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Flowering and Fruit Set of Improved Population of *Jatropha curcas* L. under Climate of Northern Lombok, Indonesia

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Abstract. In order to understand flowering and fruiting of an improved population of *Jatropha curcas* L. grown in Northern Lombok Island, flower and fruit development timeline is needed to solve the low fruit set problems. The investigation was carried out by daily observations on bud and flower initiation, anthesis of male and female flowers, floral morphology, fruit initiation and development, fruit-set, and flower characterization. Anthera dehiscence and stigma receptivity were also observed. For floral and fruit development, observations were based on macro morphological changes, and vegetative shoots were tagged and observed for their developmental changes up to fruiting stage. The results show that, there was significantly different in fruit set between improved populations of IP-1 NTB and IP-2 NTB, but not for most flowering and fruiting variables. The genotypes are monoecious, the male and female flowers were found separately in the same inflorescence. The male floral bud developed earlier (20 to 23 days) than the female ones (22 to 25 days) to bloom. Maximum flower opening was occurred from 07.00 to 09.00 A.M for male flowers, and 08.00 to 09.00 A.M for female ones. The anthera dehiscence was observed between 07.00 and 09.00 hours. The receptivity of stigma started toopenfrom08.00 to 11.30 A.M. The number of days required for fruit development and maturity after anthesis ranged from 45 to 60 days. Average of fruit set for IP-1 NTB and IP-2 NTB under open pollination was 56.40% and 68.54%, respectively.

Keywords: anthesis, biofuel, inflorescence, maturity, phenology

INTRODUCTION

At present, the insufficiency of energy resources due to the high demand for transportation and industrial uses leads to the global energy crisis. The trend of increasing energy demand will continue and is expected to raise 1.5 fold world wides and almost double in Asia by the year 2035 [1]. At the current rate of fossil fuel utilization, oil and gas will be exhausted in 2050 [2]. The supplementary and alternative energy sources are urgent needs, then has brought researchers to find renewable source especially plant-based fuel as an alternative source, in order to face the energy crisis. *Jatropha curcas* L. is considered as the most potential biodiesel source in the tropic and sub-tropic due to its drought tolerance and non-edible oil.

In Indonesia, currently, the plant is an interesting energy source for biofuel production. It has been cultivated around the country as one of the government programs to diversify the renewable energy sources. It is also easily cultivated under climatic conditions of Indonesia, including West Nusa Tenggara Province (Lombok and Sumbawa islands). However, a major limitation to commercialize this plant is the lack of high yielding varieties for oil content and yield [3]. Therefore, to make the production of this plant profitable and sustainable, genetic improvement of seed and oil yield, as well as quality are required urgently. To commercialize physic nut, its seed yield should reach, at least, 4–5 $tha^{-1}year^{-1}$ (about 2.0 kg $plant^{-1}$ $harvest^{-1}$) [4].

Efforts for genetic improvement of Jatropha, especially West Nusa Tenggara genotype, have been carried out and have produced superior genotypes, namely *Improved Population* (IP-1 and IP-2 NTB) which have higher yield potential than the initial (wild) population. However, the variation in the number of harvested fruit per inflorescence among plants and within the plants was still quite high. Those phenomena were also reported by Santoso *et al.* [5] that, there was low fruit set of *Jatropha* West Nusa Tenggara ecotypes, although the number of female flowers is

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high. It was due to the phenomenon of the fertilization and pollination failure or flower and fruit set during its growth and development, and unbalanced soil fertility. Therefore, phenology the plant flowering should be investigated in order to obtain basic information to make the fruit (seeds) yield more profitable, and at the same time to create a superior variety throughout breeding programs.

Information regarding time and span of flowering, phonological behavior, and anthesis pattern stages are prerequisite for planting and developing breeding strategies [6] and also for cultivation techniques especially to increase the fruit set. Thus, increasing the number of female flowers seems critical to improving the seed yield. Earlier studies on pollination ecology and fruiting behavior reported that *Jatropha* flower was monoecious and protandrous [7]. As Parthiban *et al*. [8] reported that flowering depends on location and agro climatic conditions. It was also indicated that the number of inflorescence per plant was significantly correlated with the number of fruits per plant, and with male to female flower ratio. The pollination studies also showed that the ratio of male to female flowers was 29:1, both flower sexes opened synchronously, the sexual system facilitates geitonogamy and xenogamy, and the pollinators included bees, ants, thrips and flies. This article elaborated the study, which aimed to understand the floral phenology and fruit set of improved population Jatropha grown in Northern Lombok, Indonesia.

METHODS

Plant Material and Experimental Site Condition

Crops stand was improved population genotypes of West Nusa Tenggara (IP-1 NTB and IP-2 NTB) with three and two years old grown in the research field station at Amor-Amor Village, District of Kayangan, North Lombok, West Nusa Tenggara, Indonesia, located at 8°16'15.02"S 116°17'34.02"T elevation 120 m. The field investigation was initiated during reproductive period from rainy season to early dry season (January to July 2015).

The soil was sandy loam Entisols and composed of sand (69%), silt (25%), and clay (5%), with 1.8% organic carbon, 0.2% total N, the pH was 5.9-6.3, and cation exchange capacity of the soil measured 7.2-10.4 cmol.kg^{-1}. Climate condition during investigation was 723 mm of rain fall; four months of rainy months; 52 days of rainy days; 26.1 °C and 35.7 °C of the minimum and maximum air temperature; 72 % of relative humidity.

The plantation was maintained throughout agronomic practices, such as fertilizer was applied at planting time of 5,000 kg of manure ha^{-1} (2 kg tree^{-1}) and 25 kg of Urea ha^{-1} (10 g tree^{-1}), 150 kg of SP-36 ha^{-1} (60 g tree^{-1}), and 30 kg of KCl ha^{-1} (12 g tree). The second Urea application (25 kg Urea ha^{-1} (10 g tree^{-1}) was applied one month after planting. At two and three years old, the plants were given 25 kg of Urea ha^{-1} (10 g tree^{-1}), 150 kg of SP-36 ha^{-1} (60 g tree^{-1}), and 30 kg of KCl ha^{-1} (12 g tree^{-1}). Weeding was done in radius one meter from the stem base. Unproductive shoots were pruned regularly every two weeks. Irrigation was applied weekly up to one month after planting, and thereafter, no irrigation was applied.

Floral and Fruit Biology and Development

Daily observation regarding bud initiation, flower initiation, anthesis of male and female flowers, fruit initiation and development, and fruit set was recorded from 50 tagged inflorescences (three to five inflorescences per tree) at the rainy season production cycle of trees (January to May 2015). The other group of 50 inflorescences was sent to laboratory to be subjected for the floral morphology and flower characterization. Another set of 20 tagged inflorescences was examined visually in the field using hand lens at an hour interval started from 06.00 A.M. for anther dehiscence and stigma receptivity. The presence of pollen grain on the anther surface was considered to be an anther dehiscence, and receptivity of stigma was considered when it was glossy and shiny, but dull and dry surface was considered as non-receptive.

For floral and fruit development, the observations were based on macro morphological changes, and vegetative shoots were tagged and observed for their developmental changes up to fruiting stage.

Data were subjected to analyze the mean along with their standard deviation. They were computed for each parameter using Minitab-14 statistical program.

RESULTS AND DISCUSSION

Floral Biology and Flowering

The inflorescence of The West Nusa Tenggara (IP-1 NTB and IP-2 NTB) genotype germinated from the top of branch, with the upper end of the branch stops growing upward due to inflorescence formation. The plant produced flowers in racemose inflorescence with dichasial cyme pattern. The flowers were unisexual, with male (Fig. 1A) and female flowers (Fig. 1B). They were produced in the same inflorescence. The inflorescences produced a central female flower surrounded by a group of male flowers (Fig. 1C). In few, the places where female flower develop were substituted by male flower, and it was very rare to be substituted by hermaphrodite flowers. From each inflorescence grew and developed 5-7 individual inflorescences as a secondary inflorescence, and then, another 4-8 more tertiary inflorescence. The size of the inflorescence growing up at the beginning of the rainy season (Fig. 1D and 1F) was thinner and longer compared to that at the end of the rainy season (Fig. 1E and Fig. 1G).

Both populations of flowers were pale green in color. They had five petals, five glands, and five sepals. The male flowers had ten stamens arranged in two tiers of five flowers, while the female flower had one ovary terminating in a three-lobed stigma and surrounded by five nectarines, and three styles (Table 1). The male flower was smaller than female flower regarding to the length and diameter. The male flower was 1.19±0.1 cm in length and 0.68±0.13 cm in diameter for IP-1 NTB; 1.12±0.12 cm in length and 0.64±0.12 cm in diameter for IP-2 NTB; and the female flower was 1.43±0.10 cm in length and 0.98±0.10 cm in diameter for IP-1 NTB; 1.15±0.09 cm in length and 0.97±0.12 cm in diameter for IP-2 NTB (Table 2).

The observation on flowering of the populations under climate of Northern Lombok also indicated that, the number of male flowers varied. It was 133.02±42.07 for IP-1 NTB and 136.72±41.92 for IP-2 NTB. They were no significantly different. However, the number of female flowers was fewer and there were significantly different between IP-1 NTB (16.20±5.23) and IP-2 NTB (18.64±5.00). The ratio of male/female flower was no significantly different between IP-1 NTB (8.73±3.67) and IP-2 NTB (8.63±3.17) (Table 2). In addition, the number of hermaphrodite flowers was 0.9±1.27 for IP-1 NTB and 0.7±0.91 for IP-2 NTB.

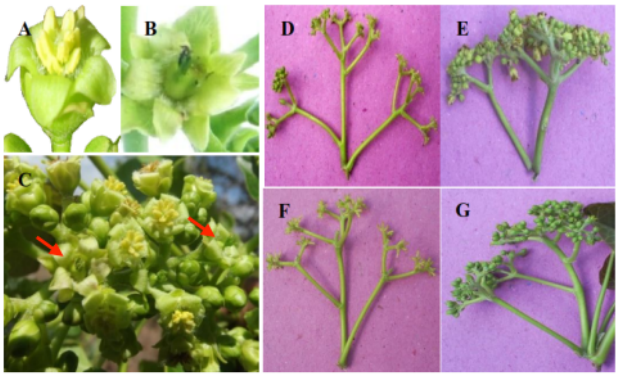

FIGURE 1.The male (A), female (B) flower, female flowers (arrow sign) surrounded by male flowers (C), and type of inflorescences at early (D, F) and at late (E, G) of rain season

TABLE 1. The component of male and female flowers of the improved populations

Component	Male Flower♂		Component	Female Flower♀	
	IP-1 NTB	IP-2 NTB		IP-1 NTB	IP-2 NTB
Stamens	10	10	Ovariums	1	1
Petals	5	5	Stylus	3	3
Glands	5	5	Petals	5	5
Sepals	5	5	Glands	5	5
			Sepals	5	5

In summary, both genotypes had higher number of male flowers (133 and 137) than female flowers (16 and 19), and very rare of hermaphrodite flowers. Raju and Ezradanam [7] reported that, numerically, 1-5 female flowers and 25-93 male flowers are produced per inflorescence, with average male to female flower ratio is 29:1. Related to hermaphrodite flower, Juhasz *et al*. [9] did not find hermaphrodite flowers in *Jatropha curcas* cultivated in North Minas Gerais. The occurrence of hermaphrodite flowers and the variation in the ratio between male and female flowers may be related to the genetic and environmental factors, and their interaction [10].

The flowering observation result also showed that, in the same inflorescence, male flower opened two to three days earlier than female flowers (Table 3). This was also observed by Raju and Ezradanam [7], Wei *et al*. [11], and Camellia *et al*. [12]. However, different condition that female flowers opened before male flowers, was reported by Juhasz *et al*. [9] and Saturnino *et al*. [13].

The hourly observations on tagged inflorescence showed that anthesis period of male flowers was occurred earlier and longer (07.00 to 09.00 A.M.) than female flowers (08.00 to 09.00 A.M.) (Table 3). The male flowers started to open after 06.30 A.M. and the peak male flower opening was noticed at 07.30 to 08.00 A.M. The female flowers anthesis occurred later than male flowers. It was at 08.00 to 09.00 A.M., and the peak number was occurred around at 08.30 A.M. The anthera dehiscence was observed between 07.00 and 09.00 A.M. The stigma became receptive from 08.00 to 11.30 A.M (Table 3). Earlier studies have shown that the anthesis of male and female flower was from 06.00 to 07.00 A.M, and 07.00 to 08.00 A.M, respectively, from 07.30 to 08.30 A.M., as reported by [14], and from 05.30 to 06.30 A.M., as reported by [7].

Male flowers in an inflorescence of both IP-1 NTB and IP-2 NTB will bloom for5-8 days, while female flowers blooming for 7-11 days for IP-1 NTB, and 7-10 days for IP-2 NTB (Table 3). Peak time for male flower to bloom was3-4 days, while female flowers were 2-3 days. This study contrasts the finding of Camellia *et al*. [12] that male flower open for 8-11 days while female flower open for 3-4 days. There was a situation at the same inflorescence that there is no source of pollen for the fertilization, so there is a need of pollen resources from other plants or other inflorescence. It means that, this situation of improved population *Jatropha* flowering promotes cross-pollination.

TABLE 2. The physical characteristics of flower, number of female and male flower, and the ratio of male-female flowers of the improved populations

Characters	Male flower ♂			Female flower ♀		
	IP-1 NTB	**IP-2 NTB**	**T-test**	**IP-1 NTB**	**IP-2 NTB**	**T-test**
Length (cm)	1,19±0,10	1,12±0,12	NS	1,43±0,10	1,15±0,09	NS
Minimum	1,00	0,90		1,20	1,19	
Maximum	1,40	1,40		1,70	1,75	
Diameter (cm)	0,68±0,13	0,64±0,12	NS	0,98±0,10	0,97±0,12	NS
Minimum	0,50	0,50		0,80	0,79	
Maximum	1,00	0,90		1,10	1,12	
Number of flower	133,02±42,07	136,72±41,92	NS	16,20±5,23	18,64±5,00	S
Minimum	67,00	86,00		8,00	9,00	
Maximum	221,00	219,00		25,00	26,00	
	IP-1 NTB	**IP-2 NTB**				
Ratio ♂/♀	8,73±3,67	8,63±3,17	NS			
	3,80	4,20				
	24,60	22,70				

Explanation: ± = standard error, S = significant, NS = no significant

TABLE 3. The time of anthesis, periode of anthesis, viability of flower of the improved populations

	Male flower♂		Female flower♀	
	IP-1 NTB	**IP-2 NTB**	**IP-1 NTB**	**IP-2 NTB**
Period of anthesis[1)] (days)	6.46±1.01	6.58±0.97	9.16±1.06	10.64±3.59
Minimum	5.0	5.00	7.00	7.00
Maximum	8.0	8.00	11.00	10.00
Time of anthesis	07.00–09.00	07.00–09.30	08.00–09.00	08.00–09.00
Time of anthera dehiscence	07.00–08.30	07.00–09.00		
Time of stigma receptive			08.00–11.00	08.00–11.00

Explanation: 1) days after bud inflorescence formation

FIGURE 2. Flower development of the improved populations. Phase of intiation (A), phase of buds development (B-C), phase of bloom (D-E), phase of dried and fall (F), and phase of fruit formation and development (G)

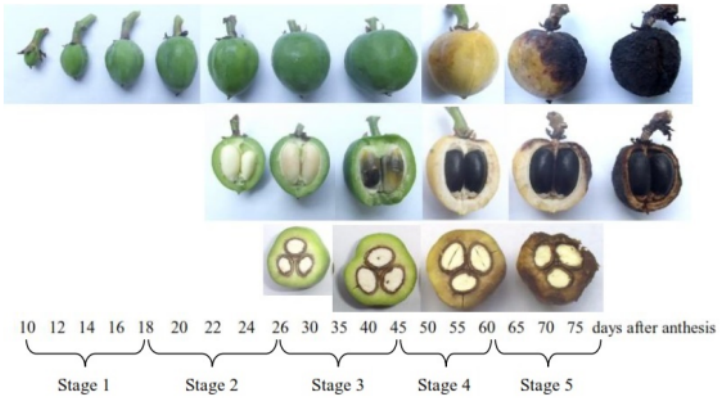

FIGURE 3. The fruit development of the improved populations under climate of Northern Lombok

Figure 2 shows the flower development of improved population *Jatropha* grown under climate of Northern Lombok. Few numbers of flower bud could be seen when inflorescence stalks were about 0.3-0.5 cm lengths, and the crown (corolla) still closed, flower bud about 5-7 mm lengths, green color of flower buds (Fig. 2A), then by the start time to blooms its color changed to yellowish green. Number of flowers was more than 150-155 buds per inflorescence (Fig. 2B-C). Male and female flower buds could be already recognized. The phase of A-C took 10-12 days. During the phase of flowers bloom, flower organs such as the sepals, petals, stamens and pistil are visible, flowers diameter between 0.5-1 cm, yellowish green in color, leaves around the flower does not exist anymore, the amount flower (males and females) varied between 45-155 per inflorescence. This phase ranged 14-20 days (Fig. 2D-E). All organs of male flowers dried and then fall, while the female flowers and hermaphrodite, only crowns are loss. Ovary in the female and hermaphrodite flowers started to grow and will develop into fruit. This phase ranged from 7 to 8 days (Fig. 2F) until a very small fruit visible (Fig. 2G). In brief, flower development takes approximately 20 days (male) and 22 days (female) to complete the cycle from the initiated flower bud to anthesis.

Fruit Set and Development

In general, fruit development was considered to start after anthesis, with the initial event being pollination followed by fertilization, growth, maturation, and ripening. Due to the pattern of flower maturity was occurred at different time that lead to different period of male and female flower opening will also cause subsequent maturation of fruit within inflorescence. All stages of fruit development associated with changes on the cellulair and metabolic levels. Figure 3 shows fruit development of improved population (IP-1 NTB and IP-2 NTB) *Jatropha curcas* L. under climate of Northern Lombok, devided into 5 stages e.g. stage 1, a periode of very rapid cell division, but very little increase in fruit size; stage 2, a periode of rapid increase in size; stage 3, fruit reaches physiological maturity,

increase in size slows and then stop when the fruit reaches its full size; stage 4, fruit come to ripening, change in color (yellowing), and stage 5, senescense phase of fruit, decaying fruit, decrease in weight.

The fruit development of IP-1NTB and IP-2NTB genotypes was no significantly different. In the current study also showed that, terminal stems of the bear flowers then fruited after the vegetative stage. It then took about a month from the vegetative flush to initiation of visible flower buds. After that, flower buds would be visible to flower anthesis. Once the anthesis had begun, the flowers were then opened daily. About 6-8 days, it was appear a very small fruit, and then followed by 22-25 days for fruit development to immature, then 35-40 days to mature. Fruit senescence occurred 20-25 days after fruit maturity. The complete cycle of final fruit set and development required approximately 65-70 days. Fruit formation started about24-30 days after flower bud formation, or 7-9 days after anthesis.

The development of fruits set was illustrated in Table 4, which elaborate the development of fruit set (number of fruit exist in inflorescence) from very small fruit to harvested fruit. Final fruit set was total percentage of harvested fruit to total number of female and/or hermaphrodite flowers. There was no significant difference between numbers of female flowers in both improved population genotype, but there was a significant difference in level of fruit drop during fruit development between the two genotypes. This study also shows that, the final fruit set in IP-1 NTB (56.40 percent) was significantly lower, compared to that of the IP-2 NTB (68.54 percent). Table 4 also indicates that there were3-4 flowers failed to form fruit at the beginning of its development. It means that the process of pollination failed. This could be caused by the differences in time period of male and female flowers bloom in the same inflorescense. Bhattacharya *et al.* [10] reported that only 50% of female flowers set to fruit in Lucknow, India. The initial fruit set of *Jatropha* was high (92%) as reported by Camellia *et al*. [12], but no information for final fruit set. The amount of fruit harvested was depending on the amount of flower, and at the same time, depend on inflorescense branching. As Camellia *et al*. [12] stated that, the main shoot terminate in a flower, while growth continues through lateral axes produced below the terminal flower, and then lateral axes again form terminal flowers and this process was repeated several times.

Related to fruit set, Santoso *et al*. [15] reported that, using plant material of local superior selected *Jatropha curcas* genotypes of West Nusa Tenggara, the number of capsules produced per inflorescence ranged from 10.8 to 13.8. The number of capsules produced per inflorescence depended on the ratio between the male and female flowers, which ranged from 8.6 to 12.7. In this study, the amount of harvested fruit ranged from 4 to 11 (9.1 in average) on the IP-1NTB, and from 8 to 16 (11.4 in average) on IP-2NTB. This means that inflorescenses of both improved population had more structure of cymes, than the amount discovered by Camellia *et al*. [12], that was 6-10 compound cymes. Figure 4 explained most of the structure cymes of both improved population in this study.

A high percentage of fruit drop occurred during the early phases of fruit development. Abscission of flowers or failure of the flower to form fruit was a critical phase causing a reduction in the fruit set. This was due to the failure of the stigma (female flower) pollinated by pollen grains. The cause of the failure of pollination may be due to differences in time of maturity, both male and female flowers. There was also fruit drop during their development since small fruit to mature fruit, which can be due to a physiological disorder caused by lack of nutrient elements, water, and also other aspects of plant metabolism. This finding then agreed to Camellia *et al*. [12] that continues flowering and differences in period of male and female flower to bloom were the factors causing the wide range of fruit development. Therefore, the main constrain in *Jatropha curcas* cultivation especially for the improved

TABLE 4. The development on fruit set of the improved populations

		Number of ….. (fruit set development)					Final fruit Set (%)
		Flower ♂+ ♂/♀*	Very small fruits	Im-mature fruits	Mature fruits	Harvested fruits	
IP-1 NTB	x	17.9	13.8	10.8	9.4	9.1	56.40
	Std	5.15	3.78	2.48	1.70	1.41	15.81
	Min	9.00	7.00	4.00	4.00	4.00	36.00
	Max	26.00	21.00	16.00	12.00	11.00	100.00
IP-2 NTB	x	18.5	15.5	13.2	11.9	11.4	68.54
	Std	5.04	4.18	3.21	2.26	2.09	11.67
	Min	9.00	9.00	8.00	8.00	8.00	50.00
	Max	26.00	22.00	19.00	16.00	16.00	100.00

Explanation: there were significantly different between IP-1 NTB and IP-2 NTB, except number of flower*

FIGURE 4. The most of cymes (inflorescense) structure and final fruit set of the improved populations

populations in Northern Lombok was the low enough percentage of fruit set from the high enough total number female and hermaphrodite flowers. Agreed on this research obtained by the fact that the increase in fruit set can still be done through agronomic practices. From the condition of this study, to increase fruit set could be completed with the application of growth regulator substances. The use of growth regulator substances intended to increase the number of female flowers, harmonized time of flowers bloom, or to improve the success of fertilization and pollination, and also to prevent flowers and fruit fall during its growth and development. The extent to which the success of the use of growth regulators substances require attention and further study in order to induce high yield (seed) from the cultivation of *Jatropha curcas*. As Liu *et al*. [16] stated that, the study of plant flowering was not only beneficial to species conservation and management of genetic resources, but also to provide evidence for reasonable cultivation management measures, so as to achieve the aim of increasing plant yield.

CONCLUSIONS

Flowering and fruiting are two important stages in *Jatropha curcas* L. seed production because their time difference can affect pollen flow, mating pattern, ultimate success rate of pollination and then seed setting rate. Significantly different on the fruit set between improved population such IP-1NTB and IP-2NTB but not for most variables of flowering and fruiting was clearly evident of this study. Those *Jatropha* genotypes are monoecious. The male and female flowers took different length of time for developing. Flower (female and male) and fruit development took approximately 85 days to complete the cycle from floral initiation stage until fruit maturity. The number of days required for fruit development and maturity after anthesis ranged from 45 to 60 days. The average of fruit set for IP-1NTB and IP-2NTB under open pollination was 56.40% and 68.54% respectively from the total flowers of 17.9 of IP-1NTB and 18.5 of IP-2NTB. The pattern of flower maturity or continuous flowering was occurred at different time lead to different period of male and female flower opening which will also cause subsequent maturation of fruit within inflorescence.

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