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# Combining ability analysis and evaluation of heterosis in *Jatropha curcas* L. F<sub>1</sub>-Hybrids

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# Abstract

With the aim of estimating genetic parameters and recognizing superior *Jatropha curcas L*.combinations, ten superior plants were selected based on seed yield and oil content, and were crossed among them in a 10 x 10 half-diallel mating design to produce 45  $F_1$ -hybrids. The experiment was conducted in nursery stage using a randomized complete block design (RCBD) with three replications. Analysis of variance for the combining ability indicated that GCA and SCA variance were significant at 1% probability for plant height, collar diameter and number of leaves in nursery stage. The low ratio of GCA/SCA exhibited the non-additive effects. Broad sense heritabilies were high for plant height, collar diameter and number of leaves were low. Percentages of heterosis and heterobeltiosis values for plant height, collar diameter and number of leaves ranged from negative to positive. This showed that the existence of dominance or non-additive gene actions might be present in the hybrids. On the basis GCA and SCA effects, parents, Ph1.2 and In2.1 and hybrids Ph1.2 (3) × In1.2 (8), Ph1.1 (9) × My2.2 (10) and My2.1 (1)× My2.2 (10) could be used for future breeding program.

**Keywords:** *Jatropha curcas L*; Diallel crosses; General and specific combining ability; Additive effects; Non-additive effects; Heterosis.

**Abbreviations:** GCA- General combining ability; SCA- Specific combining ability; DAP- Days after planting;  $h_n^2$ - Narrow-sense heritability;  $h_b^2$ -Broad-sense heritability.

# Introduction

The employment of energy crops as a source of renewable fuels is a concept with great relevance to current ecological and economic issues at both national and global scales. Biodiesel, an alternative diesel fuel, is produced from renewable biological sources such as vegetable oils and animal fats (Fangrui and Milford, 1999; Kumar and Sharma, 2008, Rafii et al. 2012a). Oil yielding crops produce different types of oil, said vegetable oils. Recently, biofuels extracted from plant species has been a major renewable source of energy such as Jatropha curcas L. (Mukherjee et al., 2011; Sharma et al., 2011). J. curcas, also known in Peninsular Malaysia area as "Jarak Pagar" is a euphorbiaceous plant with many uses around the world with great potential (Milford, 1999; Shah et al., 2004; Basha and Sujatha, 2007; Gohil and Pandya, 2008; Fangrui and Ganesh Ram et al., 2008; Singh et al., 2010; Ghosh and Singh, 2011; Rafii et al. 2012b). However, Jatropha is native to Central America, and it exists as a crop with good adjustment in both the Old and the New World. Many advantages exist for J. curcas more than other oily species, due to fast growing, tolerance to drought and easily adapts to marginal lands (Achten et al., 2008; Rao et al., 2008; Basha et al., 2009; Divakara et al., 2010; Ovando-Medina et al., 2011; Sharama et al., 2011). However, in Malaysia as well as other countries, its properties such as oil content, seed yield and other traits are still needed to be improved (Heller, 1996; Kaushi and

Kumar, 2004; Divakara et al., 2010; Shabanimofrad et al., 2011). The early views that hybridization would be an elixir for all crop improvement problems are strongly driven. Heterosis is rare far from a common phenomenon in hybrids (Rameeh, 2011a). Can the more favourable and beneficial contribution of hybridization is in transferring desirable genes from one to another accession (Amiri Oghan et al., 2007). Same another tree, Jatropha breeding is a time consuming process due to perenniality. Diallel mating designs provide useful genetic information for breeding programs, such as general combining ability (GCA) and specific combining ability (SCA) to help them design appropriate breeding and selection strategies (Johnson and King, 1998). Diallel mating design was used generally in crops including rapeseed, oleaginous and Griffing's approaches were employed more frequently (Banumathy and Patil, 2000; Machikowa et al., 2011; Rameeh, 2011b; Sharma et al., 2011). Utilizing diallel mating with different parents, oil content, some agronomic traits, seed yield, and seed oil yield of Jatropha were investigated for gene effects, combining ability (GCA and SCA), heritability and heterosis. Better understanding and realizing of the mode of inheritance of the traits leads to improve in breeding programs, this current study was managed to estimate the genetic parameters and the mode of inheritance for some morphological traits of J. curcas in a set of half diallel crosses. From this study, better parents and hybrids could be selected for future breeding program.

# Results

## Plant height

Results regarding to plant height is presented in the Table 1 and Table S1. Among parents and hybrids, the highest plant height at 15, 30, 60 and 90 DAP were recorded in My2.1 (1) × My2.2 (10) (19.cm), My2.1 (1) × My2.2 (10) (20.7cm), In2.1 (2) × Ph1.1 (9) (44.7cm) and In2.1 (80.7cm) respectively. While, the shortest plant height were recorded in My2.1 (1) × Id1.2 (5) (9.7 cm), In1.1 (7) × In1.2 (8) (11.7cm), In1.1 (7)  $\times$  In1.2 (8) (21.7cm) and In1.1 (7)  $\times$ Ph1.1 (9) (29.7cm) at 15, 30, 60 and 90 DAP, respectively (Table S1). The highest positive GCA effects were found for parents Ph1.2, Ph1.2, In2.1 and In2.1 for plant height at 15, 30, 60 and 90 DAP, respectively (Table 2). Parents Ph1.2 and In2.1 could be provided good plant height. Estimates of SCA effects for plant height were positive and significantly differed in some hybrids as shown in Table S2. Hybrids My2.1 (1) × My2.2 (10), In1.1 (7) × My2.2(10), Ph1.2 (3) × In1.2 (8) and Ph1.2 (3)  $\times$  In1.2 (8) gave the higher positive significant SCA effects for plant height. Broad and narrow sense heritabilities are presented in Table 3. Estimates for broad sense heritability values were higher for plant height and ranged from 76.24 to 95.71%. Even, narrow sense heritability was low for plant height and ranged from 2.31 to 13.45%. Broad sense heritability was higher than that of narrow sense for plant height. Percentages heterosis and heterobeltiosis values for the 45 hybrids are presented in the Table S3. Some hybrids had the values more than plant height averages of their parents. The heterosis for plant height at 15, 30, 60 and 90 DAP ranged from 0 to 49.84% (Table S3). The highest heterosis for plant height at 15, 30, 60 and 90 DAP were found in the hybrids Id1.1 (4)  $\times$  In1.1 (7), In1.1 (7)  $\times$  My2.2 (10), Ph1.2 (3)  $\times$  In1.1 (7) and Ph1.2  $(3) \times In1.2$  (8), respectively. The heterobeltiosis of plant height ranged from 0 to 34.50%. The highest value was obtained from the crosses between parents My2.1 (1)  $\times$ My2.2 (10), In1.1 (7) × My2.2 (10), Ph1.2 (3) × In1.1 (7) and Ph1.2 (3)  $\times$  In1.2 (8) for plant height at 15, 30, 60 and 90 DAP, respectively. The percentages for heterosis and heterobeltiosis for plant height in some hybrids were significantly higher indicating the high degree of genetic diversity existed among the parents. Crosses, In1.1 (7)  $\times$ My2.2 (10),  $3 \times \text{In1.1}$  (7) and Ph1.2 (3)  $\times \text{In1.2}$  (8) gave both the highest heterosis and heterobeltiosis values (Table S3).

# Collar diameter

The highest collar diameter was recorded in My1.1 (7.2mm), My1.1 (9.7mm), In2.1 (16.9mm) and My1.1 (24.1cm) at 15, 30, 60 and 90 DAP, respectively (Table S1). Significant differences ( $p \le 0.01$ ) were observed among collar diameter for genotypes (Table 1). Analysis of variance for the combining ability indicated that GCA and SCA variance were significant at 1% probability level for collar diameter in nursery stage. The ratio of GCA/SCA ranged from 0.01 to 016 (Table 3). Parents Id1.1, Ph1.2, Id1.1 and My2.2 presented the highest positive GCA effects for collar diameter at 15, 30, 60 and 90 DAP respectively. While, parent In1.1 presented the highest negative GCA effect for collar diameter at 15, 30, 60 and 90 DAP (Table 2). Estimates of SCA effects for collar diameter were positive and differed significantly in some hybrids as shown in the Table S2. Higher SCA values suggested that parents for these hybrids

were more diverse in collar diameter than the parents of other hybrids. So hybrids having large SCA values may be developed as candidate hybrid varieties. Hybrids My2.1 (1)× My1.1 (6), 5×8, My2.1 (1)× In1.2 (8) and Ph1.1 (9) × My2.2 (10) gave the highest positive significant SCA effects for collar diameter at 15, 30, 60 and 90 DAP, respectively (Table S2). The values of broad sense heritability for collar diameter ranged from 76.24 to 95.71%. The narrow sense heritability was low for collar diameter and ranged from 2.31 to 13.45% (Table 3). Broad sense heritability was higher than narrow sense ones for collar diameter. The heterosis for collar diameter ranged from 0 to 46.8% (Table S4). The highest heterosis for collar diameter were found in hybrids My2.1 (1)× Id1.2 (5), My2.1 (1)× Id1.2 (5), In1.2 (8)× My2.2 (10) and In2.1 (2)× My2.2 (10) at four different days after planting, respectively. Some hybrids did better than the average plant height of their parents.

The heterobeltiosis of collar diameter ranged from 0.6 to 34.88%. The highest heterobeltiosis value was obtained from crosses between parents, In1.1 (7) × In1.2 (8), 1×5, In1.1 (7) × Ph1.1 (9) and 1× Ph1.1 (9) at 15, 30, 60 and 90 DAP, respectively (Table S4). The percentages of heterosis and heterobeltiosis for collar diameter in some hybrids were significantly higher indicating that the high genetic diversity existed among the parents. Two crosses, In2.1 (2) × In1.1 (7) and Ph1.2 (3) × Id1.1 (4) gave both the highest heterosis and heterobeltiosis.

# Number of leaves

Significant differences between genotypes with respect to the number of leaves at 15, 30, 60 and 90 DAP were observed (Table 1). Among parents the highest number of leaves was recorded in My2.2 (4.0), My2.2 (7.0), In2.1 (13.3) and Id1.1 (20.3) at 15, 30, 60 and 90 DAP, respectively. Among hybrids the highest number of leaves was recorded in In2.1 (2) × Ph1.2 (3), Ph1.1 (9) × My2.2 (10), Ph1.1 (9) × My2.2 (10) and My2.1 (1)  $\times$  My2.2 (10), In2.1 (2)  $\times$  Ph1.2 (3), Ph1.1 (9) × My2.2 (10), Ph1.1 (9) × My2.2 (10) and My2.1  $(1) \times My2.2$  (10) at 15, 30, 60 and 90 DAP, respectively (Table S1). Analysis of variance (ANOVA) on number of leaves indicated significant differences were existed among parents and hybrids at  $P \le 0.01$  (Table 1). Variance of GCA and SCA were significant at 1% probability level for number of leaves in nursery stage except SCA for number of leaves after 15 days after planting (Table 1). The ratio of GCA/SCA ranged from 0.05 to 014 (Table 3). The low ratio of GCA/SCA revealed the non-additive effects and played a more important role than additive effects. Parents Ph1.2, Ph1.2, In2.1 and My2.1 had the highest positive GCA effects for number of leaves presented at 15, 30, 60 and 90 DAP, respectively while parents In1.1, In1.1, In1.1 and Id1.1 had the highest negative GCA effect at similar intervals (Table 2). Estimates of SCA effects for number of leaves were positive and differed significantly in some hybrids as shown in Table S2. Hybrids Id1.2 (5) × In1.1 (7), Id1.2 (5) × In1.2 (8), Ph1.1 (9)  $\times$  My2.2 (10) and My2.1 (1)  $\times$  My2.2 (10) gave the highest positive significant SCA effects while hybrids My1.1  $(6) \times My2.2$  (10), In2.1 (2) × Id1.2 (5), In2.1 (2) × My2.2 (10) and  $2 \times$  My2.2 (10) gave highest negative significant SCA effects for number of leaves at four different days after planting, respectively (Table S2). Estimates for broad sense heritability of the studied genotypes displayed high heritability values for number of leaves at days after planting and ranged from 33.82 to 86.08%. Narrow sense heritability

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Source of	d.f						Mean	square					
variation		Plant height				Collar diameter				No. of leaves			
		15 DAP	30 DAP	60 DAP	90 DAP	15DAP	30 DAP	60DAP	90DAP	15DAP	30DAP	60DAP	90DAP
Blocks	2	16.06**	16.61**	62.52**	143.13**	1.64 <sup>ns</sup>	0.54 <sup>ns</sup>	5.52*	13.16**	2.01 <sup>ns</sup>	1.32*	13.17**	5.01 <sup>ns</sup>
Genotypes	54	12.31**	11.86**	120.59**	430.07**	1.47**	2.28**	8.38**	25.96**	0.40**	0.91**	10.75**	37.67**
GCA	(9)	14.90**	2511**	223.04**	594.69**	2.59**	4.71**	21.09**	29.10**	0.85**	1.89**	17.3**	56.41**
SCA	(45)	11.80**	9.24**	100.25**	397.24**	1.25**	1.81**	5.83**	25.36**	0.30 <sup>n.s</sup>	0.75**	9.45**	33.93**
Error	108	3.54	2.91	11.96	23.33	0.70	0.81	1.50	3.58	0.25	0.41	1.75	6.35

Table 1. Mean squares from analysis of variance, general combining ability (GCA) and specific combining ability (SCA) for three characters of diallel cross involving 10 parents of Jatropha curcas.

Note : DAP-Days after planting. <sup>ns</sup>, \* and \*\* non-significant, significant at the 0.05 and 0.01 levels respectively

Table 2. Estimate of general combining ability effects (GCA) of ten Jatropha parents for measured traits in nursery stage.

Parents		Plant l	neight			Collar diameter				No. of leaves			
	15DAP	30DAP	60DAP	90DAP	15DAP	30DAP	60DAP	90DAP	15DAP	30DAP	60DAP	90DAP	
My2.1 (1)	0.18 <sup>n.s</sup>	0.6**	2.43**	3.55**	0.17**	-0.07 <sup>n.s</sup>	-0.43**	-1.08**	-0.36*	-0.37**	0.46**	2.12**	
In2.1 (2)	$0.07^{n.s}$	0.24*	4.59**	8.63**	0.01 <sup>n.s</sup>	0.12*	0.41**	-0.24*	-0.31 <sup>n.s</sup>	0.16**	1.02**	2.10**	
Ph1.2(3)	0.79**	1.16**	0.62**	0.88*	0.12*	0.45**	0.42**	0.28*	1.53**	0.30**	0.49**	-0.62**	
Id1.1 (4)	0.32*	0.57**	1.23**	-4.26**	0.39**	0.26**	0.69**	-0.24*	$-0.22^{n.s}$	0.13*	-0.21*	-1.53**	
Id1.2(5)	-0.46**	-0.76**	-2.68**	-5.08**	-0.27**	-0.17**	-0.35**	0.22 <sup>n.s</sup>	-0.38 <sup>n.s</sup>	-0.12*	-0.71**	-1.65**	
My1.1 (6)	-0.09 <sup>n.s</sup>	-0.06 <sup>n.s</sup>	-1.96**	-1.7**	0.26**	0.21**	0.37**	1.22**	1.56**	0.11 <sup>n.s</sup>	-0.26*	-0.65**	
In1.1 (7)	-1.18**	-1.48**	-3.18**	-2.36**	-0.52**	-0.79**	-1.57**	-1.37**	-0.72**	-0.39**	-1.07**	-0.34 <sup>n.s</sup>	
In1.2 (8)	-0.76**	-1.15**	-0.38 <sup>n.s</sup>	1.52**	-0.31**	-0.47**	-0.62**	-0.79**	-0.5**	-0.31**	-0.43**	-0.04 <sup>n.s</sup>	
Ph1.1 (9)	0.18 <sup>n.s</sup>	0.15 <sup>n.s</sup>	1.51**	-0.92**	0.01 <sup>n.s</sup>	0.01 <sup>n.s</sup>	0.61**	0.69**	-0.31 <sup>n.s</sup>	0.19**	0.66**	-0.18 <sup>n.s</sup>	
My2.2 (10)	0.93**	0.74**	-2.18**	-0.25 <sup>n.s</sup>	0.15**	0.44**	0.45**	1.32**	-0.28 <sup>n.s</sup>	0.30**	0.04 <sup>n.s</sup>	0.77**	
SE (g)	0.09	0.07	0.30	0.58	0.02	0.02	0.04	0.09	0.01	0.01	0.04	0.16	
SE (gi-gj)	0.20	0.16	0.67	1.30	0.04	0.05	0.08	0.20	0.01	0.02	0.10	0.35	

Note: DAP- Days after planting .\* and \*\* non-significant, Significant at the 0.05 level and Significant at the 0.01 level respectively

Table 3. Quadratic components of GCA ability, SCA ability, narrow and broad sense heritability and ratio of GCA/SCA.

	1				5								
		Plant height				Collar d	liameter			No. of leaves			
	15DAP	30DAP	60DAP	90 DAP	15DAP	30DAP	60DAP	90DAP	15DAP	30DAP	60DAP	90DAP	
$\sigma^{2}_{SCA}$	2.75	2.11	29.43	124.64	0.18	0.33	1.44	7.26	0.02	0.11	2.57	9.19	
$\sigma^2_{GCA}$	0.09	0.44	3.41	5.48	0.04	0.08	0.42	0.10	0.02	0.03	0.22	0.62	
$\sigma^2_{D}$	11.01	8.44	117.72	498.55	0.72	1.33	5.77	29.04	0.07	0.45	10.27	36.77	
$\sigma^2_A$	0.34	1.76	13.64	21.94	0.15	0.32	1.70	0.42	0.06	0.13	0.87	2.50	
$\sigma_{e}^{2}$	3.54	2.91	11.96	23.33	0.71	0.81	1.50	3.58	0.25	0.41	1.75	6.35	
$h^2_{b}$	76.24	77.81	91.66	95.71	55.03	67.15	83.28	89.16	33.82	58.59	86.42	86.08	
$h_n^2$	2.31	13.45	9.52	4.03	9.43	13.07	18.90	1.26	16.18	12.79	6.77	5.48	
GCA/SCA	0.02	0.12	0.09	0.04	0.09	0.12	0.16	0.01	0.14	0.11	0.06	0.05	

Note: DAP- Days after planting, SCA variance ( $\sigma^2_{SCA}$ ), GCA variance ( $\sigma^2_{GCA}$ ), dominant variance ( $\sigma^2_D$ ), additive variance ( $\sigma^2_A$ ), broad sense heritability ( $h^2_b$ ), narrow sense heritability ( $h^2_n$ ).

was low for number of leaves and ranged from 5.45 to 16.18% (Table 3). Broad sense heritability was higher than that of narrow sense heritability. The heterosis for number of leaves ranged from 0.0 to 43.8% (Table S5). The highest heterosis for number of leaves were found in the cross between parents My2.1 (1) × In1.1 (7), Id1.2 (5) × In1.2 (8), My2.1 (1) × Id1.2 (5) and Ph1.2 (3)× In1.2 (8) at 15, 30 60 and 90 DAP, respectively. The heterobeltiosis of number of leaves ranged from 0.0 to 32.61% (Table S5). The highest heterobeltiosis value was obtained in hybrids My2.1 (1) × In1.1 (7), Id1.2 (5) × In1.2 (8), My2.1 (1) × In1.1 (7), Id1.2 (5) × In1.2 (8), My2.1 (1) × In1.1 (7), Id1.2 (5) × In1.2 (8), My2.1 (1) × Id1.2 (5) and My2.1 (1) × My2.2 (10) at four collecting intervals, respectively.

### Phenotypic correlation between characters

Phenotypic correlations between traits of hybrids are showed in the Table 4. Phenotypic correlations between plant heights were positive and highly significant with collar diameter and number of leave in most of the collecting intervals. Collar diameter also showed highly significant and positive relationship with number of leave in most of the cases.

### Discussion

Ten superior parents were crossed in a diallel manner to produce 45 hybrids. These hybrids and their parents were evaluated for combining abilities and heterosis in green house. Analyses of variance presented significant differences among parents and hybrids for plant height, collar diameter and number of leaves in nursery stage. In our study estimation of GCA and SCA effects were highly significant for plant height collar diameter and number of leaves in nursery stage except SCA for number of leaves after 15 days after planting. Estimates of GCA effects indicated that only one parent, namely Ph1.2 showed significant positive GCA effects for plant height, collar diameter and number of leaves at 15, 30, 60 and 90 days after planting, indicating that it is a good general combiner for these traits and should be used in breeding program. Analysis of specific combining ability in this study exhibited that some hybrids presented significant SCA effects for each trait. Hybrids My2.1  $\times$  My2.2, In1.1  $\times$ My2.2, Ph1.2× In1.2 and Ph1.2× In1.2 gave the higher positive significant SCA effects for plant height. According to Sprague and Tatum (1942) the SCA is controlled by non additive gene action. The SCA effect is an important criterion for the evaluation of crosses. Machikowa et al. (2011) reported that SCA were highly significant for plant height in sunflower and revealed that non additive effects were important for plant height. This reported had same trend with our study for plant height in the above hybrids. In our study, the low ratios of GCA/SCA were observed for plant height, collar diameter and number of leaves. Vaghela et al. (2011) also reported the variance due to SCA was higher than that of variance due to GCA for some characters. This revealed the role of non additive gene action in the inheritance of these traits. This result is in agreement with our study. Rego et al. (2009) and reported low GCA/SCA ratio for pepper plant canopy width and show non additive effects is more important than additive effects. Tchiagam et al. (2011) reported that the values of GCA /SCA ratios and the variance components showing the preponderance of SCA for studied traits, demonstrated the higher influence of non additive gene effects in cowpea. High broad sense heritability was observed for plant height, collar diameter and number of leaves in present study. Ginwal et al. (2005) presented high broad sense heritability for plant height after 6 and 24 months-old

plants of Jatropha. Ginwal et al. (2004) observed an increase in heritability related to height of the same plants from one year to another. Rao et al. (2008) also showed high broad sense heritability (88%) for plant height in 34 month old plants. The high values of broad sense heritability were associated with higher selective precision, revealing the possibility of high accuracy in the selection. Cilas et al. (1998) reported that broad sense heritability values for collar diameter is significantly greater than zero and greater than the narrow sense heritability which is not significantly greater than zero for coffee tree (Coffea Arabica). Genotypic variance was observed very close to phenotypic variance accompanied by high broad sense heritability in collar diameter of J. curcas under nursery condition (Ginwal et al., 2005). High broad sense heritability was computed by Tchiagam et al. (2010) indicated that phenotypic selection may be effective and that there may be little or no cropping system effect. In the present study, low narrow sense heritability was observed for plant height, collar diameter and number of leaves. The low narrow sense heritability of some traits also was reported in other crops also (Peyman et al., 2012). On the other hand, high estimates of broad sense heritability for these traits revealed that other types of genetic effects such as dominance or epitasis might be involved in their variation (Bolanos-Aguilar et al., 2001; Pahlavani et al., 2007) The high heterosis and heterobeltiosis values were observed in our study. Several researchers observed significant positive heterotic effects involving mid-parent and high parent heterosis for studied traits but for different number of crosses of rapeseed. Parental genotypes with best GCA and its utilization as one of the parents produced superior hybrid combinations having valuable SCA determination for seed oil yield. The result of this study could be an excellent indicator to recognize the most promising genotypes to be exploited either as F1 hybrids or as a resource population for further selection in J. curcas improvement.

Parents Ph1.2 and In2.1 had positive GCA effect for plant height, collar diameter and number of leaves. Finally, the hybrids Ph1.2× In1.2, Ph1.1 × My2.2 and My2.1× My2.2 showed positive SCA effects for these characters. Heterosis and heterobeltiosis were found higher for those characters. Therefore, these parents and hybrids could be used for future breeding program. To our knowledge, this is first report about diallel mating design in *J. curcas*. Therefore, the outcomes of this part of the study could not compare to any other sources in the Jatropha research activities.

### Material and methods

### Description of the experimental site

The study was conducted in the experimental field of Universiti Putra Malaysia in 2010. The experimental site is located at 3.0059 N, 101.71655 E and at an altitude of 88 meter above sea level. The area receives 2429 mm rainfall per year. The monthly average minimum and maximum temperature of UPM ranged from 21-23°C and 31-33°C, respectively.

### Parental lines and crossing scheme

Ten superior plants were selected based on seed yield and oil content for a half diallel cross from six *J. curcas* populations after one year evaluation in the farm (Table 5). The superior plants were crossed in a half-diallel mating design  $(10 \times 10)$ . A half diallel cross were produced 45 hybrids. The

Table 4. Coefficients of phenotypic correlation between traits of Jatropha in diallel crosses involving ten parents in nursery stage.

Т	Traits Plant height				Collar o	liameter	No. of leaves					
		15DAP	30DAP	60DAP	90DAP	15DAP	30DAP	60DAP	90DAP	15DAP	30DAP	60DAP
Plant height	30 DAP 60 DAP 90 DAP	0.81** 0.23 <sup>ns</sup> 0.07 <sup>ns</sup>	0.35** 0.14 <sup>ns</sup>	0.75**								
Collar diameter	15 DAP 30 DAP 60 DAP 90 DAP	0.75** 0.74** 0.13 <sup>ns</sup> 0.03 <sup>ns</sup>	0.61** 0.71** 0.22 <sup>ns</sup> 0.06 <sup>ns</sup>	0.14 <sup>ns</sup> 0.08 <sup>ns</sup> 0.47** 0.38**	-0.01 <sup>ns</sup> -0.01 <sup>ns</sup> 0.34** 0.61**	0.83** 0.08 <sup>ns</sup> -0.01 <sup>ns</sup>	0.12 <sup>ns</sup> 0.04 <sup>ns</sup>	0.22 <sup>ns</sup>				
No. of leaves	15 DAP 30 DAP 60 DAP 90 DAP	0.53** 0.55** 0.07 <sup>ns</sup> 0.14 <sup>ns</sup>	0.53** 0.64** 0.2 <sup>ns</sup> 0.15 <sup>ns</sup>	0.04 <sup>ns</sup> 0.35** 0.70** 0.66**	-0.1 <sup>ns</sup> 0.27* 0.72** 0.89**	0.52** 0.45** 0.03 <sup>ns</sup> 0.11 <sup>ns</sup>	0.57** 0.56** 0.06 <sup>ns</sup> 0.01 <sup>ns</sup>	0.22 <sup>ns</sup> 0.07 <sup>ns</sup> 0.38** 0.19 <sup>ns</sup>	-0.06 0.40** 0.60** 0.55**	0.32* 0.02 <sup>ns</sup> -0.07 <sup>ns</sup>	0.39** 0.25 <sup>ns</sup>	0.65**
	Note: DAD days after planting <sup>B.S.</sup> * and ** non significant significant at the 0.05 level and significant at the 0.01 level respectively.											

Note: DAP - days after planting; <sup>n.s</sup>, \* and \*\* non-significant, significant at the 0.05 level and significant at the 0.01 level, respectively.

Table 5. Code and origin of ten superior plants accession (based on higher seed yield and oil content).

Parents no.	Parents name	Parents origin
1	My2.1	Malaysia
2	In2.1	Indonesia
3	Ph1.2	Philippines
4	Id1.1	India
5	Id1.2	India
6	My1.1	Malaysia
7	In1.1	Indonesia
8	In1.2	Indonesia
9	Ph1.1	Philippines
10	My2.2	Malaysia

Table 6. Expected mean square and genetic interpretation of a half diallel.

Source of variation	Degrees of freedom	Error mean square
Replications	(r-1)	$\sigma_e^2 + g\sigma_r^2$
Genotypes	${[n(n-1)/2]+n}-1$	$\sigma_{e}^{2} + r\sigma_{g}^{2}$
GCA	[n-1]	$\sigma^{2}_{e} + r\sigma^{2}_{SCA} + r(n+2) \sigma^{2}_{GCA}$
SCA	[n(n-1)/2]	$\sigma_{e}^{2} + r\sigma_{SCA}^{2}$
Error	$(r-1) \{\{[n(n-1)/2]+n\}-1\}$	$\sigma_{e}^{2}$

Where: r and n refer to number of replications and parents per diallel respectively.

pollinations were carried out shortly after emasculation by using fresh pollen collected from male parents and then the pollinated female flower were covered for protecting from other pollens. Two inflorescences with about five female flowers each was employed for making cross.

#### Field experimental design and data collection

All nine hundred ninety seeds of parental accessions and hybrids (Eighteen seeds from each parental accessions and hybrids) were planted in polyethylene bags to produce seedlings. Polyethylene bags filled with soil mixture (clay, sand and organic matter in a 1:1:1 ratio). After planting, the seedbed was prepared carefully and a small amount of N, P and K fertilizers were applied in the soil. The seedbed was watered for ensuring the germination of seeds. Experiment was conducted using a randomized complete block design with three replications. For morphological traits, data on plant height, collar diameter and number of leaves was collected at 15, 30, 60 and 90 days after planting (DAP).

# Statistical analysis

For morphological traits, analysis of variance was conducted to determine significance of variability among the parents and hybrids. Mean, range, standard deviation and coefficient of variation for each characteristic was determined using SAS version 9.1 (SAS Institute Inc, 2005). Parental accessions were selected from different Jatropha populations. Diallel analysis was performed to calculate general combining ability (GCA) and specific combining ability (SCA) according to the Griffing's (1956) method 2, model 1, using SAS program (GLM procedure) (Zhang et al., 2005).

The below model was considered for statistical analysis in this experiment:

$$\begin{split} \hat{Y_{ijk}} &= \hat{\mu} + G_i + G_j + S_{ij} + R_k + E_{ijk} \\ \text{Where: } Y_{ijk} &= \text{the observed value for a hybrid between the } ith \end{split}$$
and *j*th parents in the *k*th replication;  $\mu$  = population mean; G<sub>i</sub> and  $G_i$  = GCA effect of the *i*th and *j*th parents;  $S_{ii}$  = SCA effect for the hybrid between the *i*th and the *j*th parents;  $R_k$ = effect of the kth replication;  $E_{iik}$  = the error associated with

the  $ijk^{th}$  hybrid (the residual) (Johnson and King, 1998). The expected mean squares and genetic interpretations of a half diallel design are presented in Table 6.

Estimation of the additive and dominance genetic variance is simple after estimating the GCA variance ( $\sigma^2_{GCA}$ ) and SCA variance ( $\sigma^2_{SCA}$ ) (Zhang *et al.*, 2005). The relationships are as follows:

When inbreeding coefficient (F) of parents = 0 (no inbreeding)

 $\sigma_{A}^{2} = 4\sigma_{GCA}^{2}$ 

 $\sigma_D^2 = 4\sigma_{SCA}^2$ 

Heterosis and heterobeltiosis were computed as the percentage of superiority of the hybrid over its mid parent value (MP%) or better parent value (BP%), respectively. Heritability expresses the proportion of the total variance that is attributable to the average effects of genes. Broad and narrow sense heritability was estimated based on the variance components in the ANOVA Table 6.

Broad sense heritability values were obtained using GCA and SCA values.

 $H^{2}b = \sigma_{g}^{2} / \sigma_{p}^{2}$  Where:  $H^{2}b =$  Heritability;  $\sigma_{g}^{2} =$  Total genetic variance=  $4\sigma_{GCA}^{2} + 4\sigma_{SCA}^{2}$  and  $\sigma_{p}^{2} =$  Phenotypic variance =  $4\sigma_{GCA}^{2} + 4\sigma_{SCA}^{2} + \sigma_{E}^{2}$ 

Narrow sense heritability was computed from the variance components in the ANOVA for the analysis of combining ability (Table 6).

 $h_N^2 = 4\sigma_{GCA}^2 / 4\sigma_{GCA}^2 + 4\sigma_{SCA}^2 + \sigma_E^2$  Where:  $h_N^2 = narrow$  sense heritability;  $4\sigma_{GCA}^2 = variance$  due to GCA;  $4\sigma_{SCA}^2 = variance$  due to SCA and  $\sigma_E^2 = variance$  due to residual error. Correlation mentioned to a change in one trait that is accompanied by a change in another. Phenotypic correlation was obtained from the following formula:

 $\begin{array}{l} r_p=\ cov_p\ /(\sigma_{p(x)}\ \sigma_{p(y)}) \\ \text{Where:}\ r_p\ =\ phenotypic\ correlation \\ \text{between traits } x\ and\ y;\ cov_p\ =\ phenotypic\ covariance\ and\ \sigma_{p(x)} \\ \sigma_{p(y)}\ =\ the\ root\ of\ the\ genetic\ variance\ of\ x\ and\ y\ respectively. \\ Phenotypic\ correlation\ was\ computed\ from\ the\ combined \\ analysis\ data\ based\ on\ genotype\ values\ over\ environment\ and \\ replications. \end{array}$ 

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### References

- Achten W, Verchot L, Franken YJ, Mathijs E, Singh VP, Aerts R, Muys B (2008) Jatropha bio-diesel production and use. Biomass Bioenergy 32(12): 1063-1084
- Amiri Oghan H, Shariati F, Asadi M, Tusi F, Kamyab F (2007) Study of heterosis and combining ability of yield and yield components in spring rapeseed. Seed and Plant Improvement Institute, Iran
- Basha S, Francis G, Makkar H, Becker K, Sujatha M (2009) A comparative study of biochemical traits and molecular markers for assessment of genetic relationships between *Jatropha curcas* L. germplasm from different countries. Plant Sci. 176(6): 812-823
- Basha S, Sujatha M (2007) Inter and intra-population variability of *Jatropha curcas* (L.) characterized by RAPD and ISSR markers and development of population-specific SCAR markers. Euphytica 156(3): 375-386
- Cilas C, Bouharmont P, Boccara M, Eskes A, Baradat P (1998) Prediction of genetic value for coffee production in Coffea arabica from a half-diallel with lines and hybrids. Euphytica 104(1): 49-59

- Bolanos-Aguilar E, Huyghe C, Djukic D, Julier B, Ecalle C 2001) Genetic control of alfalfa seed yield and its components. Plant Breeding 120(1): 67-72
- Divakara B, Upadhyaya H, Wani S, Gowda C (2010) Biology and genetic improvement of *Jatropha curcas* L.: A review. Appl Energ 87(3), 732-742
- Fangrui M, Milford AH (1999). Biodiesel production: a review. Bioresource Techno, 70(1), 1-15.
- Ganesh Ram S, Parthiban K, Senthil Kumar R, Thiruvengadam V, Paramathma M (2008) Genetic diversity among Jatropha species as revealed by RAPD markers. Genetic Resour Crop Ev 55(6): 803-809
- Ghosh L, Singh L (2011) Variation in seed and seedling characters of *Jatropha curcas* L. with varying zones and provenances. Trop Ecol 52(1): 113-122
- Ginwal H, Phartyal S, Rawat P, Srivastava R (2005) Seed source variation in morphology, germination and seedling growth of *Jatropha curcas* Linn. in central India. Silvae Genét 54(2): 76-79
- Ginwal H, Rawat P, Srivastava R (2004) Seed source variation in growth performance and oil yield of *Jatropha curcas* Linn. in central India. Silvae Genét 53(4): 186-191
- Gohil R, Pandya J (2008) Genetic diversity assessment in physic nut (*Jatropha curcas* L.). Int J Plant Prod 2(4): 321-326
- Griffing B (1956) Concept of general and specific combining ability in relation to diallel crossing systems. Aust J Biol Sci 9(4): 463-493
- Heller J (1996) 'Physic nut, *Jatropha curcas* L: Promoting the conservationaluse of underutilized and neglected crop.' (Bioversity International: Rom, Italy: International Plant Genetic Resources Institute (IPGRI).
- Johnson G, King J (1998) Analysis of half diallel mating designs. Silvae Genet 47: 74-79
- Kaushik N, Kumar S (2004) *Jatropha curcas* L. Silviculture and Uses. (Agrobios: India, Jodhpur)
- Kumar A, Sharma S (2008) An evaluation of multipurpose oil seed crop for industrial uses (*Jatropha curcas* L.): A review. Ind Crop Prod 28(1): 1-10
- Machikowa T, Saetang C, Funpeng K (2011) General and Specific Combining Ability for Quantitative Characters in Sunflower. J Agr Sci 3(1): p91
- Mukherjee P, Varshney A, Johnson TS, Jha TB (2011) *Jatropha curcas*: a review on biotechnological status and challenges. Plant Biotech Rep: 1-19
- Ovando-Medina I, Espinosa-García F, Núñez-Farfán J, Salvador-Figueroa M (2011) State of the art of genetic diversity research in *Jatropha curcas*. Sci Res Es 6(8): 1709-1719
- Pahlavani M, Saeidi G, Mirlohi A (2007) Genetic analysis of seed yield and oil content in safflower using F1 and F2 progenies of diallel crosses. Int J Plant Prod 1: 129-140
- Peyman S., Safari Motlagh M. R. and Aminpanah H. (2012) Diallel analysis for salinity tolerance in rice traits at germination stage. African J of Biotechnol 11 (14): 3276-3283.
- Rafii MY, Shabanimofrad M, Wahab PEM, Latif MA (2012a) Analysis of the genetic diversity of physic nut, *Jatropha curcas* L. accessions using RAPD markers. Mol Biol Rep 39 (6):6505-6511
- Rafii MY, Arolu IW, Omar MHA, Latif MA (2012b) Genetic variation and heritability estimation in *Jatropha curcas* L.for seed yield and vegetative traits. J Med Plants Res 6: 2178-2183

- Rameeh V (2011a) Heritability and other genetic parameters assessment for flowering associated stress indices in oil seed rape varieties. Int J Plant Breeding Genet 5(3): 268-276
- Rameeh V (2011b) Heterosis and Combining Ability Assessment for Phenological Traits, Plant Height and Grain Yield in Winter×Spring Combinations of Rapeseed Varieties. Int J Plant Breeding Genet 5: 349-357
- Rao G, Korwar G, Shanker AK, Ramakrishna Y (2008) Genetic associations, variability and diversity in seed characters, growth, reproductive phenology and yield in *Jatropha curcas* (L.) accessions. Trees-structfunct 22(5): 697-709
- Rêgo ER, Do-Rêgo MM, Finger FL, Cruz CD, Casali VWD. (2009) A diallel study of yield components and fruit quality in chilli pepper (*Capsicum baccatum*). Euphytica, 168(2): 275-287
- Shabanimofrad M, Yusop MR, Saad MS, Megat Wahab PE, Biabanikhanehkahdani A, Latif MA (2011). Diversity of physic nut, *Jatropha curcas* in Malaysia–application of DIVA-GIS and cluster analysis. Aust J Crop Sci 5(4):361-368
- SAS Institute Inc (2005) SAS/STAT<sup>®</sup> Users Guide. Version 9.1. SAS Institute Inc. Cary, NC.
- Shah S, Sharma A, Gupta M (2004) Extraction of oil from *Jatropha curcas* L. seed kernels by enzyme assisted three phase partitioning. Ind Crop Prod 20(3): 275-279

- Sharma S, Kumar N, Reddy MP (2011) Regeneration in *Jatropha curcas*: Factors affecting the efficiency of in vitro regeneration. Ind Crop Prod 34:943-951
- Sharma S, Sudheer Pamidimarri D, Vijay Anand K, Reddy MP (2011) Assessment of genetic stability in micropropagules of *Jatropha curcas* genotypes by RAPD and AFLP analysis. Ind Crop Prod 34: 1003-1009
- Singh A, Reddy MP, Chikara J, Singh S (2010) A simple regeneration protocol from stem explants of *Jatropha curcas*-A biodiesel plant. Ind Crop Prod 31(2): 209-213
- Sprague G, Tatum LA (1942) General vs. specific combining ability in single crosses of corn. J Am Soc Agron 34(10): 923-932.
- Tchiagam JBN, Bell JM, Nassourou AM, Njintang NY, Youmbi E (2011) Genetic analysis of seed proteins contents in cowpea (*Vigna unguiculata* L. Walp.). Afr J Biotech 10(16): 3077-3086
- Vaghela P, Thakkar D, Bhadauria H, Sutariya D, Parmar S, Prajapati D (2011) Heterosis and combining ability for yield and its component traits in Indian mustard [*Brassica juncea* (L.)]. J Oilseed Brassica 2(1): 39-43
- Zhang Y, Kang MS, Lamkey KR (2005) Diallel-SAS05: a comprehensive program for griffing's and gardner-eberhart analyses. Agron J 97(4): 1097