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Selection of High Yielding *Jatropha curcas* L. Accessions for Elite Hybrid Seed Production

(Pemilihan Aksesi Hasil Tertinggi *Jatropha curcas* L. untuk Penghasilan Benih Hibrid Elit)

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ABSTRACT

Phenotypic selection of individuals is the first step in a selective breeding program for elite hybrid seed production. In this study, a total of 295 Jatropha curcas *individuals raised from cuttings representing 21 accessions, collected from eight different countries were evaluated for growth performance. The evaluation was done at the Biodiesel Research Station of Universiti Kebangsaan Malaysia, Kuala Pilah from December 2012 to December 2013. Individual plants from each accession were observed on several agronomic and yield related traits and all the data were recorded periodically. Performance of each accession was analyzed using Statistical Analysis System (SAS) 9.4. Four traits which were plant height (PH), number of flowers per inflorescence (NFI), number of female flowers per inflorescence (NFFPI) and hundred seed weight (HSW) showed significant differences among the accessions after one year of planting. Maximum values for each trait were 115.5 cm for PH, 6 for number of branches per plant (BPP), 9 for number of inflorescences per plant (NIPP), 25 for number of fruits per plant (NFPP), 5 for number of fruits per inflorescence (NFPI), 191 for NFI, 10 for NFFPI, 81.0 g for HSW and 70 for number of seeds per plant (NSPP). Accession number 1 from Thailand showed the best performance for most traits. A highly significant and positive correlation was found between NFPP and NSPP. Based on superior trait values for NIPP, NFPP, NFPI, NFI, NFFPI and HSW, five plants from accession UKMJC 01, 04, 05, 13 and 14 have been selected for generating elite intraspecific hybrids.*

Keywords: Hybrid seed; Jatropha curcas*; yield influencing traits*

ABSTRAK

Pemilihan fenotip secara individu merupakan langkah pertama dalam program pembiakan memilih untuk menghasilkan benih hibrid elit. Kajian ini menilai prestasi pertumbuhan sebanyak 295 individu daripada Jatropha curcas *yang diperoleh daripada keratan batang. Individu ini mewakili 21 aksesi yang diperoleh dari lapan buah negara dinilai untuk prestasi pertumbuhan. Penilaian ini telah dilakukan di Stesen Penyelidikan Biodiesel Universiti Kebangsaan Malaysia, Kuala Pilah bermula dari Disember 2012 hingga Disember 2013. Individu daripada setiap aksesi telah diperhatikan berdasarkan beberapa ciri agronomi dan ciri yang mempengaruhi hasil. Kesemua data yang diperoleh dicatatkan secara berkala dan prestasi setiap aksesi diuji untuk melihat kewujudan perbezaan bererti menggunakan Sistem Analisis Statistik (SAS) 9.4. Empat perlakuan iaitu ketinggian pokok (PH), bilangan bunga setiap jejambak (NFI), bilangan bunga betina setiap jejambak (NFFPI) dan berat seratus biji (HSW) menunjukkan perbezaan bererti antara aksesi selepas setahun penanaman. Nilai maksimum untuk setiap ciri ialah 115.5 sm bagi PH, 6 bagi bilangan dahan setiap pokok (BPP), 9 bagi bilangan jejambak setiap pokok (NIPP), 25 bagi bilangan buah setiap pokok (NFPP), 5 bagi bilangan buah setiap jejambak (NFPI), 191 bagi NFI, 10 bagi NFFPI, 81.0 g bagi HSW dan 70 bagi bilangan biji setiap pokok (NSPP). Aksesi nombor 1 dari Thailand menunjukkan prestasi yang paling baik bagi semua ciri. Korelasi yang positif dan bererti tinggi didapati antara NFPP dan NSPP. Berdasarkan ciri yang unggul bagi NIPP, NFPP, NFPI, NFI, NFFPI dan HSW, lima pokok daripada aksesi UKMJC 01, 04, 05, 13 dan 14 telah dipilih untuk menjana hibrid intraspesies elit.*

Kata kunci: Benih hibrid; ciri yang mempengaruhi hasil; Jatropha curcas

INTRODUCTION

Jatropha curcas L. (2n = 2× = 22), a semi-evergreen, perennial, multipurpose shrub, resistant to high degree of drought is one of the most promising bioenergy crops used for commercial production of biodiesel (Dahmer et al. 2009). As a non-edible seed due to high content of phorbol ester, this crop has received a worldwide attention for its richness of oil (Ahmed & Salimon 2009). This perennial plant can be found throughout most of the tropics. It can grow up to 6 m (20 ft) and can withstand low soil moisture content (Pérez-Vázquez et al. 2013). It is believed to be originated from Mexico and Central America with Mesoamerican region as a center of its diversity (Heller 1996; Ovando-Medina 2011). Current distribution of *Jatropha* is concentrated in a drier region of the tropics with annual rainfall of 250 to 1200 mm (Katwal & Soni

2003). It occurs mainly at lower altitudes (0-500 m) but can grow at higher altitudes and tolerate slight frost.

There are 175 species of *Jatropha* and the major species include *J. curcas*, *J. multifida*, *J. integerrima* and *J. podagrica*. *J. multifida* is preferred for its large fruit size, *J. integerrima* for having profuse flowers whereas *J. podagrica* for its high oil yield (Ratha & Paramathma 2009). Artificial hybridization between *J. curcas* and *J. integerrima* has been done and the hybrids produced more seed, have higher yield potential, resistant to diseases and showed early flowering (Parthiban et al. 2009; Sujatha & Prabakaran 2003). However, interspecific hybridization of *J. podagrica* with the other *Jatropha* species did not yield any hybrids (Basha & Sujatha 2009). Intraspecific hybridization of *J. curcas* also produced hybrids with high yield and early maturity (Islam et al. 2011; Tar et al. 2011).

Oil content in *J. curcas* varies from 28 to 41% in its seeds (Gubitz et al. 1999; Kaushik et al. 2007; Wang et al. 2008) and 50 to 63% in the kernel (Akhbar et al. 2009; Gubitz et al. 1999). According to Chawla (2010), one hectare of *Jatropha* plantation, depending on density, can produce 158-396 gallons of oil and 1000 g of *Jatropha* oil can produce about 94% g of pure biodiesel (Alkabbashi et al. 2009).

Some studies recorded that the other species which is *J. integerrima* also produces oil but their oil quality is inferior as the result of its lower oleic–linoleic acid ratio (Popluechai 2010). *J. curcas* oil has been used by adding directly in diesel engines, adding to diesel fuel as an extender or transesterified with methanol to biodiesel fuel. The oil content is higher than linseed (46%) (Green & Marshal 1981) and soybean (22%) (Miranda et al. 1998). Its oil contains less saturated fatty acids (21.6%) and more unsaturated fatty acids (78.4%) with the dominating fatty acids such as oleic acid (44.7%) and linoleic acid (32.8%) (Akhbar et al. 2009). The dominating monounsaturated fatty acid in *J. curcas* especially oleic acid makes it most desirable for jet fuel application. This fatty acid helps in reducing the emission of nitrogen oxides (NO_x) in the atmosphere and has better oxidative stability (Chhetri et al. 2008; Durrett et al. 2008; Tat et al. 2007).

Most of the current planting materials of *J. curcas* come from open pollinated seed with low seed yield, low number of inflorescence per year and uneven maturity. Progress in *Jatropha* breeding may be limited, mainly due to its low or poor genetic diversity (Rosada et al. 2010; Siju et al. 2014; Yue et al. 2013). Thus, the objective of the current study was to identify high yielding accessions for elite hybrid seed production. The results can be used by plant breeders interested in producing some new cultivars of this species.

MATERIALS AND METHODS

A total of 21 accessions were raised from cuttings in a polybag containing a mixture of soil and compost in the ratio of 1:1. The cuttings were watered three times a week. The accessions originally came from eight different countries. Nine accessions were from Malaysia (UKMJC02, 03, 08, 09, 10, 11, 12, 17, 21), three from Indonesia (UKMJC 13, 18, 20), two each from Thailand (UKMJC01, 04), India (UKMJC14, 19) and the Philippines (UKMJC07, 15) and one each from South Africa (UKMJC16), Cape Verde (UKMJC05) and Vietnam (UKMJC06). Four months old cuttings were transplanted in the field in a randomized complete design. The spacing used was 3 m between plants and 2.5 m between rows. Chicken manure (2-3 kg) was applied at four and eight months after transplanting. Fertilizers (20 g urea, 120 g single super phosphate and 16 g muriate of potash) were applied at two and six months after transplanting (Punia 2007). Irrigation was applied when needed to supplement the natural rainfall and care was taken to avoid plants from being infested with pests and diseases. All plants were maintained under suitable agricultural management practices. A total of 295 individual plants which survived after the transplanting, were evaluated periodically until the plants were one year old. The data were recorded on nine different traits as summarized in

TABLE 1. Growth parameters recorded for *J. curcas* accessions

No			Growth parameter
1	PH	=	average height from the ground level to the highest tip of the inflorescences at the time of harvesting was measured
2	BPP	=	branches per plant (total number of branches growing from the main stem at different node positions or primary branches)
3	NIPP	=	no of inflorescences per plant was counted and recorded
4	NFPP	=	total number of fruits per plants were counted and recorded
5	NFPI	=	average number of fruits per inflorescence were counted and recorded
6	NFI	=	average number of flowers (male + female flower) per inflorescence were counted and recorded after emergence of 80 % flowering
7	NFFPI	=	average number of female flowers per inflorescence were counted and recorded
8	HSW	=	weight of 100 dried, cleaned seeds were measured and recorded in grams
9	NSPP	=	no of seeds per plant

Table 1. This experiment was conducted from December 2012 to December 2013 at the Biodiesel Research Station, Universiti Kebangsaan Malaysia in Kuala Pilah, Negeri Sembilan, Malaysia.

STATISTICAL ANALYSIS

All traits measured were subjected to analysis of variance. Once an F-test for difference between treatments was declared significant, the treatment means were compared with Fisher's least significant difference (LSD) tests. Then, the significant traits were calculated for correlation coefficient (r). All statistical analysis was performed using the Statistical Analysis System (SAS) 9.4 software.

RESULTS AND DISCUSSION

VARIABILITY IN AGRONOMIC AND YIELD RELATED TRAITS OF *J. CURCAS*

Analysis of variance (ANOVA) for all traits showed that three out of nine traits showed significant differences at p=0.05. The traits were PH, NFI and NFFPI whereas HSW showed significant differences at p=0.01 (Table 2). This indicated that the HSW trait has a high variability compared to the other traits. Significant variation in HSW was also observed in wild accessions of *J. curcas* in eleven different districts in India (Rao et al. 2008). On the other hand, no significant differences were observed in the remaining five traits.

Maximum plant height of 102.0 cm was recorded in UKMJC12 followed by UKMJC01 which was 99.9 cm (Table 3). Low ranking group of less than 80.0 cm consisted of eight accessions which were UKMJC10, 06, 09, 07, 03, 18, 04 and 19 (Figure 1(a)). The results also showed that the number of flowers per inflorescence with a high degree of variability. For example, UKMJC05 had 139 flowers and was almost eight times higher compared to the lowest rank which was UKMJC15 with only 17 flowers. Accessions UKMJC05, 04, 12, 06, 10, 02, 21 and 16 recorded more than 100 flowers per inflorescence whereas UKMJC14, 18, 17 and 15 recorded the least number of flowers of 66, 49, 26 and 17, respectively (Figure 1(b)). The average number of flowers (male + female flowers) per inflorescence observed in this study were comparable to the results obtained by Chang-Wei et al. (2007) and Raju and Ezradanam (2002) with the number of flowers per inflorescence varying between plants and male:female ratios of 24.5:1 and 29:1, respectively.

For the number of female flowers per inflorescence, the highest was seen in UKMJ04 and UKMJC05 with both having six female flowers per inflorescence. Three accessions with the lowest value for this trait were UKMJC19, 17 and 15 with only one female flower per inflorescence. These three accessions showed significant differences to UKMJC04. However, accessions UKMJC12, 02 and 03 did not show any significant differences between them. Despite this, all the accessions were producing flowers almost throughout the year and the same observation was also made by Ahoton and Quenum (2012) and Ghosh and Singh (2008). Hundred seed weight in this study varied from 26.5 g to 69.5 g. UKMJC04, 13, 02 and 08 showed high significant differences when compared to UKMJC15. Hundred seed weight for ten accessions were more than 60.0 g which were UKMJC04, 13, 02, 08, 09, 06, 10, 01, 05 and 03. The low ranking accessions with less than 50.0 g were UKMJC14, 18, 12, 17, 07 and 15.

The number of branches per plant ranged from two to four with accessions UKMJC12, 17, 10 and 18 having the highest value and accessions UKMJC04, 05 and 07 having the lowest value. The number of inflorescence per plant was highest in four accessions which were UKMJC04, 13, 02 and 01 with each having six inflorescences. Accession UKMJC15 had the least number of inflorescences i.e. 2 inflorescences per plant. A maximum of 16 fruits per plant was recorded in UKMJC01, followed by UKMJC13 with 14 fruits per plant. The minimum value recorded in this trait was three fruits per plant for accession UKMJC15. However, ten out of 21 accessions had more than ten fruits per plant. The accessions were UKMJC01, 13, 04, 14, 02, 12, 08, 06, 05 and 03. The highest value recorded for the number of fruits per inflorescence was three for five accessions (Figure 1(c)). The five accessions were UKMJC01, 04, 14, 08 and 06. Several accessions (UKMJC12, 17, 16, 19, 07, 18 and 15) fell into the low ranking group with only one fruit per plant. Most of the accessions had two fruits per inflorescence. Accessions UKMJC01, 04, 14 and 13 had high numbers of seeds per plant (>30). The least number of seeds (9) was recorded in UKMJC15. Most of the other accessions fell into the group of 21 to 30 seeds per plant. The maximum values recorded for each trait were 115.5 cm for PH, 6 for BPP, 9 for NIPP, 25 for NFPP, 5 for NFPI, 191 for NFI, 10 for NFFPI, 81.0 g for HSW and 70 for NSPP.

GENETIC CORRELATION AMONG AGRONOMIC AND YIELD RELATED TRAITS

The number of fruits per plant exhibited a significant and positive correlation with the number of seeds per plant, number of fruits per inflorescence, number of inflorescences per plant, number of flowers per inflorescence, number of female flowers per inflorescence and hundred seed weight at 0.01 levels (Table 4). This trait also showed a significant and positive correlation with the number of branches per plant at p=0.05 (Table 3). This significant correlation suggests that high number of branches tend to develop more flowers and fruits and selection can be made on the basis of these traits. Similar observation was reported by Rao et al. (2008) and Tar et al. (2011).

However, fruits per plant showed no evident correlation with plant height. Plant height exhibited no evident correlation with all the others traits and the r values were negative in the case of the number of fruits per inflorescence, the number of female flowers per inflorescence and hundred seed weight. In contrast, the number of seeds per plants exhibited a high correlation with the number of fruits per inflorescence, the number

TABLE 2. Mean square of analysis of variance (ANOVA) for all traits

Source of variation	Degree of freedom (d.f.)	PH	BPP	NIPP	NFPP	NFPI	NFI	NFFPI	HSW	NSPP
Accession	20	251.13*	0.90^{ns}	3.42^{ns}	30.68^{ns}	1.48^{ns}	3024.05*	7.04*	406.39**	209.02^{ns}
Error	42	132.48	0.69	2.66	25.77	0.99	1303.36	3.64	177.73	188.67

*= significant at the 0.05 level, **= significant at the 0.01 level, ns= non-significant
PH= plant height (cm), BPP= number of branches per plant, NIPP= number of inflorescence per plant, NFPP= number of fruit per plant, NFPI= number of fruit per inflorescence, NFI= number of flower per inflorescence, NFFPI= number of female flowers per inflorescence, HSW= hundred seed weight (g), NSPP= number of seeds per plant

TABLE 3. Mean values for different agronomic and yield related traits of *J. curcas*

Country of origin	Accession no.	PH	BPP	NIPP	NFPP	NFPI	NFI	NFFPI	HSW	NSPP
Malaysia	UKMJC02	88.9	3	6	11	2	112	3	67.2	30
	UKMJC03	76.3	3	4	10	2	78	3	60.1	25
	UKMJC08	81.7	3	4	10	3	87	2	65.8	27
	UKMJC09	76.8	3	4	8	2	72	2	65.3	23
	UKMJC10	78.1	4	5	8	2	115	4	62.1	22
	UKMJC11	92.4	3	5	7	2	95	3	57.8	21
	UKMJC12	102.0	4	5	11	1	120	4	44.5	30
	UKMJC17	90.6	4	3	8	1	26	1	38.4	17
	UKMJC21	92.5	3	5	8	2	111	4	54.0	22
Indonesia	UKMJC13	88.5	3	6	14	2	95	2	67.8	33
	UKMJC18	74.5	4	4	4	1	49	2	45.0	13
	UKMJC20	84.6	3	3	7	2	71	3	50.2	19
South Africa	UKMJC16	80.7	3	4	6	1	109	3	56.2	18
Thailand	UKMJC01	99.9	3	6	16	3	91	4	61.0	43
	UKMJC04	72.4	2	6	13	3	127	6	69.5	35
Cape Verde	UKMJC05	83.2	2	4	10	2	139	6	60.8	27
Vietnam	UKMJC06	76.9	3	4	10	3	116	5	64.2	25
India	UKMJC14	89.3	3	4	13	3	66	2	47.2	35
	UKMJC19	69.2	3	4	5	1	70	1	50.1	16
The Philippines	UKMJC07	76.7	2	3	5	1	82	3	37.3	15
	UKMJC15	95.5	3	2	3	1	17	1	26.5	9
Maximum value		115.5	6	9	25	5	191	10	81.0	70
Minimum value		53.7	2	1	1	0	0	0	18.2	1
LSD at 0.05		18.97	1.37	2.69	8.36	1.64	59.49	3.15	21.97	22.63

PH= plant height (cm), BPP= number of branches per plant, NIPP= number of inflorescence per plant, NFPP= number of fruit per plant, NFPI= number of fruit per inflorescence, NFI= number of flower per inflorescence, NFFPI= number of female flowers per inflorescence, HSW= hundred seed weight (g), NSPP= number of seeds per plant

FIGURE 1. *Jatropha curcas* L. (a) One year old of *J. curcas* plants, (b) Male and female flowers in the same inflorescence, and (c) Fruits at the beginning of brown pigmentation

TABLE 4. Pearson correlation coefficient for the nine traits in *J. curcas*

Traits	PH	BPP	NIPP	NFPP	NFPI	NFI	NFFPI	HSW
BPP	0.57**							
NIPP	0.21^{ns}	0.31**						
NFPP	0.16^{ns}	0.24*	0.56**					
NFPI	-0.08^{ns}	-0.05^{ns}	0.13^{ns}	0.66**				
NFI	0.13^{ns}	0.17^{ns}	0.53**	0.50**	0.42**			
NFFPI	-0.01^{ns}	-0.02^{ns}	0.34**	0.48**	0.42**	0.78**		
HSW	-0.09^{ns}	-0.09^{ns}	0.55**	0.35**	0.45**	0.57**	0.43**	
NSPP	0.14^{ns}	0.20^{ns}	0.57**	0.99**	0.66**	0.52**	0.51**	0.37**

*= significant at the 0.05 level, **= significant at the 0.01 level, ns= non-significant
PH= plant height (cm), BPP= number of branches per plant, NIPP= number of inflorescence per plant, NFPP= number of fruit per plant, NFPI= number of fruit per inflorescence, NFI= number of flower per inflorescence, NFFPI= number of female flowers per inflorescence, HSW= hundred seed weight (g), NSPP= number of seeds per plant

of inflorescences per plant, the number of flowers per inflorescence, the number of female flowers per inflorescence and hundred seed weight at p=0.01. The number of flowers per inflorescence and the number of female flowers per inflorescence were highly correlated too (r=0.78 at p=0.01).

Conclusion

Eight out of the nine traits chosen to evaluate the 21 accessions in this study were able to differentiate and rank the accessions for growth performance. Based on the high values for six traits (NIPP, NFPP, NFPI, NFI, NFFPI) and significant correlation among traits (NFPP and NSPP), accessions UKMJC 01, 04, 05, 13 and 14 have been selected as parents for generating elite intraspecific hybrids.

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