Journal of Tropical Crop Science Vol. 1 No. 2, October 2014
www.j-tropical-crops.com

RESEARCH ARTICLE

Reproductive success and compatibility among accessions of *Jatropha curcas* in Indonesia.

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Abstract

Large scale exploitation of *Jatropha curcas* for biofuel production is restrained by low productivity hence economically not profitable. One of the main constraints is due to limited number of female flowers in an inflorescence which eventually develop into fruits that bear seed. Investigation on floral biology and reproductive potential of the species had been carried out, but seems to vary among sites. The research was conducted to study reproductive success and the compatibility among Indonesian *Jatropha* accessions. The research was set up in two experiments. The first experiment was conducted at the *Jatropha* seed orchard, Pakuwon, Sukabumi, West Java from April to July 2007 using four-year- old trees of accessions from Lampung, Banten, West Java, and Central Java. The second experiment was conducted at Leuwikopo Experimental Station of Bogor Agricultural University from April to July 2008 using one-year-old trees of accessions from Lampung, Bengkulu, Palembang, and Kediri. Flowers were obtained from controlled self and cross-pollinated as well as left for open-pollination. The result showed that reproductive success varied among accessions; the greatest success was 0.73 obtained from a West Java accession and the least was 0.53 from Banten accession. The *Jatropha* accessions were categorized as partially self-incompatible as indicated by index of self-incompatibility (ISI) that ranged 0.93-0.99. Banten accession produced highly viable and vigorous seeds regardless of pollination methods; accessions of Central Java produced higher viability seeds when cross-pollinated within accession, whereas those from West Java and Lampung produced higher viability seeds when self-pollinated.

Keywords: accessions, *Jatropha curcas*, self-pollination, self-incompatibility, seed viability

Introduction

The need of renewable energy has encouraged many parties to find sources of biofuel. *Jatropha curcas* is considered as one of the potential species not only for biofuel production but also biogas and compost (Openshaw, 2000, Tiwari et al., 2007, Achten et al., 2008). Drought tolerant species can survive in marginal land thus avoid competition with food crops which can only grow in fertile land. However, large scale exploitation is restraint to low productivity due to the low number of female flowers, i.e. averaged 10 female flowers per inflorescence (Adikarsih and Hartono, 2007). Moreover a long-term systematic breeding program has not been set up which make the species is still considered as wild and exhibits variability in productivity among individuals (Achten et al., 2008).

The biodiesel development program in Indonesia has been set up to grow plantations consisting of 1.5 million ha of oil palm, 1.5 million ha of *Jatropha*, 0.75 million ha of sugar cane and 1.5 million ha of cassava. The development of 1.5 million ha of *Jatropha* plantations during 2007-2010 required about 900 ton of seeds (Hasnam, 2007) or about 0.6 kg.ha^{-1}. Seed procurement is also facing several limitations that contributed to low seed viability such as time of harvest in relation to fruit maturity, seed processing and drying (Sudjindro, 2008).

The project was started with the exploration of *Jatropha* germplasm in nine provinces which consisted of 54 districts and 200 000 stem cuttings and seeds had been collected from these areas. The collected germplasm were then grouped into 12 accessions, i.e. West Sumatera, Lampung, Banten, West Java, Central Java, East Java, West Nusa Tenggara, East Nusa Tenggara, South Sulawesi, Gorontalo and Maluku. Variations in plant morphology including leaf thickness, leaf shape and size, leaf color, pedicel length, fruit shape and size, seed weight and size, number of seed per capsule were found among the accessions (Hasnam, 2007).

The germplasm were then planted in three locations: Pakuwon, Sukabumi, West Java representing wet areas, Asembagus, Situbondo, and East Java representing dry areas, and Muktiharjo, Pati Central Java representing moderately dry areas. After a mass selection the first generation of improved-population was obtained in 2006, i.e. IP-1P (Pakuwon), IP-1A (Asembagus), and IP-1M (Muktiharjo) with productivity of about four to five $t.ha^{-1}.year^{-1}$ at the fourth year after planting. The second generation of the improved-population (IP-2) had a higher productivity of about six to seven $t.ha^{-1}.year^{-1}$ at the fourth year after planting. The third generation (IP-3) was expected to have productivity of about eight to ten $t.ha^{-1}.year^{-1}$ after the fourth year. After the third generation of improved-population a long-term breeding plan will be required to increase productivity further, using either conventional or molecular techniques in order *Jatropha* to become a more economically attractive (Heliyanto et al., 2008).

Conventional technique for plant improvement through controlled pollination or hybridization requires information on flowering phenology and pollination system as well as compatibility among accessions. Hartati (2008) studied the flowering phenology of *Jatropha* in Pakuwon, Sukabumi during April-October and reported that pollination was best conducted in either late morning (when the stigma has completely splayed), or afternoon on the first day of anthesis, although the pistillate (female) flower lasts for several days. This research was aimed at studying flowering biology, reproductive success of several accessions and the compatibility among accessions of *Jatropha* in Indonesia.

Materials And Methods

The research was set up in two experiments. The first experiment was conducted at the *Jatropha* Seed Orchard, Pakuwon, Sukabumi, West Java, about 450 m above sea level, with Latosol soil and type B climate (Oldeman) from April to July 2007. Four-year-old plants from four accessions were used i.e. accessions from Lampung, Banten, West Java, and Central Java with 20 plants from each accession. The second experiment was conducted at Leuwikopo Experimental Station, Bogor Agricultural University, about 250 m above sea level, from April to July 2008. One-year-old plants of accessions from Lampung, Bengkulu, Palembang, and Kediri (from stem cuttings) taken from the seed orchard in Pakuwon were used with 20 plants from each accession.

Type of inflorescence, ratio of male to female flowers, as well as time and duration of blooming were observed. Reproductive success, which was defined as the number of ovules that develop into viable seeds (Wiens et al., 1987) was observed in open-pollinated population during the first and second experiment which was started with the number of inflorescence per plant and number of female or hermaphroditic flower per inflorescence (Fl/Infl). Fruit set (Fr/Fl) was calculated as the ratio of female/hermaphroditic flowers that developed into mature fruits in an inflorescence, whereas number of ovule per flower (O/Fl) was counted as averaged of 20 flowers per accession, which resulted in three ovules per flower. Therefore seed set (Sd/O) was calculated as the proportion of the three ovules that developed into viable seeds in a fruit. Reproductive success was calculated from Fr/Fl ratio and Sd/O ratio.

The flowers were tagged with color threads when once opened. The number and time of flower or fruits abortion were observed every alternate day. The experiment was arranged in a completely randomized design and analyzed using Duncan's multiple range tests (DMRT) to compare the reproductive success among accessions.

The compatibility among accessions in the first experiment (four-year-old plants) was recorded from four types of hand controlled-pollinations, i.e.:

1. Intra-self-pollination (SP-intra): pistillate flowers were pollinated with pollen from staminate flowers of the same inflorescence.
2. Inter-self-pollination (SP-inter): pistillate flowers were pollinated with pollen from staminate flowers of different inflorescence of the same plant.
3. Intra-cross-pollination (CP-intra): pistillate flowers were pollinated with pollen from staminate flowers of other plants within the same accession.
4. Inter-cross-pollination (CP-inter): pistillate flowers were pollinated with pollen from staminate flowers of other accession. Accession from South Sulawesi was used as pollen donor for the four selected accessions because it had high staminate flower production.

All pistillate flowers were bagged at 06.00-07.00hrs in the morning and pollinated during 10.00-12.00hrs. Based on the results from the first experiment self- and cross-pollination of the second experiment (on one-year-old plants) were arranged as follows:

1. Self-pollination: pistillate flowers were pollinated with pollen from staminate flowers of the same or different inflorescence of the same plant.
2. Cross-pollination: pistillate flowers were pollinated with pollen from staminate flowers of other accession. IP-1P was used as pollen donor for the four selected accessions.

The observation on each accession included fruit set from self- and cross-pollination, index of self-incompatibility. Seed quality was determined based on germination percentage, and germination speed was analyzed according to ISTA (2007). The data was analyzed using split-plot design, with accession as the main plot and pollination type as the sub-plots.

Index of self-incompatibility (ISI) was defined as the ratio of fruit set from self-pollination to cross-pollination. The ISI values were then classified into: ISI≥1: self-compatible; 0.2<ISI<1: partially self-incompatible; ISI<0.2: mostly self-incompatible; ISI=0: completely self-incompatible (Zapata and Arroyo, 1978).

RESULTS AND DISCUSSION

The first inflorescence of the one-year-old plants was commonly unisexual. Staminate inflorescences were observed in Lampung and Kediri accessions whereas pistillate inflorescences in Bengkulu and Palembang accessions. The subsequent inflorescences in all plants were bisexual. In most cases staminate and pistillate flowers were born on the same inflorescence (monoecious) and rarely staminate and hermaphroditic (andromonoecious) (Figure 1).

Most of the plants from Central Java produced staminate and hermaphroditic flowers. The flower sex was indicated by the size of the flower bud; the hermaphroditic flower buds was the largest followed by pistillate and staminate flower buds. The staminate flowers of the four-year-old plants usually bloomed around six days earlier than the pistillate flowers and flowering continued for several days. The pistillate flowers bloomed only for two to five days and the flowering period coincided with that of staminate flowers. On the other hand, the pistillate flowers of the one-year-old plants bloomed 2 days earlier than the staminate flowers and last for 2-6 days, followed by the staminate flowers for several days. In this case only late blooming pistillate flowers coincided with the blooming staminate flowers. Raju and Ezradanam (2003) reported that *Jatropha* is a cross pollinating species, therefore different blooming time of male and female flowers within an inflorescence is beneficial to prevent selfing. Battacharya et al. (2005) added that *Apis* spp. were the most frequent visitor of *Jatropha* flowers at the same time they deposited pollen grains on the stigmatic surface, thus generating cross-pollination.

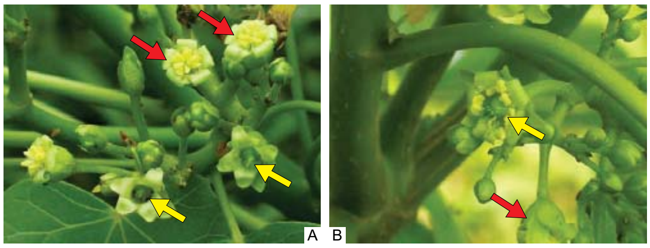

Figure 1. *Jatropha* inflorescence: (A) male (red arrow) and female (yellow arrow) flowers (B) male (red arrow) and hermaphroditic (yellow arrow) flowers

The number of inflorescence of the four-year-old plants varied among accessions and was fewer compared to that of the one-year-old plants. However, the

Table 1. Reproductive characters and reproductive success of each accession (from open pollination)

Accassion	Infl/Pl[1]	St. Infl.(%)[2]	Fl/Infl[3]	Fr/Infl[4]	Fr/Fl[5]	Sd/Fr[6]	Sd/O[7]	Rs[8]
				Four-year-old plants				
Lampung	3.90b	14.1	1.93	3.80	0.77ab	0.36	0.78	0.61b
Banten	5.15a	21.4	5.33	3.69	0.69bc	2.27	0.76	0.53b
West Java	2.65c	15.1	3.50	2.62	0.75bc	2.28	0.76	0.59b
Central Java	3.40bc	17.1	4.63	3.98	0.86ab	2.57	0.85	0.73a
Average		16.9	4.60	3.52		2.37	0.79	
				One-year-old plants				
Lampung	5.57	1.7	8.88a	5.63	0.62	2.74	0.88	0.56
Bengkulu	6.02	0	7.35ab	5.82	0.80	2.76	0.94	0.74
Palembang	6.29	0	9.10a	6.46	0.73	2.80	0.92	0.68
Kediri	5.02	0.9	6.34b	4.75	0.73	2.66	0.86	0.66
Average	5.73	0.7		5.67	0.72	2.74	0.90	0.66

Note: Numbers of the same set of experiment and the same column followed by different letters were significantly different based on DMRT at = 0.05

[1]Number of blooming inflorescence per plant
[2]Percentage of staminate inflorescence: inflorescence that produces only staminate flower
[3]Number of female or hermaphroditic flowers per inflorescence
[4]Number of maturing fruits per inflorescence
[5]Fruit to flower ratio (female or hermaphroditic): the proportion of female or hermaphroditic flower that develop into mature fruit
[6] Number of filled seeds per fruit
[7] Seed to ovule ratio: the proportion of ovule that develop into viable seed (based on three ovules per flower)
[8] Reproductive success

percentage of staminate inflorescence was higher in the older (averaged 16.9%) than the younger plants (averaged 0.7%) (Table 1). The four-year-old plants were planted in the field with 2 x 2 m spacing; while the one-year-old plants were planted in polybags. It is possible that the higher light intensity in the field led to the formation of more male flowers. Investigation on environmental factors affecting sex ratio will be valuable to increase production of female flowers which could lead to an increase in fruit yield.

Accession from Banten produced the greatest number of inflorescence whereas that of West Java had the fewest. The number of pistillate or hermaphroditic flower per inflorescence as well as the number of fruits per inflorescence was not significantly different among accessions, ranged 3.5-5.33 in the four year-old and ranged 6.3-9.1 in the one-year-old plants (Table 1). Again, the data showed that the performance of the one-year-old was better than the four-year-old plants. However, the fruit to flower ratio (fruit set) from both plants was similar (Table 1), ranged 0.62-0.86 indicating that the number of pistillate or hermaphroditic flower per inflorescence was a major yield component and at the same time the limiting factor of kernel production.

The data implied that increasing the number of pistillate flower per inflorescence would be the key to increase the productivity. Not all pistillate or hermaphroditic flowers can develop into mature fruits. Bhattacharya et al (2005) reported that fruit set from pistillate flowers was only 50%, due to fruit abortion during development. In this research, abortion at flowering stage, i.e. flowers that aborted within 4 days after anthesis, was around 5% in the four-year-old and 1% in the one-year-old plants. The abortion during fruit and seed development ranged 9-33%.

The average number of seeds per fruit in both experiments ranged 2.27-2.57 and 2.66-2.80 although on average three ovules were found in one ovary. Hermaphroditic flowers in this experiment can produce up to four seeds per fruit. The seed to ovule ratio (seed set) was 0.76-0.85 in the four-year-old plants and 0.86-0.92 in the one-year-old plants, and did not vary among accessions. The data indicated that seed set was reasonably high although not all ovules developed into viable seeds. Insufficient pollination could be the cause of the less than maximum seed set and could be overcome by increasing the population of the insect pollinators. Social bees, i.e. *Apis cerana* and *Apis dorsata* were reported as effective pollinators for *Jatropha* in Indonesia (Rianti et al., 2010), hence could be employed to increase seed production. In Nigeria, however, where the most effective *Jatropha* insect pollinators were not social bees, i.e. *Chrysomya chloropyga* (Diptera-Calliphoriade) and *Eristalis tenax* (Diptera-Syrphidae) (Alamu et al., 2013), introduction and maintenance of the insect population could be more difficult.

Data on reproductive success showed similar range from the four-year-old and the one-year-old plants, between 0.53-0.74, meaning that from 100 ovules produced, only 53-74 developed into viable seeds. This data indicated that seed production was lower than the yield potential; therefore cultural techniques need to be explored to increase fruit and seed set.

Abortion at the flowering stage following controlled pollination was low, i.e. < 3% in cross-pollination and self-pollination during both experiments. However, abortion at the fruit development stage was as high as 32% in the four-year-old and 20% in the one-year-old plants. Fruit abortion in self-pollination was similar to that in cross-pollination. The data indicated that the abortion was not due to incompatibility as fruit set from self-pollination was not significantly different from that of

Table 2. Fruit set and seed set from self and cross-pollination

Accession	Self-pollination		Cross-pollination		ISI[2]
	Fruit set (%)	Seed set (%)[1]	Fruit set (%)	Seed set (%)	
Four-year-old plants					
Lampung	74.3	54.3	65.7	51.4	1.13
Banten	61.4	42.9	65.7	40.5	0.93
West Java	70.0	59.5	64.3	51.4	1.09
Central Java	64.3	44.8	78.6	57.1	0.82
One-year-old plants					
Lampung	81.1	88.3	85.1	86.2	0.95
Bengkulu	80.2	89.1	80.2	91.0	1.00
Palembang	72.0	90.2	78.1	86.1	0.92
Kediri	75.2	87.1	89.3	86.0	0.84

Note: [1]Seed set was calculated from the number of sound seed divided by the number of total ovule assuming that there were three ovules per flower
[2]ISI (index of self-incompatibility): ratio of fruit set from self-pollination to cross-pollination

cross-pollination (Table 2). Fruit abortions occurred at different stages of development, but mostly were during the early stages when the size was less than half of the mature fruit. Observation on the causes of the abortion would be useful to find measures to increase kernel production.

Fruit set and seed set of the four pollination types in the four-year-old plants (SP-intra, SP-inter, CP-intra and CP-inter) were not significantly different, so the data was presented as self- and cross-pollination (Table 2). Fruit set did not vary among accessions either in the first or second experiments. It was surprising that cross pollination did not result in higher fruit and seed set than self-pollination, which indicated that *Jatropha* was not an obligate cross pollinated species. These results were in contrast to that reported by Raju and Ezradanam (2002) in India that fruit set from hand cross-pollination was higher (96%) than that of hand self-pollination (77%). Kaur et al (2011) also reported that in the central region of Punjab the fruit set from cross-pollination was higher (93.2%) than that of self-pollination (72.2%). The average index of self-incompatibility in this research was 0.99 and 0.93 in the four-year-old and one-year-old plants, respectively, so *Jatropha* is categorized as a partially self-incompatible species, supporting the research result of Wang and Ding (2012) that *J. curcas* has both self-pollination and cross-pollination systems. This is contradictory to Heller (1996) who stated that *Jatropha* was a cross-pollinating species. Given the data it is possible that the seeds produced in any *Jatropha* plants in Indonesia were the results of self-pollination with ants as the effective pollinator (Luo et al. 2012).

The germination percentage of the self-pollination seeds was not significantly different from the cross-pollination seeds, supporting the idea that *Jatropha* was not an obligate cross pollinating species. The four-year-old accession from Banten produced highly viable seeds from both self- and cross-pollination. Lampung and Central Java accessions produced highly viable seeds (> 80%) from self-pollination, whereas cross pollinated seeds from West Java accession had higher viability than self-pollinated (Table 3).

Slightly different results were obtained from the one-year-old plants in which cross-pollination produced better quality seeds than self-pollination as indicated by germination rate of > 80% and germination speed of ≥ 9.46 $\%.day^{-1}$ (Table 4). In general Banten, Bengkulu and Kediri accessions produced highly viable seeds regardless of pollination systems. It would be interesting to learn the reproductive success of the self- and cross-pollinated progenies. A study in Malaysia showed positive heterosis from crosses of particular superior parents (Islam et al, 2011) which could potentially lead to the development of hybrid varieties of *Jatropha curcas*.

Table 3. Seed germination (%) and germination speed ($\%.day^{-1}$) based on pollination systems and accessions from four-year-old plants

Accession	SP-intra	SP-inter	CP-intra	CP-inter
	Germination (%)			
Lampung	96.67a	82.22abcde	81.82abcde	69.84e
Banten	90.48ab	92.31abcde	90.91abcde	97.44a
West Java	72.22de	73.33cde	89.58abcde	77.08bcde
Central Java	95.83a	88.89abcd	85.97abcde	40.48f
	Germination speed ($\%.day^{-1}$)			
Lampung	8.53ab	7.47bcd	7.42bcd	6.55cd
Banten	8.00abc	8.18a	8.13abcde	9.30a
West Java	6.60cd	6.39d	8.33ab	7.20bcd
Central Java	8.34ab	7.99abc	8.24ab	3.53e

Note: Numbers followed by the same letter of the same column and row were not significantly different based on DMRT at α = 0.05

Table 4. Germination (%) and germination speed (%.day^{-1}) based on pollination system and accessions from one-year-old plants

Accession	Open-pollination		Self-pollination		Cross-pollination	
	Germ[1]. (%)	GR[2] (%.day^{-1})	Germ[1]. (%)	GR[2] (%.day^{-1})	Germ[1]. (%)	GR[2] (%.day^{-1})
Lampung	80.54	9.67	73.71	8.73	86.41	10.34
Bengkulu	85.02	10.35	84.90	8.54	85.28	10.32
Palembang	80.98	9.82	78.61	9.53	81.20	9.46
Kediri	78.49	9.84	85.42	9.72	87.51	10.78

Note: [1]Germination percentage indicating seed viability
[2]Germination speed indicating seed vigor

The germination speed, which is an indication of seed vigor, showed similar trends to germination percentage in which self- and cross-pollination resulted in non-significantly different rates. The one-year-old plants tend to demonstrate higher germination speed than the four-year-old plants. Banten accession germinated at a faster rate (Table 4) regardless of pollination types. Self-pollinated seeds from West Java were slower to germinate compared to cross-pollinated ones. These data were in line with data on germination percentage that cross pollination between accessions could give negative effect on seed viability. In general, although Banten accession had the lowest reproductive success, hence seed production, this accession produced high quality seeds, which is an important character for a conventional breeding program.

Conclusion

Reproductive success varied among accessions, the highest was from West Java of 0.73 and the lowest from Banten of 0.53.
Jatropha sccessions (Lampung, Banten, West Java, Central Java, Bengkulu, Palembang and Kediri) are partly self-incompatible with self-incompatibility index range of 0.93-0.99.
Pollen source did not affect fruit set and seed set, but affected seed germination and speed of germination.

Acknowledgment

We thank the Indonesian Centre for Estate Crops Research and Development for the permission to use seeds from *Jatropha* seed orchard in Pakuwon, West Java for this research.

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