied significantly for oil content (natural pollination -27.1%, and self-pollination-29.%). However, in a study by Negussie et al. (2014) in Zambia and Malawi significantly less fruit set was observed in autogamous compared to open pollination. Similarly, Raju and Ezradanam (2002) observed significant differences in *J. curcas* fruit set rates through xenogamy (96%) and geitonogamy (77%) in India. The objectives of this study were to assess the effects of different mating systems (open and artificial pollination) on fruit set and seed quality of *J. curcas*. The findings could be utilized for fruit set and seed quality enhancement of *J. curcas* planting materials.

Materials and Methods

A total of 150 female flowers from five accessions were studied under two different mating systems: open-pollination and artificial self-pollination. The mating system evaluations were performed on five-year old *J. curcas* plants grown at Biodiesel Research Station, Universiti Kebangsaan Malaysia (UKM), Kuala Pilah (2°41’13.9” N, 102°16’26.3” E). Plants comprised of accessions from Malaysia (UKMJC10, UKMJC17 and UKMJC21) and Indonesia (UKMJC13 and UKMJC20). Each plant received chicken manure (2-3 kg) at every four-month intervals and fertilizer (20 g urea, 120 g single super phosphate and 16 g muriate of potash) at every two-month intervals throughout the study period (Punia 2007). Plants were irrigated when needed to supplement the natural rainfall and care was taken regularly to prevent infestation by pest and diseases. Three different methods were used to evaluate *J. curcas* mating systems: For open-pollination, ten random female flowers from each accession were selected, marked and left undisturbed under natural conditions; For artificial cross-pollination, ten random female flowers from each accession were covered with a paper bag prior to flower opening. The male flowers within the same inflorescence were removed before fixing the bags. At the following days, when the female flower opened, pollens from the same plant were artificially applied onto the stigma of female flower by rubbing the anthers of male flowers. Next, the female flower was re-bagged and was only released when small fruit started to develop from the flower; To establish an apomixis system, ten random female flowers from each accession were bagged before anthesis after removing all the surrounding male flowers. Extra care was taken by wrapping the paper bag onto the stem using cellophane tape to ensure no insects can enter and pollinate the female flowers.

Thirty female flowers from each accession were evaluated for number of fruits per plant (NFP), number of seeds per plant (NSP), number of seeds germinated (NSG) and number of seedlings surviving after three months (NSS) under each mating system. To determine the percentage of seed germination, all seeds were collected when the fruits turned yellow to get the highest germination rate as described by Ahmad and Sultan (2015). The seed weight (SWt) was measured using a digital weighing balance (AND FY-300), whereas, the seed length (SLT), seed width

(SWH), seed thickness (STH) and seedling diameter (SDA) were measured using a Vernier caliper. The evaluation was conducted from April to September 2015.

STATISTICAL ANALYSIS

All fruit set and seed quality associated traits were subjected to two way-analysis of variance (ANOVA) to determine significant differences among the mating systems and accessions using the Statistical Analysis System (SAS) 9.4 software (SAS 2013). The SWt and SDA were categorized into four classes whereas SLT was categorized into three classes. Chi square (χ^2) test was carried out to determine the relationship of SWt, SLT and SDA on seed germination percentage and survivability (Steel et al. 1997).

RESULTS AND DISCUSSION

Our observation on 50 female flowers from five accessions showed open-pollination as the best mating system for *J. curcas* fruit set (90%), followed by geitonogamy at 74% and apomixis at 10% (Table 1). The highest number of fruits formed under open-pollination was 45, followed by 37 and 5 under geitonogamy and apomixis, respectively. The results were comparable to a study conducted in Soconusco, Chiapas, Southern Mexico which showed a similar trend in fruit set rates: open-pollination (86.3%), geitonogamy (16.2%) and apomixis (2.5%) (Rincon-Rabanales et al. 2016). The opening of female flowers first prior to male flowers and a high pollen production by anther in each flower may explain the occurrence of open-pollination (Bhattacharya et al. 2005; Cruden 1977; Rincón-Rabanales et al. 2016). A relatively high incidence (percentage) of geitonogamy in our study indicates prevalence of self-pollination, a trait which can contribute to low genetic diversity in *J. curcas* (Siju et al. 2016).

Likewise, for number of seeds, open-pollination showed the highest yield at 92%, followed by geitonogamy (87%) and apomixis (13%). Five fruits were formed without pollination, four aborted and one matured with two seeds. Low number of seeds per fruit under self-pollination and apomixis may be due to ovule abortion at the time of fruit development (Raju & Ezradanam 2002). The results corroborated with a similar study by Kaur et al. (2011), who showed a total of 1.3 and 1.6 number of seeds per fruit under the self-pollination and apomixis, respectively. Since the number of fruits produced under apomixis was too small (negligible), the data was excluded for subsequent statistical analyses. A study conducted by Nietsche et al. (2014) in South Florida showed no differences in percentage of fruit set under the natural and self-pollination systems. On the contrary, significant differences ($p \leq 0.01$) in the production of fruits and seeds among the different mating systems were found in this study (Table 2). These differences may imply occurrence of inbreeding depression during fruit and seed set of *J. curcas* under geitonogamy as suggested by Hartati and Sudarson (2014), whereby inbreeding depression was observed only in several accessions under geitonogamy.

The maximum number of fruits and seeds in UKMJC20 was 10 and 29, respectively, under open-pollination whereas the geitonogamy showed nine and 26 fruits and seeds, respectively, for the same accession. Significant

TABLE 1. Yield components of *J. curcas* accessions obtained under different mating systems

Country of origin	Accession	Open-pollination				Self-pollination				Apomixis			
		NFP	NSP	NSG	NSS	NFP	NSP	NSG	NSS	NFP	NSP	NSG	NSS
Malaysia	UKMJC10	9	22	16	16	7	19	15	14	0	0	0	0
	UKMJC17	10	27	20	14	6	14	12	10	0	0	0	0
	UKMJC21	8	24	19	18	8	20	18	12	4	0	0	0
Indonesia	UKMJC13	8	22	17	13	7	18	14	10	1	2	2	1
	UKMJC20	10	29	27	18	9	26	22	18	0	0	0	0
Total		45	124	99	79	37	97	81	64	5	2	2	1
Percentage (%)		90	92	80		74	87	84		10	13		

NFP= number of fruits per plant, NSP= number of seeds per plant, NSG= number of seeds germinated, NSS= number of seeds survived after three months

TABLE 2. Mean square of ANOVA for number of fruits, seeds and seeds characteristics of *J. curcas* accessions in two different kinds of mating system

Sources of variation	Degree of freedom (d.f)	NFP	NSP	NSG	NSS
Mating systems	1	0.96**	10.14**	4.80*	3.28ns
Accession	4	0.13ns	2.13ns	3.20*	1.60ns

*= significant at the 0.05 level, **= significant at the 0.01 level, ns= non-significant
NFP= number of fruits per plant, NSP= number of seeds per plant, NSG= number of seeds germinated, NSS= number of seeds survived after three months

differences were observed within the mating systems and accessions studied for NSG at $p \leq 0.05$. This suggests that beside soil texture that can influence the ability of seeds to germinate, type of mating system and certain accessions (UKMJC20 and UKMJC21) had better germination rate compared to others (Valdés-Rodríguez et al. 2013). However, no significant differences were observed within the accessions studied for NFP, NSP and NSS. No significant difference was also observed in the mating systems for NSS.

For all seedlings and seed-related traits, both open-pollination and geitonogamy showed high value in all of the traits studied with the highest value in SWt of 1.77 g recorded in open pollination whereas the highest value in SLT of 2.23 cm was recorded in geitonogamy (Table 3). Seeds obtained through apomixis showed a different result with the following maximum values: SWt at 0.54 g, SLT at 1.91 cm, SWH at 1.19 cm, STH at 0.90 cm and SDA at 1.08 cm. The results for the maximum SWt (1.77 g) was comparable to the average SWt (1.65 g) recorded in South Eastern Mexico under the open-pollination system (Rincon-Rabanales et al. 2016). ANOVA for seed and seed-related traits showed a significant difference for SLT trait under the two different mating systems at 0.05% (Table 4). The results were in agreement with Abdelgadir et al. (2009), who showed no significant differences in SWt between the cross- and self-pollination systems of *J. curcas* in South Africa. However, significant differences were observed in SWt and SLT traits among the different accessions at $p \leq 0.01$. The UKMJC21 showed the highest mean value for the two traits (Table 3). No significant differences were observed in SWt, SLT and SDA traits studied.

TABLE 3. Mean and standard deviation values for different seedlings and seed related traits of *J. curcas*

Mating system	Accession	SWt	SLT	SWH	STH	SDA
Open-pollination	UKMJC10	0.81±0.14	1.92±0.08	1.24±0.23	0.90±0.02	0.79±0.72
	UKMJC17	0.75±0.26	1.81±0.16	1.12±0.16	0.87±0.13	0.89±0.75
	UKMJC21	0.94±0.36	2.05±0.16	1.16±0.18	0.88±0.14	0.87±0.60
	UKMJC13	0.65±0.18	1.88±0.04	1.16±0.04	0.87±0.03	0.83±0.58
	UKMJC20	0.80±0.15	2.00±0.06	1.16±0.02	0.88±0.02	0.97±0.67
Maximum trait value		1.77	2.20	2.04	1.00	1.79
Minimum trait value		0.04	1.35	0.56	0.41	0.00
Self-pollination	UKMJC10	0.62±0.08	1.95±0.04	1.15±0.02	0.87±0.02	1.19±0.55
	UKMJC17	0.54±0.13	1.82±0.05	1.11±0.05	0.87±0.06	0.78±0.52
	UKMJC21	1.08±0.19	2.06±0.09	1.19±0.03	0.91±0.03	0.84±0.59
	UKMJC13	0.68±0.24	1.97±0.10	1.16±0.05	0.89±0.05	0.42±0.40
	UKMJC20	0.80±0.23	2.03±0.10	1.13±0.03	0.84±0.03	0.96±0.46
Maximum trait value		1.26	2.23	1.27	1.03	1.80
Minimum trait value		0.32	1.74	1.02	0.79	0.00
Apomixis	UKMJC10	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	UKMJC17	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	UKMJC21	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	UKMJC13	0.07±0.07	0.25±0.67	0.16±0.42	0.12±0.31	0.07±0.28
	UKMJC20	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Maximum trait value		0.54	1.91	1.19	0.90	1.08
Minimum trait value		0.00	0.00	0.00	0.00	0.00

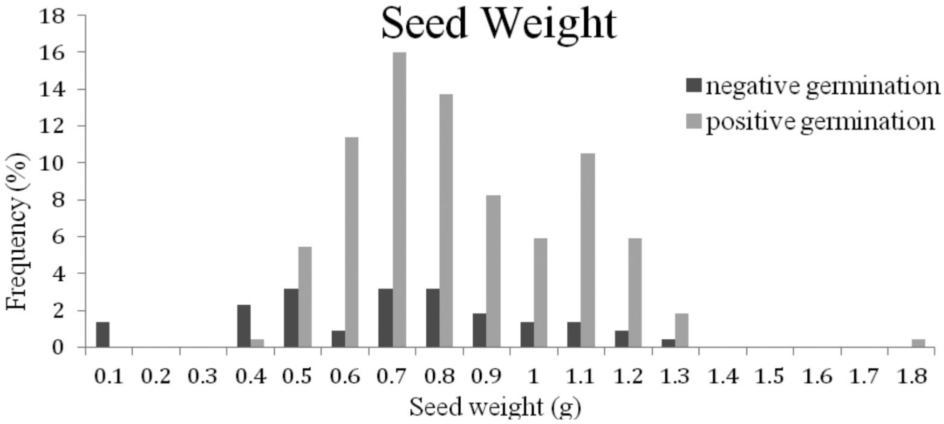
SWt = seed weight (g), SLT = seed length (cm), SWH = seed width (cm), STH = seed thickness (cm), SDA = seedlings diameter at the age of two months (cm)

TABLE 4. Mean square of ANOVA for seedlings and seed related traits

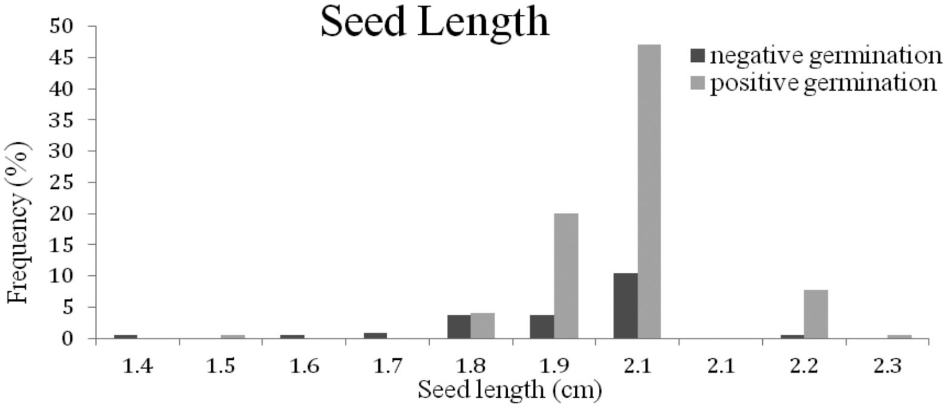
Sources of variation	Degree of freedom (d.f)	SWt	SLT	SWH	STH	SDA
Mating systems	1	0.06^{ns}	0.04^{*}	0.02^{ns}	0.00^{ns}	0.03^{ns}
Accession	4	0.63^{**}	0.23^{**}	0.03^{ns}	0.01^{ns}	0.59^{ns}

* significant at the 0.05 level, ** significant at the 0.01 level, ns = non-significant
SWt = seed weight (g), SLT = seed length (cm), SWH = seed width (cm), STH = seed thickness (cm), SDA = seedlings diameter at the age of two months (cm)

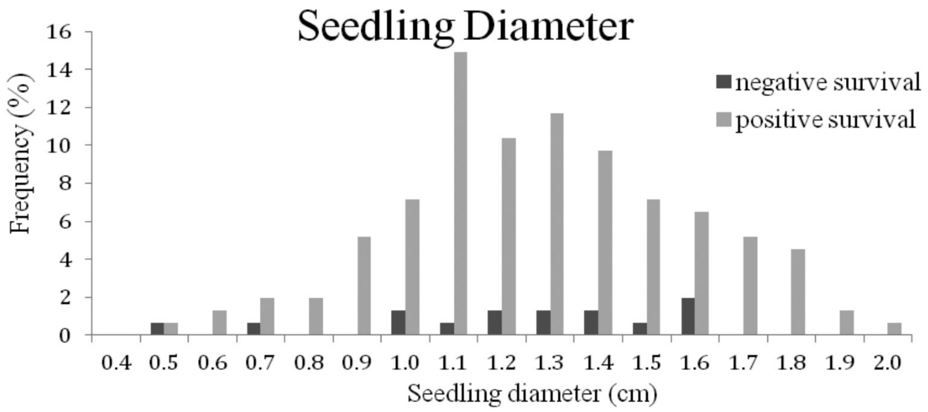
Both the frequency distribution graphs of SWt and SDA showed positive skewness with positive germination ranged at 0.4-1.8 g and positive survival ranged at 0.5-2.0 cm (Figure 1(a), 1(c)). On the contrary, frequency distribution graph for SLT showed negative skewness with positive germination ranged at 1.8-2.3 cm (Figure 1(b)). Chi-square values were significant for SWt ($p\leq0.01$) and SL ($p\leq0.05$) whereas SDA was not significant ($p\leq0.05$) (Table 5). Hence, both SWt and SLT can influence germination in *J. curcas*.



(a)



(b)



(c)

FIGURE 1. Frequency distributions of seed weight on seed germination (a) seed length on seed germination (b) and seedling diameter on seedling survival (c) in *J. curcas*

TABLE 5. Germination and survival mean of seedlings and seed related traits of *J. curcas*

Seeds and seedlings	SWt	SLT	SDA
Total negative germination/survivability observed	44	44	15
Total positive germination seeds/seedlings observed	175	175	140
Chi-square value (χ^2)	27.98**	6.41*	2.82ns
Degree of freedom	3	2	3

* significant at the 0.05 level, ** significant at the 0.01 level, ns = non-significant according to Chi square (χ^2) test
SWt = seed weight (g), SLT = seed length (cm), SDA = seedlings diameter (cm)

CONCLUSION

The highest NFP and NSP were set under open-pollination in comparison to geitonogamy and apomixis. Seed quality especially SLT was higher in geitonogamy as compared to open-pollination. Thus, open-pollination could be recommended to increase seed set whereas artificial self-pollination could be utilized to increase seed germination.

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