Asian Journal of Plant Sciences

ISSN 1682-3974

Asian Journal of Plant Sciences 8 (6): 433-439, 2009
ISSN 1682-3974

Effect of Genotypes and Pre-Sowing Treatments on Seed Germination Behavior of Jatropha

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Abstract: The objective of the study was to explore the effects of pre-sowing seed treatments on germination behaviour and to assess the possibilities of increasing the germination rate of *Jatropha curcas*. Seeds of twenty jatropha accessions obtained from seven different sources were subjected to three pre-sowing treatments viz., control (T_0): unsoaked seeds directly sown in the polybag and apply water up to saturation; T_1: seed placed on filter paper in the petridis and moistened once with the water; and T_2: seeds kept under stone sand and moistened once with the water. Seeds in T_1 and T_2 were kept for 72 h before sown in the polybag. The study was conducted in the Glass House of Plant Biotechnology Laboratory, Universiti Kebangsaan Malaysia. Study revealed that pre-sowing treatments significantly ($p<0.01$) enhanced seed germination parameters of Jatropha. Seed germination started 5 days after sowing and continued up to 12 days. The highest germination percentage (95.85%) was observed in T_2 and 100% germination was observed in the genotypes viz., UKM-JC-011, UKM-JC-012, UKM-JC-014, UKM-JC-016 and UKM-JC-020 in T_1 and T_2. None of the genotypes showed 100% germination in T_0. The highest Germination Index (GI) and Seedling Vigor Index (SVI) was found in T_2 and the lowest in T_0. T_2 was found more effective in respect to faster germination, high germination percentage, germination index, seedling vigor index, speed and energy of germination. Five accessions viz., UKM-JC-012, UKM-JC-014, UKM-JC-016, UKM-JC-017 and UKM-JC-019 were found suitable in all the treatments including control.

Key words: *Jatropha curcas* L., biofuel, biodiesel, germination percentage, germination index, seedling vigour

INTRODUCTION

Jatropha curcas is a multipurpose shrub belonging to the family Euphorbeaceae with significant economic importance since its seed oil can be converted to biodiesel. It is emerging as a renewable energy source, alternative to petrodiesel. Due to an increasing demand for energy and declining fossil fuel resources (Becker and Francis, 2003), biofuels are worldwide recognized as an alternative source of energy. Several reports have demonstrated better performance of the Jatropha biodiesel compared with the conventional petrodiesel (Ghosh *et al*., 2007; Mandpe *et al*., 2005). Physic nut (*Jatropha curcas* L.) is a perennial shrub of the tropics and subtropics grow up to five meter, which produces seeds containing approximately 30% of oil (Heller, 1996; Grimm, 1996; Rockefeller Foundation, 1998) and has low requirements to soil quality and can grow under low rainfall conditions. Before being able to conduct research on generation of physic nut oil using different extraction methods and on combustion properties of the oil in the stove, it is necessary to develop high yielding variety, cultivation technology and grow a sufficient amount of physic nut to obtain the desired oil. Seed germination is one of the important factors in the production process of the crop. Genetic and environmental factors determine germination rate, speed of germination and vigor of seed and seedling in the plant (Hartmann *et al*., 1990).

One of the main problems in jatropha cultivation is the poor germination of their seeds. This comes from their seed water impermeable testas, which exerts a physical exogenous dormancy (Holmes *et al*., 1987). Jatropha is normally propagated through seeds and very much unreliable in terms of seed germination. Germination of seed varies from 10 to 95%. Jatropha therefore, will not germinate promptly when placed under condition, which are normally regarded as suitable for germination. Such seeds are said to be dormant. To overcome seed

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dormancy and obtain rapid and synchronous germination artificially before sowing, the seed must be subjected to some physical or chemical treatment. Such pre-germination treatments will quickly destroy the integrity of impermeable cover and so permit the imbibition of the embryo. Hard-seeded species like, *Jatropha curcas* require external stimuli to promote seed-coat rupture. Seeds that soaked in water over night before planting showed the highest survival and germination rate (Feike *et al*., 2008). The general purpose of the pre-sowing treatment of seed is to hydrate partially the seed which results higher germination percentage as compared to untreated directly sown seed. The stone sand acts as a buffer between seeds, improves the environment around the seed by absorbing excess moisture and helps to exchange gases between germination medium and embryo. Another advantage of the stone sand is when the seed expand and radicle pushes for emergence, the sand offers resistance to the seed coat.

There is little published information on the effects of seed pre-sowing treatment on germination of *Jatropha curcas*. It would be useful to increase the germination rate of jatropha seeds, especially for those interested in commercial production of this crop. Therefore, the objective of this study was to assess the possibilities of increasing the germination rate and contribute that knowledge to the cultivation of *Jatropha curcas* L to address the energy needs for the future.

MATERIALS AND METHODS

The study was conducted in the Glass House, Laboratory of Plant Biotechnology, Faculty of Science and Technology, University Kebangsaan Malaysia during December 2008 to February 2009. Seeds of twenty jatropha accessions obtained from seven different sources (Swaziland, Cape Verde, India, Thailand, Vietnam, Indonesia and Malaysia) were used as experimental materials. To assess the effects of genotype and pre-sowing treatment on germination behaviour of jatropha, the seeds were sown in the polybag ($18\times10\times7$ cm^3) containing sand : soil : compost in the proportion of 1: 1: 1. There were three treatments in the experiment including the control (T_0): unsoaked seeds directly sown in a depth of 3 cm (method after Henning, 2000) in the polybag and apply water up to saturation (control); T_1: seed placed on filter paper in the petridis and was moistened with water; and T_2: seeds kept under stone sand and moistened with water. Watering was done once before germination (during sowing) and three times per week after germination. Seeds in T_1 and T_2 were kept for 72 h before sown in the polybag. Pre-treatment of the seeds enhanced rapid and uniform germination of seeds by cracking each seed. Radicle protrusion occurs by cracking outer seed coat in pre-treated seeds and then seeds were sown in the polybag. One seed per polybag was sown in all treatments. The experiments were directed in completely randomized design with threefold replication and 30 seeds were tested for each replication. Data on different germination parameters were recorded in 24 h intervals and continued until no further germination occurred. The seed germination criterion was visible protrusion on the surface of soil at least 0.5 cm of the cotyledon and hypocotyls of the seedlings. The seedlings were evaluated as described in Seedling Evaluation Handbook (AOSA, 1991).

The Germination Index (GI) was calculated as described in the Association of Official Seed Analysts (AOSA, 1983) by following formula:

$$\text{Germination Index} = \sum (GT/Tt) \text{ or } \left[\frac{\text{No. of germinated seed}}{\text{Days of first count}}\right] + \ldots\ldots\ldots\ldots + \left[\frac{\text{No. of germinated seed}}{\text{Days of final or last count}}\right]$$

The vigor index was calculated according to following formula:

$$\text{Seedling Vigor Index (SVI)} = \left[\frac{\text{Seedling length (cm)}\times\text{Germination percentage}}{100}\right]$$

The speed of emergence was calculated according to following formula:

$$\text{Speed of Emergence} = \left(\frac{\text{No. of seedlings emerged 5 days after sowing}}{\text{No. of seedlings emerged 15 days after sowing}}\right)\times100$$

Energy of germination was determined as the percentage of germinating seeds five days after planting relative to the total number of seeds tested (Ruan *et al*., 2002). Number of days taken for first germination was counted from the date of treatment. Numbers of days to first and last germination for each trial were observed. Moreover, measurements of the percentage of germinated seeds were made.

Final germination percentage (%), seedling length, was recorded after 15 days of planting (Dezfuli *et al*., 2008). For statistical analysis, the data of germination percentage was transformed to arcsin$\sqrt{(100/X)}$; actual percentage are shown. Experimental data were analyzed by a statistical package SAS (2008), version 9.01. Treatments means were compared using Tukey's test at 5% level of probability (Steel and Torrie, 1980).

RESULTS

The present study was conducted to evaluate the effect of genotype and pre-sowing treatment of seed on germination behaviour and early growth of Jatropha. It was revealed from this study that different genotypes and pre-sowing treatments can have various effects on different seed germination parameters of *Jatropha curcas*. The earliest germination was observed in pre-sowing treatment, T_2 and the delayed germination was found in T_0 (Table 1). Seed germination started five days after sowing (UKM-JC-006 and UKM-JC-014) and continued up to 12 days (UKM-JC-004). The minimum days required by the genotypes UKM-JC-015, UKM-JC-017, UKM-JC-019 and UKM-JC-021 in pre-sowing treatment T_2 for first germination and maximum days taken by the genotype UKM-JC-004 in all the treatments (Fig. 1). Pre-sowing T_2 showed the fastest germination for most of the genotypes (except UKM-JC-004) than other two treatments (Table 3). The control (T_0) took most delayed germination for all the genotypes. Pre-treatment enhanced early germination of seeds in *Jatropha curcas*. Similar results have been reported by Shivanna *et al.* (2007) for Ber seeds.

The highest germination percentage (95.85%) was observed in T_2 (seeds kept under stone sand and moistened with water) which was significantly higher than control (T_0) and T_1. Similar result was obtained previously by Goda (1987), who found soaking the seeds of *Acacia nilotica* in tap water for 72 h promoted germination percentage. The lowest germination percentage (77.17%) was recorded from the control treatment (Table 2).

Table 1: Effects of pre-sowing treatments on germination parameters of *Jatropha curcas*

Pre-sowing treatment	NDFG	GP	GI	SVI	SE	SL
Control (T_0)	6.48a	77.17c	3.43c	14.08b	27.82c	18.15a
T_1	6.55a	84.24b	3.60b	13.77c	36.59b	16.53b
T_2	5.12b	95.85a	4.11a	15.59a	43.63a	16.43b
Statistical analysis						
F-value	**	**	**	**	**	**
Mean	6.05	85.75	3.71	14.48	36.01	17.03
CV (%)	8.85	1.11	2.67	1.51	2.87	2.92

**p<0.01; NDFG: No. of days to first germination, GP: Germination percentage (%), GI : Germination index, SVI: Seedling vigor index, SE: Speed of emergence and SL: Seedling length (cm); Means with the same letter are not significantly different

Table 2: Effect of genotype on different germination parameters of *Jatropha curcas*

Genotypes	NDFG	GP	GI	SVI	SE	SL
UKM-JC-002	5.67c-f	87.43d	3.72gh	13.15k	36.53d-e	15.09f
UKM-JC-003	5.44d-f	95.67b	4.19cd	15.74g	42.02c	16.57de
UKM-JC-004	9.89a	35.56g	0.94n	5.22n	9.78j	15.98e
UKM-JC-005	6.67b	67.77g	2.28m	9.37m	21.06i	14.02g
UKM-JC-006	5.11f	91.77c	4.22de	16.67cd	40.49c	18.27ab
UKM-JC-007	6.11b-e	92.07c	4.11de	16.15ef	34.69fg	17.69bc
UKM-JC-008	6.00b-f	91.78c	3.95ef	14.60i	45.52b	16.14e
UKM-JC-009	5.67c-f	75.50f	3.69g-i	13.13k	46.35b	17.74bc
UKM-JC-010	6.44bc	75.28f	2.81l	12.46l	20.70i	16.41de
UKM-JC-011	5.78b-f	96.08b	3.53ij	16.41de	31.87h	17.21cd
UKM-JC-012	5.89b-f	99.92a	4.23cd	18.22a	46.19b	18.25ab
UKM-JC-013	6.22b-d	83.40e	3.58h-j	14.55i	36.31d-f	17.78bc
UKM-JC-014	5.11f	95.72b	4.28c	17.35b	48.43a	18.30ab
UKM-JC-015	5.78d-f	88.78d	3.47j	14.42i	30.16h	16.72de
UKM-JC-016	5.22ef	99.99a	4.99b	16.93c	46.09b	17.03cd
UKM-JC-017	6.67b	95.74b	5.29a	15.34h	35.17e-g	16.02e
UKM-JC-018	5.89b-f	91.72c	4.28c	16.16ef	37.19d	17.63bc
UKM-JC-019	6.00b-f	75.44f	3.04k	13.79j	34.04g	18.63a
UKM-JC-020	5.67c-f	92.00c	3.98e	16.01fg	31.86h	17.60bc
UKM-JC-021	5.78b-f	83.39e	3.78fg	13.93j	45.42b	17.61bc
Statistical analysis						
F-value	**	**	**	**	**	**
Mean	6.05	85.75	3.71	14.48	36.01	17.03
CV (%)	8.85	1.11	2.67	1.51	2.87	2.92

**p<0.01; NDFG : Number of days to first germination, GP : Germination Percentage (%), GI : Germination Index, SVI : Seedling Vigor Index, SE: Speed of Emergence and SL : Seedling Length (cm);Means with the same letter are not significantly different

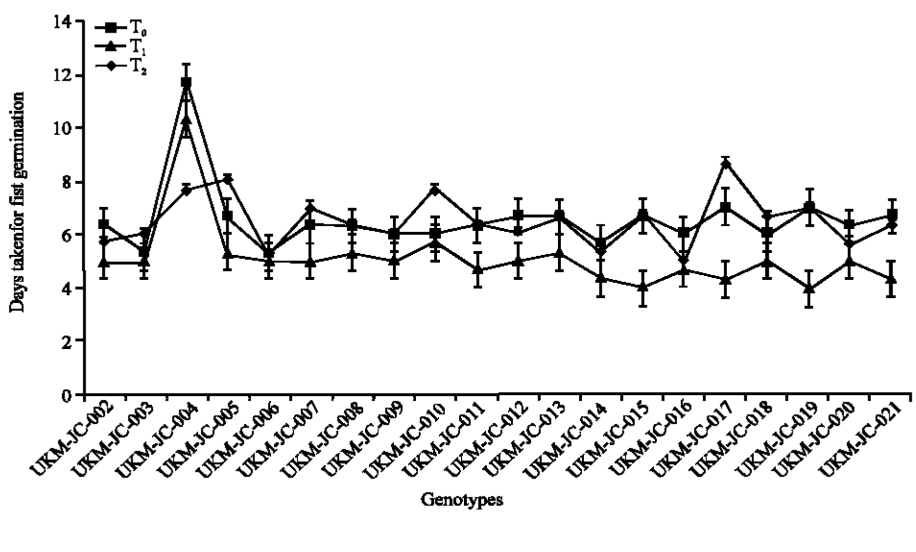

Fig. 1: Effect of genotype and pre-sowing treatment on number of days taken for first germination of *Jatropha curcas* L.

Maximum final germination was observed in genotype UKM-JC-016 (100%) followed by UKM-JC-012 (99.92%). More than 90% germination was observed in 10 genotypes. Pre-sowing treatments of the seed led to an increase in germination rate from 35 to 99.99%. Minimum germination (35%) was observed in the genotype UKM-JC-004 (Table 3). All the genotypes showed 100% germination in treatment T_2 except UKM-JC-004, UKM-JC-005 and UKM-JC-010 whereas five genotypes (UKM-JC-011, UKM-JC-012, UKM-JC-014, UKM-JC-016 and UKM-JC-020) showed 100% germination in treatment T_1 and none of the genotype showed 100% germination in T_0 (Fig. 2). So it can be concluded that seed treated at T_2 had the highest germination percentage in Jatropha followed by T_1. This result concurs with that of Magnani *et al.* (1993), who found a positive effect of pre-sowing treatments of seeds of seven *Acacia* species on their germination percentage. The germination rate of the seeds of *Acacia* species also improved when soaked in boiling water (Larsen, 1962).

Table 3: Interaction effects of genotype and pre-sowing treatment on germination index, seedling vigor index and speed of germination of *Jatropha curcas*

	GI			SVI			SE		
Genotype	T_0	T_1	T_2	T_0	T_1	T_2	T_0	T_1	T_2
UKM-JC-002	4.05	3.02	4.09	15.15	10.65	13.66	16.78	36.60	56.21
UKM-JC-003	4.02	4.36	4.20	14.46	16.25	16.50	28.02	49.50	63.17
UKM-JC-004	0.75	0.68	1.38	4.26	3.75	7.65	10.33	8.67	10.33
UKM-JC-005	2.29	2.14	2.42	9.88	8.75	9.47	16.50	20.67	26.00
UKM-JC-006	2.94	4.50	4.89	13.50	18.00	18.50	19.67	52.50	49.30
UKM-JC-007	4.49	3.33	4.50	18.82	13.46	16.17	24.56	36.87	42.55
UKM-JC-008	3.49	3.68	4.69	13.42	13.85	16.53	36.61	63.43	50.17
UKM-JC-009	2.65	3.54	4.87	9.15	12.00	18.23	41.87	49.27	76.17
UKM-JC-010	2.32	2.88	3.23	10.57	12.35	14.48	14.28	14.66	33.22
UKM-JC-011	3.89	3.44	3.26	14.51	16.72	18.00	24.89	44.00	28.37
UKM-JC-012	3.98	3.90	4.80	19.00	17.66	18.00	51.56	37.83	49.57
UKM-JC-013	3.11	3.68	3.96	12.01	14.63	17.00	24.11	43.44	41.17
UKM-JC-014	3.85	4.50	4.50	18.21	16.33	17.50	49.78	63.73	46.80
UKM-JC-015	4.03	2.44	3.93	18.60	9.66	15.00	28.33	11.30	50.67
UKM-JC-016	4.65	5.58	4.73	21.00	16.00	13.80	85.75	37.50	52.47
UKM-JC-017	5.74	4.28	5.84	18.56	13.46	14.00	12.28	71.70	48.10
UKM-JC-018	3.64	4.51	4.70	16.19	15.59	16.74	29.79	44.22	37.83
UKM-JC-019	1.69	3.45	3.98	10.00	14.13	17.23	24.78	25.26	52.20
UKM-JC-020	3.35	4.21	4.38	12.75	17.50	17.79	12.00	33.11	50.33
UKM-JC-021	3.64	3.85	3.86	11.57	14.67	15.56	41.19	43.65	51.07
Statistical analysis									
Mean	3.43	3.60	4.11	14.08	13.77	15.59	27.81	36.59	43.63
LSD (0.05)	0.247	0.407	0.235	0.569	0.712	0.737	0.028	0.027	0.023
CV (%)	2.32	3.64	1.84	1.30	1.67	1.52	1.36	1.19	0.89

GI : Germination Index, SVI:Seedling Vigor Index and SE : Speed of Emergence

Germination index increased in the pre-sowing treatment compared to control and the highest in T_2 (Table 1). The highest germination index (5.29) was observed in the genotype UKM-JC-017 followed by UKM-JC-016 (4.99) and the lowest (0.94) in UKM-JC-004. Higher value of germination index indicated the earlier germination and lower value indicated late germination.

Most of the genotypes showed higher value of germination index in T_2 compared to control (Table 3). The highest germination index was found in the genotype UKM-JC-017 in T_2 and UKM-JC-016 in T_1, the genotype UKM-JC-004 showed least germination index in all the treatments.

In this study, all genotypes of Jatropha responded differently to pre-sowing treatments for seedling vigor. Pre-sowing T_2 showed higher average seedling vigor compared to the control (Table 1). The highest (18.22) seedling vigor index was observed in the genotype UKM-JC-012 and the lowest (5.22) in UKM-JC-004. Pre-sowing treatment resulted in reduced seedling vigor index in compared to the control among the genotypes except UKM-JC-003, UKM-JC-006, UKM-JC-009, UKM-JC-011, UKM-JC-013, UKM-JC-019 and UKM-JC-021 (Table 2).

Pre-sowing treatments (T_1 and T_2) increased the speed of emergence compared with control (Table 1). The genotype UKM-JC-014 followed by UKM-JC-008, UKM-JC-009 and UKM-JC-016 showed the highest speed of emergence whereas the lowest speed of emergence was observed in the genotype UKM-JC-004 followed by UKM-JC-005 (Table 2). The speed of emergence higher in

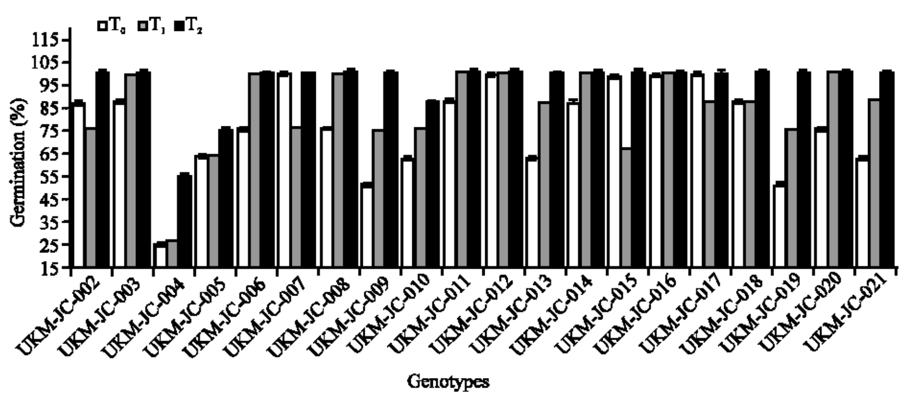

Fig. 2: Effect of genotype and pre-sowing treatment on germination (%) of *Jatropha curcas* L.

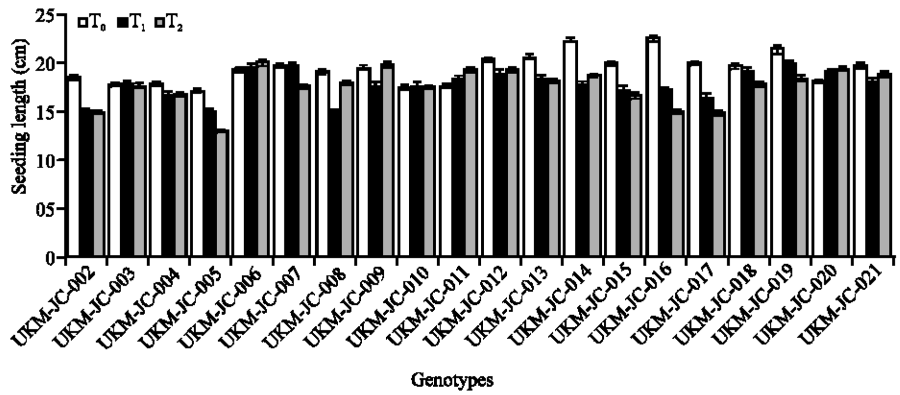

Fig. 3: Effect of genotype and pre-sowing treatment on germination behaviour of *Jatropha curcas*

all genotypes in T_2 compared to T_0 except UKM-JC-016 in T_0 (Table 2). The genotype UKM-JC-004 showed least speed of emergence in all treatments. The genotype×pre-sowing treatment interactions showed that all Jatropha accessions had the greatest speed germination when soaked in water under sand stone but had lower speed of emergence when soaked in petridish or untreated (Table 3).

Out of three pre-sowing treatments T_0 produced the longest seedling than T_1 and T_2 (Table 1). The longest (18.63 cm) seedling was achieved in the genotypes UKM-JC-019 followed by UKM-JC-006, UKM-JC-012 and UKM-JC-014 (Table 2) while the shortest seedling in UKM-JC-005 (14.02 cm). Figure 3 shows the mean seedling length of 20 Jatropha accessions in three different pre-sowing treatments. The genotype UKM-JC-016 produced the longest (21.00 cm) seedling in T_0 and UKM-JC-004 produced the shortest (15.60 cm) seedling in T_2 (Table 3).

DISCUSSION

The present study was conducted to evaluate the effect of genotype and pre-sowing treatment of seed on germination behaviour and early growth of Jatropha. It was revealed from this study that different genotypes and pre-sowing treatments can have various effects on different seed germination parameters of *Jatropha curcas*. The highest Germination Index (GI) and Seedling Vigor Index (SVI) was found in treatment T_2 and the lowest in T_0 and T_1, respectively. Treatment T_2 resulted in lower time taken to 50% germination and higher germination index, seedling vigor index and germination percentage in all accessions. On the other hand, for most of the germination parameters T_0 behaved poor than that of T_1 and T_2. Results showed that, T_2 was found more effective in respect to faster germination, high germination percentage, germination index, seedling vigor index and speed of germination than T_1 and T_0. *Jatropha curcas* have a hard seed coat, therefore, will not germinate promptly when placed under condition, which are normally regarded as suitable for germination, such seeds are said to be dormant. Mwang'Ingo *et al.* (2004) investigated the effectiveness of various seed pre-sowing treatments in enhancing germination and early seedling growth. They found complete removal of the seed coat and soaking in hot water enhanced seed germination and promoted early seedling growth and are thus recommended for adoption. The highest germination (66.5%) attained in their study was still unsatisfactory which was due to the existence of other types of dormancies such as chemical dormencies. The seed dormancy can be broken by physical or chemical treatments (Sadhu and Kaul, 1989). Jatropha genotypes showed lower germination percentage in control than those were treated before sowing. Seed germination started five days after sowing and continued up to 12 days. Seed pre-treated in the stone sand gave the higher germination than control and other treatment. Germination of seed varies from 10 to 95% and the low germination is due to physical exogenous dormancy (Holmes *et al.*, 1987). Seeds that soaked by tap water in petridis and under sand stone for 72 h before sowing showed the highest germination than directly sown seed, which is due to external stimulation of seed coat rupture (Feike *et al.*, 2008). The highest germination percentage (95.85%) was observed in T_2 and the 100% germination was observed in the genotypes viz., UKM-JC-011, UKM-JC-012, UKM-JC-014, UKM-JC-016 and UKM-JC-020 in T_1 and T_2. None of the genotypes showed 100% germination in T_0. Bohra *et al.* (1994) have also been reported that pre-treatments enhanced the germination percentage and germination energy in *Acacia tortolies* seeds. One-day seed soaking in water also accelerated and increased the germination (Grzesik and Nowak, 1998; Emmanuel and Roy, 2001). The effects of the chemicals were also similar to water treatment. The present results are also in accordance with observation of Bennett and Waters (1987) who reported that seed germination and vigor significantly enhanced by water soaking. The highest Germination Index (GI) and Seedling Vigor Index (SVI) was found in treatment T_2 and the lowest in T_0 and T_1, respectively. Treatment T_2 resulted in lower time taken to 50% germination and higher germination index, seedling vigor index and germination percentage in both genotypes. On the other hand, for most germination parameters T_0 behaved poor than that of T_1 and T_2. T_2 was found more effective in respect to faster germination, high germination percentage, germination index, seedling vigor index, speed and energy of germination. Five genotypes viz., UKM-JC-012, UKM-JC-014, UKM-JC-016, UKM-JC-017 and UKM-JC-019 were found suitable in all the treatments including control. In our study all the genotype responded differently to pre-sowing treatment. It may be concluded from present study that T_2 was better than T_1 and T_0 for high percentage of seed germination, speed of emergence, energy of germination, germination index and seedling vigor index. Further research is needed to explore the exact methods for seed germination of Jatropha.

It may be concluded from present study that T_2 was better than T_1 and T_0 for high percentage of seed germination, speed of emergence, energy of germination, germination index and seedling vigor index. This study suggested that Jatropha seeds have the ability to give more germination under pre-sowing treatment than sown directly. Further research is needed to explore the exact methods for seed germination of Jatropha.

ACKNOWLDGMENTS

The project is financed by the Universiti Kebangsaan Malaysia, under the Grant UKM-GUP-KRIB-15/2008. The authors would like to thank university authority for financial support.

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