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Germination Behaviour of 6 × 6 Diallel Hybrids of an Energy Crop *Jatropha curcas* L.

(Kelakuan Percambahan Hibrid Dialel 6 × 6 Tanaman Penghasil Tenaga *Jatropha curcas* L.)

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ABSTRACT

The seeds of 6 × 6 half diallel progenies of Jatropha curcas *were used to evaluate the effects of genotypes (parents and their hybrids) on germination traits of* jatropha *at Universiti Kebangsaan Malaysia. Germination traits were varied significantly* (p*<0.01) among the seeds of hybrids and their parents. The germination was started at four days after planting and prolonged until 15 days. Seed germination varied from 58.06 to 92.76% among the parents and 53.43 to 98.96% among the hybrids. The highest germination was observed in the hybrid* $P_2 \times P_4$ *and none of the hybrid or parent showed complete (100%) germination. The maximum* GI *(germination index) and* SVI *(seedling vigour index) were found in the hybrids* $P_1 \times P_5$ *and* $P_1 \times P_2$ *and the lowest in* $P_2 \times P_4$ *and* $P_3 \times P_6$*, respectively. For most germination parameters parents behaved poorly than that of the hybrids.*

Keywords: Biofuel; diallel; germination index; hybrid; seedling vigour

ABSTRAK

Biji benih progeni dialel separa 6 × 6 telah digunakan untuk menilai kesan genotip (induk dan hibridnya) terhadap ciri percambahan Jatropha curcas *di Universiti Kebangsaan Malaysia. Ciri percambahan didapati berbeza secara bererti (*p*<0.1) pada biji benih hibrid dan induknya. Percambahan dimulakan pada hari ke empat selepas penyemaian dan dibiarkan sehingga 15 hari. Percambahan biji benih adalah berbeza-beza dari 58.06 hingga 92.76% antara induk dan dari 53.4 hingga 98.96% antara hibrid. Percambahan tertinggi dicerap pada hibrid* $P_2 \times P_4$ *dan tiada percambahan 100% ditunjukkan oleh hibrid dan induk. Indeks percambahan* GI *dan indeks kecergasan anak benih* SVI *didapati paling tinggi masing-masing pada* $P_1 \times P_5$ *dan* $P_1 \times P_2$ *dan paling rendah pada* $P_2 \times P_4$ *and* $P_3 \times P_6$*. Kebanyakan parameter percambahan pada induk menunjukkan nilai yang kurang baik berbanding hibridnya.*

Kata kunci: Bahan api biologi; dialel; hibrid; indeks percambahan; kecergasan anak benih

INTRODUCTION

Jatropha belongs to the family of Euphorbiaceae, a non-edible oil seed plant which can be found in Central and South America, Africa, Southern Asia, and Central-Southern Peninsular Asia. *Jatropha* is a bushy plant growing up to a height of around 6 m (Grimm 1996; Heller 1996; Rockerfeller Foundation 1998). It has low requirements to soil quality and can grow under low rainfall conditions (Münch & Kiefer 1989). The seeds contain about 30% of uneatable oil (Heller 1996). *Jatropha curcas* L. is a multi-use plant with a number of features and gets attention all over the world as a prospective biofuel crop (Martin & Mayeux 1985; Openshaw 2000; Takeda 1982). Among the potential sources identified, it appears as the most potential oilseed producing plant due to its adaptability to a broad range of soil and environment (Kureel 2006; Martin & Mayeux 1985). Crop improvement through agronomic management in this species is inadequate (Dehgan 1984; Sujatha & Prabakaran 1997).

Jatropha is normally propagated through seeds. Seed germination of *Jatropha* is very much unpredictable and varies at 10-95%. The germination quality of seed is one of the most essential factors in the production system of the crop and particular conditions of genotype and environment are required to meet high seed quality (Arnold et al. 1984). Genetic and environmental factors determine the rate and speed of germination, strength of seed and seedlings (Hartmann et al. 1990). Germination capability of seed is not always associated with environmental factors (Bevington 1986; Martin et al. 1995). Several researches have established that unpredictability in germination capacity might be due to genetic factors (Hacker et al. 1984; van der Vegte 1978) and occurs in three levels: within individuals (Gutterman 1992), within populations (Hardin 1984; Pe´rez-Garcı´a 1997) or among populations (McGee & Marshall 1994). The germination traits have been found to be a complicated and its inheritance varied among the crops. There are very few reports available on germination behaviour in *Jatropha*. Diallel cross analyses leads to a fruitful result for identification of better genotypes and F_1 hybrids performing better seed germination. The main objective of the present study was to find out the genotypic effect on germination traits and to identify high heterotic combination in order to develop hybrid variety with good seed germination quality.

Materials and Methods

Germination experiment was carried out at Glass House, Faculty of Science and Technology, Universiti Kebangsaan Malaysia. Seeds of 6 ´ 6 half diallel population of *J. curcas* including six parents (P_1= CPP06, P_2= CPP18, P_3= CPP01, P_4= CPP05, P_5= CPP07 and P_6= CPP04) and their 15 hybrids were used as experimental materials. To assess the effects of genotypes (parents and their hybrids) on germination traits, the seeds of 15 F_1 progenies including their six parents were sown directly in the poly bag (18 × 10 × 7 cm) containing a mixture of soil and compost (made from kitchen waste) in the ratio 1: 1. Fifteen seeds were sown directly in the polybag to a depth of 3 cm (Henning 2000) in each replication and watered to saturation three times a week. The experiments were directed in a CRD (completely randomized) design with three-fold replication. Data were recorded on several germination traits in 24 h intervals and carried on until no more germination occurred. Seed germination standard was followed with the visible enlargement (at least 0.5 cm) of the cotyledon and hypocotyls of the seedlings on the surface of the soil. Seedling evaluation was done according to the procedure described in Handbook of Association of Official Seed Analysis (AOSA 1991, 1983).

The following equation of Ellis and Roberts (1981) was used to calculate mean germination time (MGT):

$$\text{MGT} = \Sigma\ Dn / \Sigma n$$

where n is the number of seed germinated on day D; and D is the number of days counted from the beginning of germination.

Germination index (GI) was estimated by using the formula described in the Association of Official Seed Analysts (AOSA 1983):

GI = `å´ (GT / Tt) or [(number of seed germinated / days of first count) + ……….+ (number of seed germinated / days of last count)]

Time to 50% germination (T_{50}) was estimated by using the formula described by Coolbear et al. (1984) adapted by Farooq et al. (2006):

$$T_{50} = [ti + \{(N/2) - ni\}\ (ti - tj)] / ni - nj.$$

where N is the number of seed germinated in final count and n_i; n_j cumulative number of germinated seed by adjacent counts at times t_i; and t_j when n_i<N/2< n_j.

Seedling vigor index (SVI) was estimated by using the following formula:

SVI = [seedling length (cm) × percentage of germination /100].

Speed of emergence was estimated by using the following formula:

Speed of emergence = (number of seedlings emerged 5 days after sowing / number of seedlings emerged 15 days after sowing) × 100.

Energy of germination was determined as percent of germination five days after planting relative to total number of tested seeds (Ruan et al. 2002). Percentage of germination and length of seedling was recorded after 15 days of seed sowing (Dezfuli et al. 2008). Data of germination percentage was transformed to arcsin√(100/X) for statistical analysis; actual percentage were shown. The experimental data was analyzed by a statistical package SAS, version 9.01 (SAS 2008). Means of treatments were separated by using Tukey's test (Steel & Torrie 1980).

Results

The present investigation was carried out to evaluate the effect of the parents and hybrids of *J. curcas* on germination behavior and seedling development. The analysis of variances showed highly significant (p<0.01) differences for all the characters studied, indicating the existence of wider variability among the parental genotypes and their hybrids of *J. curcas* for different germination parameters (Table 1). The response of parents and their hybrids on germination traits are described below:

NUMBER OF DAYS TAKEN FOR FIRST GERMINATION

The earliest germination was observed in the parent P_1 (4.00 days) and the hybrid $P_4 \times P_5$ (4.00 days) and delayed germination was found in P_5 and $P_4 \times P_6$ (Table 2). Seed germination started four days after seed sowing and prolonged up to seven days among the parents and hybrids (Table 2).

GERMINATION PERCENTAGE

The highest germination percentage (98.96%) was observed in the hybrid $P_1 \times P_4$ (Table 2) followed by the hybrid $P_1 \times P_2$ (96.46%). The lowest germination percentage (53.43%) was recorded from the hybrid $P_5 \times P_6$ (Table 2).

SPEED OF EMERGENCE

The highest speed of emergence (70.67) was observed in the parent P_1 and the lowest (4.80) in P_6 (Table 2). The hybrids showed higher speed of emergence in compare to their parents ranging from 3.73 to 69.47 (Figure 1). The highest speed of emergence was found in the hybrid $P_1 \times P_5$ and the lowest in the hybrid $P_2 \times P_6$.

TABLE 1. Analysis of variance for nine germination traits of 6 × 6 diallel populations of *J. curcas*

Sources of variations	df	NDTFG	GP	SE	GE	GI
Replication	2	0.40ns	10.13ns	1.86ns	0.23ns	0.07ns
Genotypes	20	2.45**	671.14**	1440.75**	1451.44**	9.02**
Parents (P)	5	17.22**	17.22**	17.22**	17.22**	17.22**
Hybrids (F_1s)	14	19.67**	19.67**	19.67**	19.67**	19.67**
P vs F_1	1	312.44**	361.24**	453.56**	667.26**	181.21**
Error	40	0.30	5.63	1.90	0.81	0.07

Sources of variations	df	MGT	T50	SL	SVI
Replication	2	0.11ns	0.08**	3.63ns	1.35ns
Genotypes	20	6.48**	4.41**	31.02**	86.87**
Parents (P)	5	17.22**	17.22**	17.22**	17.22**
Hybrids (F_1s)	14	19.67**	19.67**	19.67**	19.67**
P vs F_1	1	231.97**	273.33**	258.89**	375.8**
Error	40	0.09	0.02	2.11	1.64

*, $p<0.05$; **, $p<0.01$

NDFG = Number of days to first germination, SE = Speed of Emergence, GP = Germination Percentage (%), GE = Germination Energy, GI = Germination Index, MGT = Mean Germination Time, T_{50}= Time of 50% germination, SL = Seedling Length (cm) and SVI = Seedling Vigor Index

TABLE 2. Performance of the parents and hybrids of 6 × 6 diallel population of *J. curcas* for different germination traits

Genotypes	NDTFG	GP	GI	MGT	T50
Parents					
P_1	4.00	67.23	6.00	7.26	5.00
P_2	5.33	66.70	3.53	9.73	5.00
P_3	5.00	92.76	5.20	8.85	4.13
P_4	4.33	90.33	5.76	8.25	2.72
P_5	7.67	58.06	2.78	9.83	5.08
P_6	6.67	70.33	2.89	10.18	5.22
Hybrids					
$P_1 \times P_2$	4.67	96.46	7.63	6.55	2.00
$P_1 \times P_3$	5.33	82.10	5.61	8.50	2.00
$P_1 \times P_4$	4.33	82.60	4.66	8.84	4.00
$P_1 \times P_5$	4.33	93.10	8.63	6.14	1.80
$P_1 \times P_6$	4.33	86.96	4.37	8.51	4.78
$P_2 \times P_3$	5.33	96.40	5.21	12.48	4.59
$P_2 \times P_4$	5.00	98.96	2.31	8.27	4.50
$P_2 \times P_5$	5.00	86.90	6.35	6.51	2.21
$P_2 \times P_6$	5.00	93.00	4.36	8.61	5.00
$P_3 \times P_4$	5.00	56.10	2.63	9.25	5.00
$P_3 \times P_5$	5.00	80.33	4.19	8.00	4.73
$P_3 \times P_6$	5.33	56.23	2.59	9.56	5.00
$P_4 \times P_5$	4.00	90.00	3.83	10.49	5.00
$P_4 \times P_6$	6.33	80.66	3.73	9.15	5.23
$P_5 \times P_6$	4.33	53.43	2.46	9.75	4.64
MSD (0.05)	1.69	7.39	0.80	0.91	0.46

Means with the same letter are not significantly different, MSD = Minimum Significant Difference

GERMINATION ENERGY

The highest germination energy (53.44) was observed in the parent P_4 and the lowest (13.27) in P_5 (Table 2). The hybrid $P_1 \times P_5$ had the highest (80.00) germination energy followed by the hybrid $P_1 \times P_2$ (76.22). The lowest germination energy (13.44) was recorded from the hybrid $P_3 \times P_4$ (Figure 2).

GERMINATION INDEX

Maximum germination index (6.00) was found in the parent P_1 and the lowest (2.78) in P_5 (Table 2). The hybrids showed higher germination index in compare to their parents ranging from 2.31 to 8.63 (Table 2). The highest germination index was found in the hybrid $P_1 \times P_5$ and the lowest in the hybrid $P_2 \times P_4$.

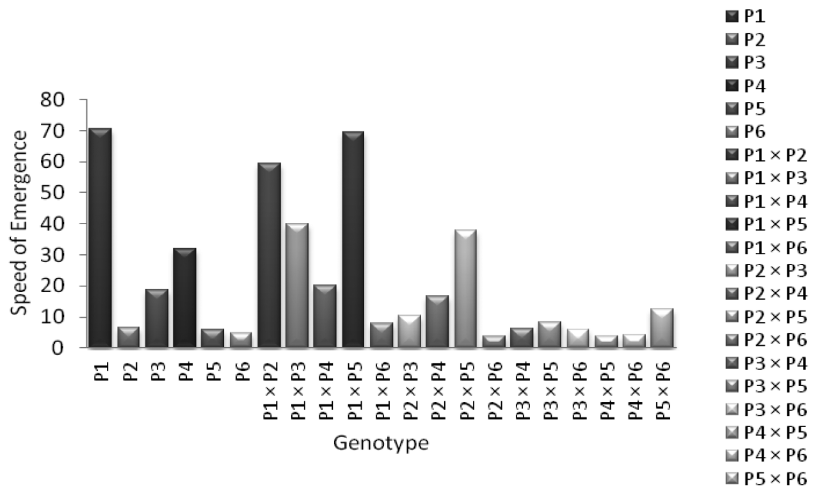

FIGURE 1. Effect of genotypes (parents and hybrids) on speed of emergence of *J. curcas* seed

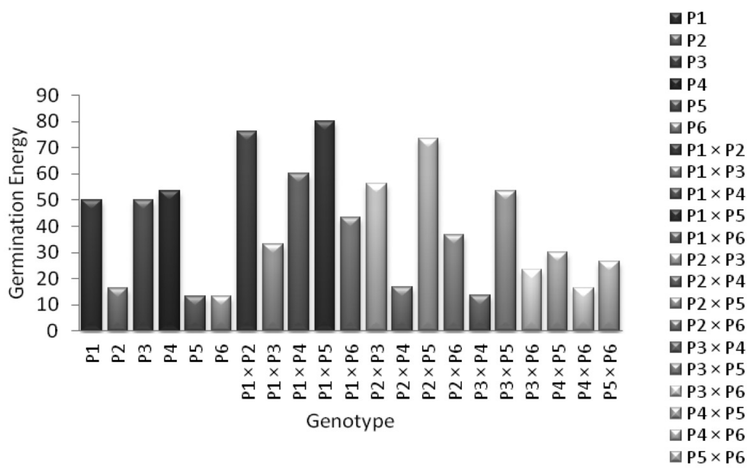

FIGURE 2. Effect of genotypes (parents and hybrids) on germination energy of *J. curcas* seed

MEAN GERMINATION TIME

The highest mean germination time was observed 10.18 days after first germination in the parent P_6 and the lowest (7.26) in P_1 (Table 2). The lowest mean germination time (6.14) was found in the hybrid $P_1 \times P_5$ and the highest (12.48) in $P_2 \times P_3$. First seed germination was taken place six days after sowing and prolonged up to 12 days among the parents and hybrids. The lowest mean germination time was counted in the hybrids $P_1 \times P_2$, $P_1 \times P_5$ and $P_2 \times P_5$ (Table 2).

TIME OF 50% GERMINATION

Higher value of T_{50} indicates late germination of seed whereas lower value of T_{50} indicates earlier germination and the highest germination rate. The lowest T_{50} value (2.72) was observed in the parent P_4 while the highest value (5.22) was observed in the parent P_6 (Table 2). The lowest T_{50} value (1.80) was found in the hybrid $P_1 \times P_5$ and the highest (5.23) in $P_4 \times P_6$. Most of the hybrids showed lower T_{50} value compared with their parents (Table 2).

SEEDLING LENGTH

Out of six parents, P_1 produced the taller seedling than the other parents (Table 2). The tallest (33.33 cm) seedling was achieved in the hybrid $P_2 \times P_5$ followed by $P_6 \times P_6$ (Figure 3) while the shortest (19.66 cm) seedling was in $P_1 \times P_4$.

SEEDLING VIGOUR INDEX

The highest seedling vigour index (6.00) was observed in the parent P_1 and the lowest (2.78) in P_5 (Table 2). The hybrids showed higher seedling vigour index in compare to their parents ranging from 2.31 to 8.63 (Figure 4). The highest seedling vigour index was found in the hybrid $P_1 \times P_5$ and the lowest in $P_2 \times P_4$.

DISCUSSION

The present research was accomplished to estimate the effect of genotype on seed germination behaviour of *J. curcas*. The mean sum of squares was due to genotypes. The parents and hybrids were highly significant for all

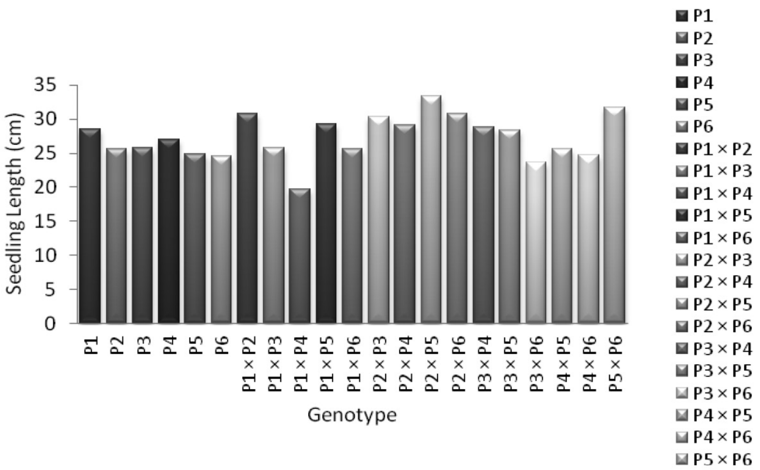

FIGURE 3. Effect of genotypes (parents and hybrids) on seedling length (cm) of *J. curcas* seed

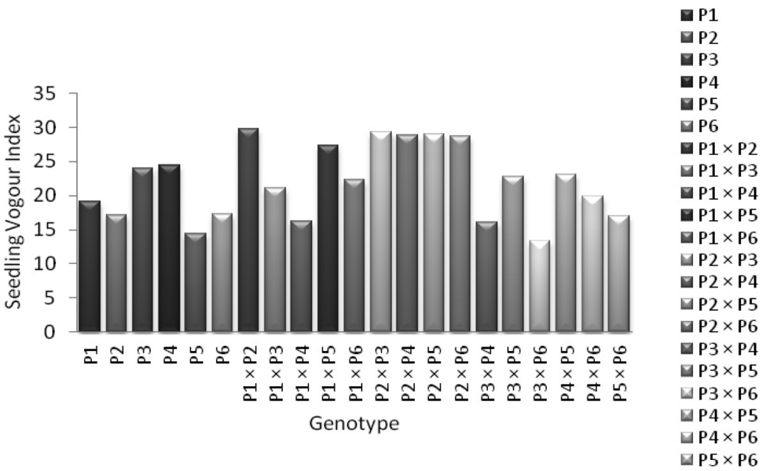

FIGURE 4. Effect of genotypes (parents and hybrids) on seedling vigour index of *J. curcas* seed

the germination traits showing that different genotypes had various effects on seed germination of *J. curcas*. Seed germination commenced five days after sowing and prolonged up to eight days. The maximum percentage of germination (98.96%) was observed in the hybrid $P_1 \times P_4$ and the lowest germination percentage was recorded for the hybrid $P_5 \times P_6$. Germination of *J. curcas* seed varies from 10 to 95% and the low germination might due to physical exogenous dormancy (Holmes et al. 1987; Islam et al. 2009; Nikolaeva 1982) and also the existence of other types of dormancies such as chemical dormancies varying from one genotype to others. The seed dormancy can be broken by physical or chemical treatments (Sadhu & Kaul 1989). The highest speed of emergence was observed in the parent P_1 and the lowest in P_6. The hybrids showed higher speed of emergence as compared with their parents and it was due to heterotic effect of combination of two parental genes. Seed germination is partly related to genetic factors associated with the physiological activity of seeds (Natarajaratnam et al. 1987) and it shows variable response between genotypes (Grange et al. 2003). The highest germination energy, germination index, seedling length and seedling vigour index was observed in the hybrid compared with their parents. Seed germination, emergence and vigour are also controlled by genetic factor (Bewley & Black 1982) and there are evidences on genotypic variation in seedling growth (Islam et al. 2013; Kumar et al. 1989; Pekşen et al. 2004). As a main criterion for rapid germination, the lowest time for MGT and T_{50} was encountered in the hybrids and comparatively higher in the parental genotypes. Out of six parents, P_1 produced the tallest seedling than the other parents. The tallest seedling was achieved in the hybrid $P_2 \times P_5$. The hybrids were found more effective in respect of faster germination, high percentage of germination, germination index, seedling vigour index, speed of germination and energy of germination compared to their parents.

CONCLUSION

Studies involving 6 × 6 half diallel populations of *J. curcas* showed significant differences among the parents and hybrids in the expression of different germination traits. The magnitude and direction of the germination traits for the parents and their hybrids provided meaningful information and this would help in future breeding program. The parents and hybrids with desirable germination traits could be used for exploitation of heterosis in *J. curcas* for seed germination and development of seedling. It is suggested that seed traits are the important quality traits for the development of *Jatropha* varieties. Further research is needed to explore the exact methods for getting higher seed germination of *Jatropha*.

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