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Hybridization and Evaluation of Jatropha (*Jatropha curcas* L.) to Improve High Yield Varieties in Indonesia

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Abstract**—***Jatropha curcas* L. is one of the crops producing non edible oil which is potential for bio-energy. Jatropha cultivation and development program in Indonesia is facing several problems especially low seed yield resulting in inefficient crop cultivation cost. To cope with the problem, development of high yielding varieties is necessary. Development of varieties to improve seed yield was conducted by hybridization and selection, and resulted in 14 potential genotypes. The yield potential of the 14 genotypes were evaluated and compared with two check varieties. The objective of the evaluation was to find Jatropha hybrids with some characters i.e. productivity higher than check varieties, oil content > 40% and harvesting age ≤ 110 days. Hybridization and individual plant selection were carried out from 2010 to 2014. Evaluation of high yield was conducted in Asembagus experimental station, Situbondo, East Java in three years (2015-2017). The experimental designed was Randomized Complete Block Design with three replication and plot size of 10 m x 8 m. The characters observed were number of capsules per plant, dry seed yield (kg/ha) and seed oil content (%). The results of this experiment indicated that all the hybrids evaluated have higher productivity than check variety IP-3A. There were two superior hybrids i.e. HS-49xSP-65/32 and HS-49xSP-19/28 with highest seed yield per hectare and number of capsules per plant during three years.

Keywords**—**Jatropha, biodiesel, hybrid, high seed yield.

I. Introduction

WORLD availability of fuel is predicted to be limited in the next few years. Therefore, it is necessary to find an alternative energy sources which does not compete with food crop. Plant oil produced by some crops including oil palm, candle nut and Jatropha (*Jatropha curcas* L.) can be processed as biodiesel. Biodiesel has a comparative advantage compared to other forms of energy, which has a higher density per volume, has a relatively clean burning character, low production cost, and friendly to environment [1].

In Indonesia, Jatropha development was limited by low seed productivity and oil content or quality. This condition initiated an inefficient cultivation cost and decreased farmer's interest to cultivate it. To cope with the problem, Jatropha breeding program is necessary to improve high yield varieties with high oil content. The success of Jatropha breeding program is depending on a great number of germplasm collections [2]. In the beginning, the main aim of jatropha breeding is to improve high oil yield per hectare which is strongly correlated with seed yield. In addition, the wide genetic diversity of Jatropha germplasm is also required to achieve high yielding varieties. The important characters required for selection in improving high yield varieties are fruit yield per hectare, oil yield, seed weight and other yield components [3].

Improvement of high seed yield varieties is normally performed by hybridization or crossing between two genotypes as parents. Jatropha hybridization is influenced by the process of castration, pollination and the age of the female flowers. Castration which is conducted in the morning, afternoon, or evening will offer the same results. Pollination should be carried out in the morning or depending on the condition of pollen and female flowers. High quality of pollen will be produced from one day old of male flowers or when the pollen color is fresh yellow. The best time for pollination is when the female flower has bloomed and three stigmas have been separated from each other [4].

To improve seed yield and oil content, Jatropha breeding in ISFCRI was started in 2010. Hybridization conducted in 2010 resulted in 72 set of F1 hybrids, and 100 seeds of each set was planted in 2011 for selection. The individual plant selection resulted in 54 genotypes which had > 250 capsules/plant in the first year of cultivation. In 2012, the selection was tightened with criterion > 600 capsules/plant/year. From this selection were obtained 48 genotypes with > 600 capsules/plant/year and 14 genotypes had oil content > 40%. The selection was continued until 2014 and found 14 genotypes with > 1,000 capsules/plant/year, oil content > 40%, and the first harvesting time ≤ 110 days. Evaluation of 14 hybrids for high yield potency was performed during three years from 2015 to 2017. The evaluation was aimed to obtain Jatropha hybrids which have higher productivity than check varieties with oil content > 40% and harvesting age ≤ 110 days.

II. Materials and Methods

This experiment consisted of three steps:

A. Hybridization to Increase Yield and Oil Content

Hybridization activity was carried out at Karangploso Experimental Station, Malang, East Java, starting from January to December 2010. The parents utilized in this hybridization were selected from the IFSCRI germplasm

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collections. 18 provenances indicating high oil content were selected as male parents: HS-1, HS-48, HS-61, HS-83, SP-33, Jatim-104, SP-103, SP-4, SP-31, SP-65, SP-89, SP-7, SP-19, SP-33, SP-54, SP-37, SP-10, and SP-13. Three improved population (IP) and one provenance, well known as high yield genotypes, were used as female parents (IP-3A, IP-3P, IP-3M, and Hs-49). Each genotype of male parents consisted of 10 plants and female parents 30 plants. These plant materials were prepared from stem cuttings. Plant distance was 2 m x 2 m with single plant per hole. Plant maintenance consisted of fertilization and insect or pest control. Fertilizer was applied twice with dosage of 10 tons/ha of manure, and 90 N kg/ha + 45 P_2O_5 kg/ha + 45 K_2O kg/ha per year. The mite and insects were controlled twice using 0.5 ml/l Imidacloprid. The offspring (F1) genotypes resulted from hybridization in 2010 were evaluated in 2011 to find out high seed yield hybrids.

B. Selection of Jatropha Hybrids

Selection of Jatropha hybrids was conducted in Asembagus Experimental Station, Situbondo, East Java, starting from January to December 2011. 72 sets of F1 hybrids resulted from hybridization between four female parents (IP-3A, IP-3P, IP-3M and Hs-49) and 18 male parents (Hs-1, Hs-48, Hs-61, Hs-83, Jatim-33, SP-104, SP-103, SP-4, SP-31, SP-65, SP-89, SP-7, SP-10, SP-13, SP-19, SP-33, SP-37, and SP-54) were selected. Each set consisted of 100 plants. For the certain sets with <100 seeds, all seeds obtained were planted for selection. Jatropha seedling was carried out by planting seed in poly bag containing soil and compost (1:1). Poly bags with 25 cm x 15 cm size or fit for one kg of soil were used for Jatropha seedling. Seedlings were maintained by watering every day for two months to avoid drought stress. Seedlings with > 25 cm height were transferred to the field with plant spacing 1 m x 1 m. The plant holes for transfer of seedlings were made by digging of the land before planting. The size of plant hole was 30 cm (length) x 30 cm (width) x 30 cm (deep). Plant maintenance consisted of fertilization and insect or pest control. Fertilizer was applied twice with dosage of 10 tons/ha of manure, and 90 N kg/ha + 45 P_2O_5 kg/ha + 45 K_2O kg/ha per year. The mite and thrips insects were controlled twice using 0.5 ml/l Imidacloprid**.**

In 2012, the selected genotypes were clonally multiplied using stem cutting and planted for further selection. The selection was conducted for three years (2012-2014) to find out the high seed yield genotypes. Each hybrid/genotype consisted of 25 plants with 2 m x 2 m plant spacing. The plant maintenance was not different from the plant maintenance of previous selection. The selection criteria were based on number of capsules/plant/year during three years, harvesting age, and oil content. Oil content was characterized by Soxhlet method [5]. The genotypes selected from this activity would be clonally multiplied using stem cutting and sown for high yield potential evaluation in 2015-2017.

C. Evaluation of High Yield Potential Hybrids

Evaluation was conducted in Asembagus Experimental Station, Situbondo, East Java for three years (2015-2017). The evaluation was designed in Randomized Block Design with three replications. The seedlings were planted in 10 m x 8 m plot size, with 2 m x 2 m plant spacing. The fertilizer used in this evaluation was 10 tons/ha of manure, and 90 N kg/ha + 45 P_2O_5 kg/ha + 45 K_2O kg/ha per year, and was applied every two months. Organic fertilizer or manure was applied once in rainy season. Weed control was done in four times a year. Insects were controlled every 20 days at the same time as water irrigation. The proper harvesting time is when the skin colour of Jatropha capsules turned from green to yellowish-green or yellow. The observation was conducted for capsules number per plant and dry seed yield per hectare per year. Seed yield per year was accumulation seed harvested from January to December. The data were analyzed using Duncan Multiple Range Test at 5% level.

III. Results and Discussions

A. Hybridization to Increase Yield and Oil Content

Hybridization conducted in 2010 resulted in 72 sets of hybrid seeds. Crossing between high productivity female and high oil content male parents produced different number of seeds (Table I). The 72 sets of hybrid seeds were planted in 2011 for selection activity. Only 100 seeds of each set were planted, but all seeds of the sets with <100 seeds were planted.

The hybrid genotypes were selected to choose the plants with high productivity. The selection was based on capsule (fruits) number per plant per year. The hybrids with high capsule number (>250 capsules per plant per year) indicated their potency of high seed yield. Selection with this criterion resulted in 54 selected genotypes of hybrid and would be used for further selection in 2012.

B. Selection of Jatropha Hybrids

Stem cutting of 54 hybrids were planted for further selection in 2012. Out of 54 genotypes of F1 hybrid, 48 genotypes had number of capsules more than 400 capsules per plant per year or equal to two tons per hectare per year in the first year (Fig. 1). This result was different from the result of selection activity conducted in 2011. The number of capsules of selected genotypes was 250-300 capsules per plant per year in the first year. The productivity of the hybrid genotypes in 2011 was lower than in 2012, this may be due to the source of plant materials planted in 2011 was different from plant materials used in 2012. In 2011, the seeds were used as plant material, but stem cuttings were used as plant materials for selection in 2012. The flowering and harvesting days of Jatropha planted from stem cuttings were earlier than from seeds.

Mostly F1 hybrid genotypes have capsule numbers higher than their parents. All F1 hybrids resulted from crossing between HS-49 female parent and 12 male parents produced more than 400 capsules per plant per year in the first year. Genetically, HS-49 is a genotype with high potency of dry seed yield. In previous experiment, HS-49 produced highest dry seed in Gunung Kidul District, Special Region of Yogyakarta Province [6]. HS-49 also showed the highest average seed yield in three locations i.e. Asembagus Experimental Station, Situbondo District, East Java Province; Muktiharjo Experimental Station, Pati District, Central Java Province, and North Lombok District, NTB Province [7].

TABLE I
NUMBER OF F1 HYBRID SEEDS PRODUCED FROM HYBRIDIZATION BETWEEN FEMALE AND MALE PARENTS CONDUCTED IN 2010

Crossing between parents				F1 hybrid seed number	Remark	Crossing between parents				F1 hybrid seed number	Remark
No.	♀		♂			No.	♀		♂		
1.	IP-3A	x	HS-1	430	*	37.	IP-3M	x	HS-1	262	*
2.	IP-3A	x	HS-48	186	*	38.	IP-3M	x	HS-48	225	*
3.	IP-3A	x	HS-61	280	*	39.	IP-3M	x	HS-61	384	*
4.	IP-3A	x	HS-83	354	*	40.	IP-3M	x	HS-83	317	*
5.	IP-3A	x	Jatim 33	245	*	41.	IP-3M	x	Jatim 33	211	*
6.	IP-3A	x	SP-104	513	*	42.	IP-3M	x	SP-104	400	*
7.	IP-3A	x	SP-103	206	*	43.	IP-3M	x	SP-103	55	√
8.	IP-3A	x	SP-4	149	*	44.	IP-3M	x	SP-4	10	√
9.	IP-3A	x	SP-31	56	√	45.	IP-3M	x	SP-31	19	√
10.	IP-3A	x	SP-65	57	√	46.	IP-3M	x	SP-65	6	√
11.	IP-3A	x	SP-89	66	√	47.	IP-3M	x	SP-89	3	√
12.	IP-3A	x	SP-7	18	√	48.	IP-3M	x	SP-7	31	√
13.	IP-3A	x	SP-19	46	√	49.	IP-3M	x	SP-19	30	√
14.	IP-3A	x	SP-33	38	√	50.	IP-3M	x	SP-33	18	√
15.	IP-3A	x	SP-37	30	√	51.	IP-3M	x	SP-37	0	√
16.	IP-3A	x	SP-54	34	√	52.	IP-3M	x	SP-54	9	√
17.	IP-3A	x	SP-10	68	√	53.	IP-3M	x	SP-10	42	√
18.	IP-3A	x	SP-13	12	√	54.	IP-3M	x	SP-13	15	√
19.	IP-3P	x	HS-1	1102	*	55.	HS-49	x	HS-1	666	*
20.	IP-3P	x	HS-48	1105	*	56.	HS-49	x	HS-48	677	*
21.	IP-3P	x	HS-61	366	*	57.	HS-49	x	HS-61	552	*
22.	IP-3P	x	HS-83	1267	*	58.	HS-49	x	HS-83	404	*
23.	IP-3P	x	Jatim 33	931	*	59.	HS-49	x	Jatim 33	714	*
24.	IP-3P	x	SP-104	658	*	60.	HS-49	x	SP-104	451	*
25.	IP-3P	x	SP-103	250	*	61.	HS-49	x	SP-103	149	*
26.	IP-3P	x	SP-4	79	√	62.	HS-49	x	SP-4	98	√
27.	IP-3P	x	SP-31	52	√	63.	HS-49	x	SP-31	23	√
28.	IP-3P	x	SP-65	86	√	64.	HS-49	x	SP-65	61	√
29.	IP-3P	x	SP-89	81	√	65.	HS-49	x	SP-89	63	√
30.	IP-3P	x	SP-7	28	√	66.	HS-49	x	SP-7	38	√
31.	IP-3P	x	SP-19	92	√	67.	HS-49	x	SP-19	138	*
32.	IP-3P	x	SP-33	90	√	68.	HS-49	x	SP-33	51	√
33.	IP-3P	x	SP-37	18	√	69.	HS-49	x	SP-37	42	√
34.	IP-3P	x	SP-54	81	√	70.	HS-49	x	SP-54	61	√
35.	IP-3P	x	SP-10	16	√	71.	HS-49	x	SP-10	69	√
36.	IP-3P	x	SP-13	228	*	72.	HS-49	x	SP-13	192	*

Notice: * : 100 seeds were planted for selection in 201; √: all seeds were planted for selection in 2011

Out of 48 F1 hybrids which had >400 capsules per plant per year, 17 genotypes showed higher oil content (≥40%-dry base) compared to their parents which were only less than 40% (Fig. 2). Oil content of Jatropha seed was influenced by several factors e.g. genetic, environment, maturity of fruit, harvesting time etc. The highest oil content was found when fruit or capsule color already turned from green to yellowish green or yellow [8].

All 54 F1 selected hybrids were categorized as early maturity genotypes because their harvesting age ≤110 days. Only one genotype i.e. HS-49XSP-33/33 had harvesting age 113 days after transplanting. Out of 54 hybrids, 22 hybrids showed their consistency in producing capsules during three years (Fig. 3). From these 22 F1 hybrids, 14 genotypes had high oil content i.e.HS-49XSP-65/32, IP-3PXSP-7/5, IP-3AXSP-65/11, IP-3AXSP-65/10, HS-49XSP-19/28, IP-3AXSP-65/9, HS-49XSP-103/24, IP-3AXSP-89/4, IP-3PXSP-65/18, HS-49XSP-65/31, HS-49XSP-10/15, HS-49XSP-10/21, HS-49XSP-65/30, and HS-49X SP-10/20. These 14 hybrid genotypes were selected and used in evaluation of high yield potential hybrids started in 2015.

C. *Evaluation of High Yield Potential Hybrids*

Data of dry seed and number of capsules per plant per year were listed in Tables II and III. The data showed that one hybrid (HS-49xSP-65/32) had highest yielding of dry seed and number of capsules per plant for three years (2015-2017). The seed yield in 2015 was 653.00 kg/ha/year and increased to 5,575.20 kg/ha/year in 2016, finally in 2017 become 6,602.63 kg/ha/year. The highest number of capsules in 2015 was only

207.22 capsules/plant/year on IP-3PxSP-7/5. The hybrid (HS-49xSP-65/32) was in second position with 181.80 capsules/plant/year. The growth of the genotypes was not optimal due to the very long period of dry climate in 2015 and there was not enough water for watering the plants. This condition was different from the time of selection, in the first year (2012) almost all F1 hybrids produced >400 capsules per plant per year in average.

In 2016, three F1 hybrids produced highest seed yield (> 5,000 kg/ha/year) i.e. HS-49xSP-65/32, HS-49xSP-19/28, and IP-3PxSP-7/5. These three genotypes also produced highest number of capsules (>1,200 capsules/plant/year). The highest seed yield and number of capsules per plant was produced by HS-49xSP-65/32 until third year.

Evaluation in 2017 resulted in four hybrids with high seed yield (>6,000 kg/ha/year) i.e. HS-49xSP-65/32, HS-49xSP-65/31, IP-3AxSP-89/4, and IP-3AxSP-65/10. Three of these hybrids also produced high number of capsules per plant per year (>1,500/plant/year) i.e. HS-49xSP-65/32, HS-49xSP-65/31, and IP-3AxSP-89/4. But statistically there was no significant difference between hybrids (Table III). Experiment data for three years of cultivation showed that the best hybrid genotype was HS-49xSP-65/32. This genotype reached the highest dry seed yield (4,276.94 kg/ha) and number of capsules per plant (1,026.98).

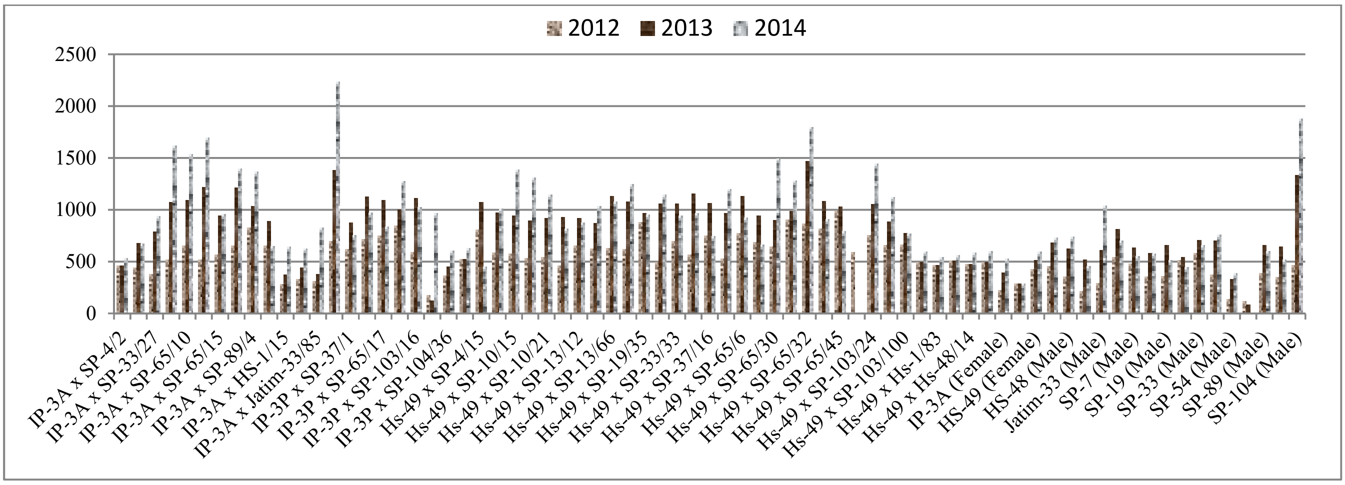

Fig. 1 Number of capsules per plant per year of 54 hybrid and 19 parent genotypes in 2012, 2013, and 2014

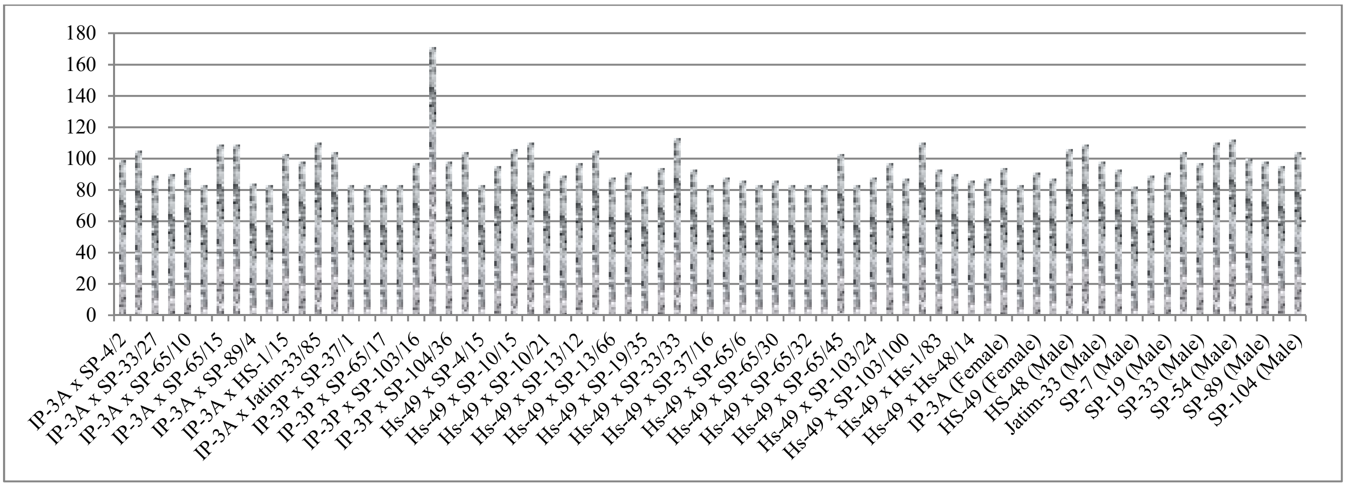

Fig. 2 First harvesting age (days) of 54 hybrid and 19 parent genotypes

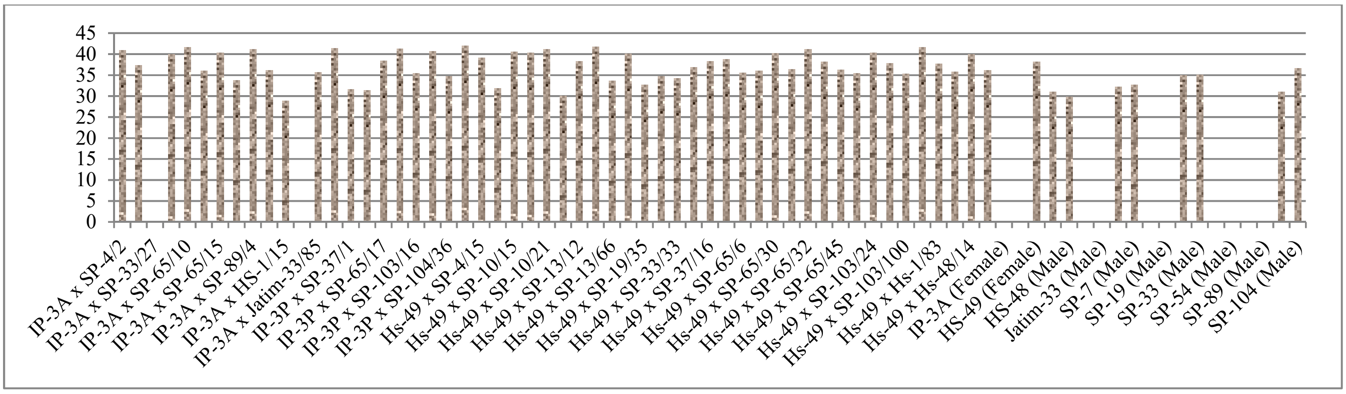

Fig. 3 Oil content (% db) of 54 hybrid and 19 parent genotypes

TABLE II
THE DRY SEED YIELD OF 14 F1 HYBRIDS AND TWO CHECK VARIETIES EVALUATED IN 2015, 2016 AND 2017

No.	Genotypes	Seed yield (Kg/ha/year)		
		2015	2016	2017
1	IP-3AxSP-65/9	547.90 abc	4,129.50 cd	5,314.63
2	IP-3AxSP-65/10	407.90 bc	4,140.00 cd	6,752.81
3	IP-3AxSP-65/11	421.10 bc	4,353.10 bcd	5,351.64
4	IP-3AxSP-89/4	519.20 abc	4,800.70 abc	6,558.43
5	IP-3PxSP-7/5	718.50 a	5,046.00 ab	4,643.33
6	IP-3PxSP-65/18	413.10 bc	4,164.30 bcd	5,289.69
7	HS-49xSP-10/15	365.70 c	2,969.40 fgh	5,279.04
8	HS-49xSP-10/20	555.70 abc	3,177.60 efg	4,402.03
9	HS-49xSP-10/21	415.80 bc	2,711.60 gh	4,544.17
10	HS-49xSP-19/28	557.40 abc	5,369.50 a	5,867.61
11	HS-49xSP-65/30	633.80 abc	3,960.20 cde	4,151.88
12	HS-49xSP-65/31	421.30 bc	4,479.60 bcd	6,147.33
13	HS-49xSP-65/32	653.00 ab	5,575.20 a	6,602.63
14	HS-49xSP-103/24	484.50 abc	3,689.50 def	5,240.71
15	IP-3P (check var)	476.90 abc	4,167.90 bcd	5,214.31
16	IP-3A (check var)	373.20 c	2,236.70 h	4,322.21
	CV	28.25	11.64	NS

Notice: The numbers in the same colon with the same letters are not significantly different at 5% of HSD. NS: not significant. CV: Coefficient of variation.

In Indonesia, the genotypes which were evaluated in this activity had a very high seed yield compared to the previous evaluation of other genotypes. In previous evaluation, nine genotypes evaluated at Asembagus, Situbondo, East Java in 2011-2013 presented the highest seed yield as only 1,651.87 kg/ha in average of three-year cultivation [7]. Other evaluation at Amor-Amor Village, District of Kayangan, North Lombok, West Nusa Tenggara, the highest seed yield ranged from 1,145.55 to 1,213.61 kg/ha in the second year and 2,389 to 2,632 kg/ha year in third year of cultivation [9]. In India, the highest seed yield was 427.21 kg/ha on five-year-old plant treated with N_{60} fertilizer [10]. Under semi-arid climatic conditions of Northwest India, the highest seed production was 472.51 kg/ha and oil yield 163.31 kg/ha when Jatropha plants were treated with both fertilizer (N_{90} K_{60}) and irrigations [11].

The interesting data were found on hybrid IP-3AxSP-89/4, the number of capsules was only 973.80 capsules per plant per year but the average seed yield was very high: 3,959.44 kg per hectare per year. This may be because of the bigger seed size or high seed quality. Unfortunately the seed size and quality were not measured in this evaluation. Some researchers suggested that seed width could be applied as a criterion for plants selection to obtain high oil yield of *J. curcas* [12].

TABLE III
THE AVERAGE NUMBER OF CAPSULES PER PLANT PER YEAR OF 14 F1 HYBRIDS AND TWO CHECK VARIETIES EVALUATED IN 2015, 2016 AND 2017

No.	Genotypes	No. of capsules/plant/year		
		2015	2016	2017
1	IP-3AxSP-65/9	157.25 a-d	1,015.45 def	1,242.00
2	IP-3AxSP-65/10	120.63 bcd	994.87 def	1,475.00
3	IP-3AxSP-65/11	130.02 bcd	1,104.73 def	1,356.00
4	IP-3AxSP-89/4	148.17 a-d	1,192.22 bcd	1,581.00
5	IP-3PxSP-7/5	207.22 a	1,287.73 abc	1,194.00
6	IP-3PxSP-65/18	119.98 bcd	1,028.62 def	1,317.00
7	HS-49xSP-10/15	92.15 d	685.40 hi	1,213.00
8	HS-49xSP-10/20	139.27 a-d	768.59 ghi	1,066.00
9	HS-49xSP-10/21	105.98 cd	624.38 i	1,063.00
10	HS-49xSP-19/28	173.48 abc	1,419.07 a	1,443.00
11	HS-49xSP-65/30	164.17 abc	933.58 efg	1,002.00
12	HS-49xSP-65/31	132.38 bcd	1,155.10 cde	1,512.00
13	HS-49xSP-65/32	181.80 ab	1,385.13 ab	1,514.00
14	HS-49xSP-103/24	127.70 bcd	891.08 fgh	1,255.00
15	IP-3P (check var)	143.12 a-d	1,042.53 def	1,313.00
16	IP-3A (check var)	105.62 cd	556.92 i	1,050.00
	CV	25.47	11.69	NS

Notice: The numbers in the same colon with the same letters are not significantly different at 5% of HSD. NS: not significant. CV: Coefficient of variation.

IV. CONCLUSION

The experiment results showed that all hybrids have higher seed yields than check variety (IP-3A). From the average seed yield per hectare and number of capsules per plant during three years, there were two superior hybrids i.e. HS-49xSP-65/32 and HS-49xSP-19/28. The hybrid HS-49xSP-65/32 even showed their consistency in highest seed yield and number of capsules production for three years.

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