Genotype by Environment Interaction of Jatropha (*Jatropha curcas* L.) Grown from Seedlings vs Cuttings

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Abstract

Jatropha (*Jatropha curcas* L.) is being focused as a non-edible oil yielding plant, especially for production of biodiesel. Two field trials were conducted at Kasetsart University, Kamphaeng Saen, Thailand to assess the agronomic diversity of 16 jatropha accessions from India, Myanmar and Thailand. The first trial was seedling propagated trial conducted from September, 2007 to June, 2008. The second trial was cutting propagated conducted from May, 2009 to February, 2010. Seed yield, yield components, and agronomic characters were recorded in all accessions from both trials. The indicators that were used to explain phenotypic variation were mean, coefficient of variability (CV), correlation coefficient, mean difference, and phenotypic clustering of the accessions. In this study, there exists considerable amount of genetic variability among the 16 accessions with respect to morphological characters. Propagation by stem cuttings showed greater mean values than those of seedling trial in most characters, except canopy height, canopy diameter, stem base diameter and number of secondary branches per plant. All characters were different across the two planting materials, except number of primary branches and number of seeds per plant. The variation in agronomic characters among both trials was caused by the combined effect of temperature, rainfall, sunshine and type of propagation. The traits that were rather stable in both trials were seed size characters. The CV was high (>20%) for number of primary and secondary branches per plant, number of fruits per plant and seed yield per plant. Seed yield per plant was highly correlated with number of fruits and number of seeds per plant. Cluster analysis grouped the accessions into 3 major clusters in both trials but with different members being grouped in each trial. The diversity identified in this study showed high genotype by environment interaction in jatropha, and even planting materials can cause a high variation.

Keywords: *Jatropha curcas*, jatropha, phenotypic variation, response to environment, G × E

Introduction

Jatropha (*Jatropha curcas* L.) (2n = 2x = 22), also known as physic nut, pig nut and purging nut, originated in tropical America and was introduced into many countries in Asia, Africa and Latin America (Soontornchainaksaeng and Jenjittikul, 2003). The plant has spread beyond its original distribution because of its hardiness, easy propagation, drought tolerant, high oil content, low seed cost, short gestation period, rapid growth, adaptation to wide agro-climatic conditions without competition for cultivated land, multiple uses of different plant parts, source of energy supply with reduction in greenhouse gas emission (Jones and Miller, 1991; Heller, 1996; Kumar and Sharma, 2008). Although the plant has many uses, the full potential of jatropha is far from being realized. There is a limited information available on its genetics and agronomy. A limited number of studies were conducted to examine the relationship between agronomic traits of jatropha grown in either the same or different environments (Divakara et al., 2010).

Kaushik et al. (2007) recorded coefficients of variation between 24 Haryana provenances, India, which indicated that environment has comparatively low influence on the seed traits and oil content. It was observed that 100 seed weight had positive correlation with seed length, seed width, seed thickness and oil content. Ginwal et al. (2005) reported an effect of seed source on variability in morphology, germination and seedling growth. Rao et al., (2008) evaluated genetic association and variability in seed and growth characters and found that male/female ratio had the highest positive relationship with seed yield, followed by number of branches and number of days from fruiting to maturity. Comprehensive work on collection of germplasm and evaluation of growth, morphology, seed characteristics and yield traits is still in its infancy.

Jatropha can be propagated by different methods. Effect of different propagation methods on survival and vegetative development was conducted in Cape Verde and in Senegal (Heller, 1996). The following methods were compared: direct seeding; transplanting of bare root plants (pre-cultivated in seed bed or in the wild); transplanting of plants with root ball (pre-cultivated in polyethylene bags); transplanting of pre-cultivated cuttings and direct planting of cuttings. Both vegetative cultivation methods and methods of generative pre-cultivation were more successful than direct seeding. Since interaction was not significant, different environments showed no specific influence on the plants propagated by different methods. The first seed yield of cuttings of >30 mm diameter was significantly higher than that of pre-cultivated plants. No significant differences were found between pre-cultivated plants and the other two treatments or among the cutting treatments. In the second harvest, no significant differences could be determined. This experiment is still lack in combined information on the effect of accessions and propagation methods.

The objectives of this study was is to examine the phenotypic variation of 16 jatropha accessions from India, Myanmar and Thailand planted in two different propagation methods.

Materials and Methods

The experiment was conducted at the field of Kasetsart University, Kamphaeng Saen, Thailand. The plant material comprised 16 jatropha accessions, of which six were procured from the Department of Agricultural Research, Myanmar, nine from Kasetsart University, Thailand and one from India (Table 1).

There were two trials conducted in this experiment. The first trial was conducted by transplanting of jatropha seedlings with root ball (pre-cultivated in polyethylene bags). In this trial, seeds from 16 jatropha accessions were sown on September 2007 in 8 inch plastic bags filled with 2 kg of loamy soil. The seedlings were transplanted in an experimental field of Kasetsart University, Kamphaeng Saen, Thailand on December 2007 in a randomized complete block design with 4 replications. After data collection of agronomic characters in May 2009, all plants were pruned and a cutting of ~30 cm long was prepared from each plant as the propagation unit for the second trial. The cuttings were planted in the plastic bags on May 2009 and transplanted into the field on July 2009 using the same experimental design as the first trial. In both trials, the plot size for each accession was 12 m × 4 m. with row-to-row × plant-to-plant distance of 2 m × 2 m. Each accession had 12 plants in each plot. Drip irrigation was provided as needed. Compound chemical fertilizer (15:15:15) was applied once basally at the rate 375 kg ha^{-1}. Herbicide (glyphosate) was applied together with hand-weeding to control weeds throughout the experiments. Insecticides (methidathion and abamectin) were sprayed at the recommended rates to control leaf minors, bugs and aphids throughout the growing season.

Four plants from each plot were randomly selected for measurement and averaged as a per plant basis. The agronomic characters were recorded as follows:

1. Canopy height (cm)
2. Canopy diameter (cm)
3. Stem base diameter (cm)
4. Number of primary branches
5. Number of secondary branches
6. Fruit length (mm)
7. Fruit width (mm)
8. Seed length (mm)
9. Seed width (mm)
10. Seed thickness (mm)
11. Number of fruits per plant
12. Number of seeds per plant

Table 1 Accession codes, names and origins of 16 jatropha accessions used in this study.

Accesion no.	Code (name)	Country of origin	Province of origin
1	D1	India	*
2	M1 (Namalatt)	Myanmar	Shan
3	M2 (Taungue)	Myanmar	Pegu
4	M3 (Magwe)	Myanmar	Magwe
5	M4 (Raw-Ngan)	Myanmar	Shan
6	M5 (YNC-36)	Myanmar	Magwe
7	M6 (D2)	Myanmar	Mandalay
8	T1 (kubp-20)	Thailand	Mukdahan
9	T2 (kubp-21)	Thailand	Mukdahan
10	T3 (kubp-27)	Thailand	Ubon Ratchathani
11	T4 (kubp-33)	Thailand	Surin
12	T5 (kubp-34)	Thailand	Amnat Charoen
13	T6 (kubp-78)	Thailand	Nakhon Si Thammarat
14	T7 (kubp-80)	Thailand	Phattalung
15	T8 (Chainat)	Thailand	Chai Nat
16	T9 (Korat)	Thailand	Nakhon Ratchasima

* Data not available

13. One hundred seed weight (g)
14. Seed yield per plant (g)

The mean difference between the above agronomic traits as the result of planting materials (seedling vs cutting) was adapted from the formula proposed by Chen et al. (2008).

Mean difference (%) = (trait mean from cutting – trait mean from seedling) * 100/ (trait mean from cutting)

The trait mean from cutting was used as the standard in this experiment because it was the usual method of jatropha propagation. A paired t-test was used to compare between agronomic characters observed from seedling and cutting of the same plant. The data were also analyzed using R program version 2.8.1. XLSTAT 7.0 (Addinsoft Inc.) to classify jatropha accessions into different groups based on their agronomic traits.

Results and Discussion

Mean Difference and Coefficient of Variability of Agronomic Characters

The cutting trial showed greater mean values than those of seedling trial in most characters, except canopy height, canopy diameter, stem base diameter and number of secondary branches per plant (Table 2). All characters except number of primary branches per plant and number of seeds per plant were significantly different across the two planting materials (Table 3). Difference in paired comparison between plants grown from seedling vs cutting was detected in 12 of 14 traits, except number of primary branches and number of seeds per plant. This revealed high genotype × environment interaction of these traits, especially planting materials, and weather condition (temperature, rainfall, sunshine). The average temperature during the growing season ranged

Table 2 Mean and CV (%) of agronomic characters of jatropha accessions from India, Myanmar and Thailand planted with seedlings vs cuttings.

Character[1/]	Planting material	Means				CV (%)			
		India	Myanmar	Thai	All materials	India	Myanmar	Thai	All materials
canht	Cutting	111.75	115.61	121.78	118.84	11.25	7.57	9.86	9.03
	Seedling	142.19	138.13	150.78	145.50	3.59	5.61	4.97	6.49
candia	Cutting	92.33	98.54	97.82	97.75	17.44	11.07	9.82	9.76
	Seedling	120.93	117.96	121.16	119.94	14.76	12.28	8.03	9.24
basedia	Cutting	4.55	4.55	4.87	4.73	11.70	6.11	7.99	7.70
	Seedling	5.93	5.89	6.03	5.97	7.67	4.14	4.86	4.46
primbr	Cutting	4.83	5.24	4.68	4.90	15.80	12.73	8.77	11.45
	Seedling	4.87	4.67	4.98	4.85	21.53	14.17	24.75	20.39
secbr	Cutting	2.90	3.71	4.17	3.92	39.77	34.36	30.61	31.62
	Seedling	11.70	11.81	13.03	12.49	23.41	20.55	32.50	27.65
fruitlen	Cutting	27.83	28.29	28.59	28.43	2.17	0.84	2.43	2.00
	Seedling	21.10	22.91	22.69	22.67	12.20	4.74	3.88	4.40
fruitwi	Cutting	25.76	25.61	25.97	25.82	2.26	1.17	2.55	2.10
	Seedling	19.63	21.37	21.33	21.24	12.33	4.14	4.28	4.44
seedlen	Cutting	17.83	17.89	17.66	17.75	1.21	0.41	2.51	1.95
	Seedling	16.80	17.11	16.29	16.63	3.06	3.18	5.61	5.05
seedwi	Cutting	11.38	11.40	11.25	11.34	1.78	0.38	1.74	1.44
	Seedling	10.80	11.00	10.43	10.67	2.56	3.06	8.78	7.04
seedthi	Cutting	8.76	8.69	8.63	8.66	3.08	0.96	1.20	1.14
	Seedling	8.05	8.21	7.72	7.92	0.63	2.15	10.61	8.25
fruitno	Cutting	78.00	66.02	75.88	72.31	18.62	30.52	19.62	23.11
	Seedling	51.52	60.74	56.46	57.76	12.98	28.82	44.47	36.54
seedno	Cutting	198.82	164.98	192.48	182.56	14.92	32.32	17.75	23.05
	Seedling	130.37	157.10	149.09	150.92	19.02	31.06	46.17	38.44
100sw	Cutting	60.65	59.21	59.25	59.32	10.48	4.04	3.20	3.35
	Seedling	51.93	51.47	49.95	50.64	2.99	7.52	7.24	7.02
yield	Cutting	117.91	99.50	114.68	109.19	16.62	33.87	20.51	24.82
	Seedling	61.35	74.67	68.71	70.48	8.76	34.76	54.78	44.74

[1/] canht = canopy height, candia = canopy diameter, basedia = stem base diameter, primbr = number of primary branches per plant, secbr = number of secondary branches per plant, fruitlen = fruit length, fruitwi = fruit width, seedlen = seed length, seedwi = seed width, seedthi = seed thickness, fruitno = number of fruits per plant, seedno = number of seeds per plant, 100sw = hundred seed weight, yield = seed yield per plant.

between 20-35^{o}C in both experiments. Temperature plays an important role in both flower bud initiation and differentiation in various fruit crops. Although the average temperature was not much different between both trials, the critical temperature was observed at flowering time. The temperature was higher (34-35^{o}C) in seedling trial than in cutting trial (31-33^{o}C) (Figure 1). The high temperature can cause pollen or stigma abnormality and poor fruit or seed set in seedling trial (Table 2) (Thompson, 1996). Seedling trial was faced with rainfall at harvesting time that seriously reduced seed quality and 100 seed weight because harvesting time in jatropha spanned 2-3 months. Total sunlight also

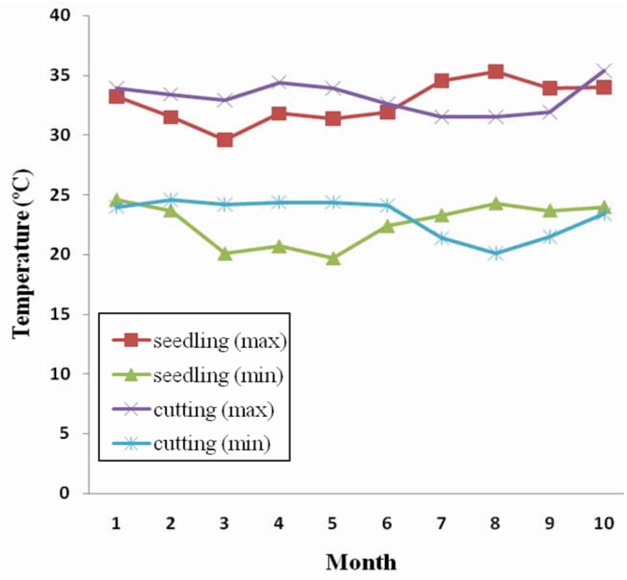

Figure 1 Monthly maximum and minimum temperatures (°C) from two trials during 10 month period (seedling trial: September, 2007 to June, 2008; cutting trial: May, 2009 to February, 2010).

gave greater values in seedling trial than in cutting trial and thus may affect plant growth characters such as canopy height, canopy diameter and stem base diameter (Figure 2). This resulted in greater growth characters from seedling trial than the cutting trial (Table 2).

The coefficient of variability (CV) of agronomic characters in cutting and seedling trail is shown in Table 2. The CV was high (>20%) for number of primary and secondary branches per plant, number of fruits per plant and seed yield per plant. Intermediate CV (7-20%) was observed in canopy height, canopy diameter and stem base diameter. The other traits having low CV (<7%) were fruit and seed size, and 100 seed weight. Most traits of seedling trial had greater CV than the cutting trial, except canopy height, canopy diameter, stem base diameter and number of secondary branches per plant.

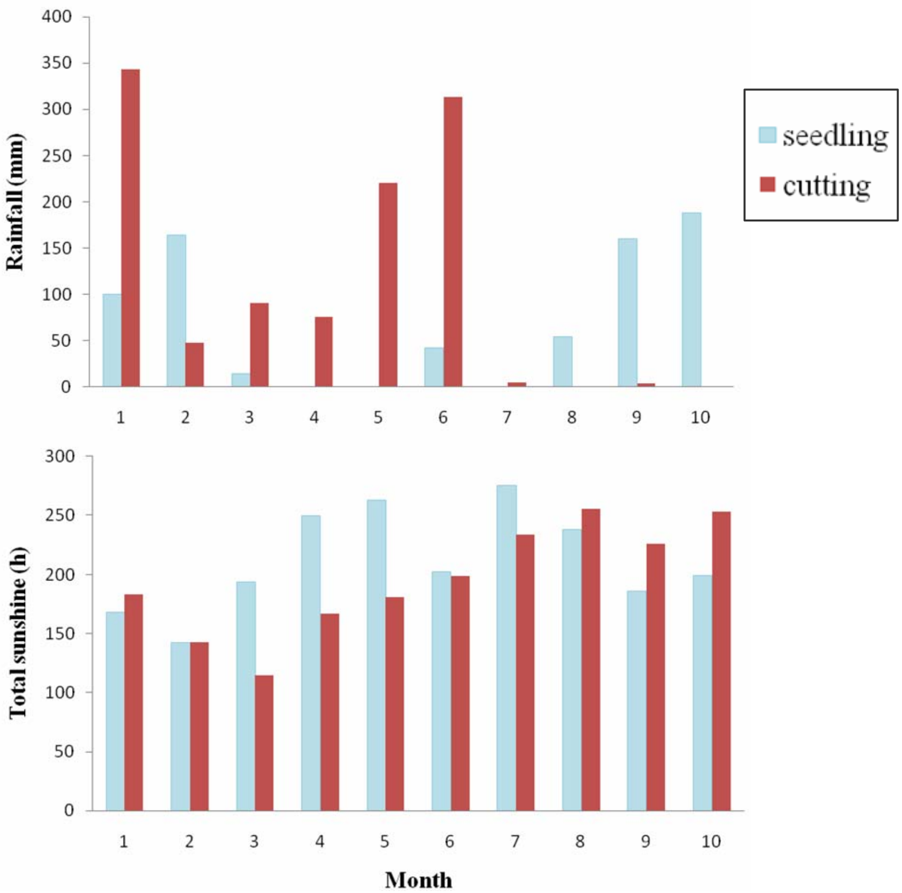

Figure 2 Monthly rainfall (mm) and monthly sunshine (h) from two trials during 10 month period (seedling trial: September, 2007 to June, 2008; cutting trial: May, 2009 to February, 2010).

Mean difference percentage among agronomic characters of all jatropha accessions across both trials can be ranked indescending order as: number of secondary branches per plant, seed yield per plant, stem base diameter, canopy diameter, canopy height, fruit length, number of fruits per plant, fruit width, 100 seed weight, seed thickness, seed length, seed width and number of primary branches (Table 3). The mean difference of the Myanmar accessions was ranked as: number of secondary branches per plant, stem base diameter, seed yield per plant, canopy height, canopy diameter, fruit length, fruit width, 100 seed weight, number of primary branches, number of fruits per plant, seed thickness, seed length and seed width. The ranks of mean difference from Thailand and India accessions were of the same trend.

In general, the diversity of agronomic traits from Thailand and India accessions was a little higher than Myanmar accessions (Table 3). The accessions originated from Myanmar were more uniform compared with the other two groups, and showed a tendency to perform better when planted by seedlings. Seed length, seed width and seed thickness showed less variation among planting with different materials. Number of secondary branches per plant showed the highest different among the trials, while the other traits were found with intermediate variation. It was confirmed that planting by cutting promoted better growth and development in all traits observed, except canopy height, canopy diameter, stem base diameter and number of secondary branches per plant. Considering overall traits of all accessions, a slight advantage in total sunlight of the seedling trial could not compensate with the advantage of favorable temperature and rainfall of the cutting trial.

Correlation between Seed Yield and Yield Components

Coefficients of correlation between all characters are shown in Table 4. Most agronomic characters observed in this study showed significant relationship

Table 3 Mean difference (%) and paired comparison t-test of agronomic characters of jatropha accessions from India, Myanmar and Thailand planted with seedlings vs cuttings.

Character[1/]	India	Myanmar	Thailand	All materials
canht	-27.24	-19.48	-23.81	-22.43**[2/]
candia	-30.98	-19.71	-23.86	-22.71**
basedia	-30.39	-29.27	-23.94	-26.25**
primbr	-0.83	11.00	-6.41	0.92
secbr	-303.45	-218.38	-212.46	-218.76**
fruitlen	24.18	19.03	20.66	20.27**
fruitwi	23.80	16.56	17.85	17.74**
seedlen	5.78	4.36	7.72	6.33**
seedwi	5.10	3.48	7.28	5.71**
seedthi	8.11	5.58	10.55	8.53**
fruitno	33.95	7.99	25.59	20.13*
seedno	34.43	4.78	22.54	17.33
100sw	14.38	13.08	15.71	14.64**
yield	47.97	24.96	40.09	35.45**

[1/] canht = canopy height, candia = canopy diameter, basedia = stem base diameter, primbr = number of primary branches per plant, secbr = number of secondary branches per plant, fruitlen = fruit length, fruitwi = fruit width, seedlen = seed length, seedwi = seed width, seedthi = seed thickness, fruitno = number of fruits per plant, seedno = number of seeds per plant, 100sw = hundred seed weight, yield = seed yield per plant.

[2/] *, ** significantly different between seedling and cutting trials at 0.05 and 0.01 level of probability, respectively.

Table 4 Correlation coefficients between yield, yield components and agronomic characters of jatropha accessions from India, Myanmar and Thailand planted with seedlings vs cuttings.

Trait[1/]	planting material	candia	basedia	primbr	secbr	frulen	fruwid	seedlen	seedwid	seedthi	fruitno	seedno	100sw	yield
canht	cutting	0.71**	0.78**	0.06	0.20	0.68**	0.50**	0.45**	0.22	0.25	0.56**	0.56**	0.44	0.58**
	seedling	0.73**	0.81**	0.25*	0.40**	0.46**	0.53**	0.25*	-0.01	0.10	0.27*	0.30*	0.47**	0.37**
candia	cutting		0.74**	0.37*	0.51**	0.38*	0.31*	0.48**	0.34*	0.25	0.68**	0.67**	0.38**	0.69**
	seedling		0.81**	0.50**	0.52**	0.51**	0.52**	0.24	-0.03	0.09	0.48**	0.49**	0.50**	0.56**
basedia	cutting			0.13	0.42**	0.53**	0.48**	0.31*	0.10	0.25	0.70**	0.71**	0.39**	0.73**
	seedling			0.39**	0.51**	0.42**	0.47**	0.34**	0.08	0.16	0.35**	0.36**	0.69**	0.46**
primbr	cutting				0.05	-0.01	0.02	0.09	0.22	-0.06	0.08	0.06	-0.09	0.06
	seedling				0.57**	-0.04	-0.03	-0.31*	-0.49**	-0.42**	0.06	0.68	0.30*	0.12
secbr	cutting					0.07	0.08	0.14	0.15	0.13	0.49**	0.46**	0.18	0.46
	seedling					0.07	0.11	-0.17	-0.35**	-0.25*	0.30*	0.30*	0.33**	0.34*
frulen	cutting						0.87**	0.32*	0.13	0.25	0.33*	0.33*	0.52**	0.38**
	seedling						0.94**	0.57**	0.41**	0.45**	0.58**	0.59**	0.33**	0.61**
fruwid	cutting							0.22	0.14	0.24	0.40**	0.40**	0.42**	0.44**
	seedling							0.55**	0.41**	0.46**	0.57**	0.59**	0.35**	0.62**
seedlen	cutting								0.74**	0.65**	0.25	0.25	0.41**	0.29*
	seedling								0.89**	0.88**	0.38**	0.39**	0.48**	0.41**
seedwid	cutting									0.42**	0.16	0.15	0.09	0.16
	seedling									0.95**	0.25*	0.24	0.26*	0.25*
seedthi	cutting										0.21	0.21	0.56**	0.27
	seedling										0.29*	0.28*	0.31*	0.31*
fruitno	cutting											0.99**	0.42**	0.99**
	seedling											0.99**	0.19	0.98**
seedno	cutting												0.43**	0.99**
	seedling												0.18	0.98**
100sw	cutting													0.52**
	seedling													0.29*

[1/] canht = canopy height, candia = canopy diameter, basedia = stem base diameter, primbr = number of primary branches per plant, secbr = number of secondary branches per plant, fruitlen = fruit length, fruitwi = fruit width, seedlen = seed length, seedwi = seed width, seedthi = seed thickness, fruitno = number of fruits per plant, seedno = number of seeds per plant, 100sw = hundred seed weight, yield = seed yield per plant.

with seed yield, except seed size and primary branches per plant in both trials. Among these traits (for cutting and seedling trials, respectively), number of fruits per plant (r = 0.99 and 0.98) and number of seed per plant (r = 0.99 and 0.98) were strongly correlated with seed yield. The other characters with weak correlation with seed yield are canopy height (r = 0.58 and 0.37), canopy diameter (r = 0.69 and 0.56), stem base diameter (r = 0.73 and 0.46), secondary branches per plant (r = 0.46 and 0.34), fruit length (r = 0.38 and 0.61) and fruit width (r = 0.44 and 0.62), with respect to seedling and stem cutting propagation trials. Rao et al. (2008) reported that female to male flower ratio had highest positive direct relationship with seed yield, i.e. more female flowers produce more fruits per plant. Similarly, number of seeds per plant depends on number of fruits per plant, which determines seed yield per plant. This trait can be used in selecting high yielding jatropha. Number of primary branches per plant was not correlated with yield in both trials because number of secondary branches can compensate for the loss of primary branches. Canopy height was highly correlated with canopy diameter (r = 0.71 and 0.73) and stem base diameter (r =0.78 and 0.81) in both planting materials. Canopy diameter was also correlated with stem base diameter (r = 0.74 and 0.81). Fruit length was highly correlated with fruit width (r = 0.87 and 0.94) and seed length, width and thickness were highly correlated with each other. Number of fruits was related with number of seeds per plant (r = 0.99 and 0.99). Thus, accessions with high number of fruits per plant should be chosen for jatropha varietal improvement in the future.

Clustering of Jatropha Accessions

The jatropha accessions can be broadly classified based on average agronomic characters, into three clusters in the seedling trial (Figure 3). Cluster I has eleven accessions comprising five accessions each from Myanmar and Thailand and one accession from India. Cluster II has two accessions comprising one from Myanmar and Thailand. Cluster III has three accessions from Thailand.

In the cutting trial, the jatropha accessions can also be grouped into three clusters with some different in members of the clusters as compared to the seedling trial. Cluster I has five accessions, comprising three from Myanmar, one from Thailand and one from India. Cluster II has only one accession from Thailand. Cluster III includes ten accessions of which three of them are from Myanmar and seven from Thailand.

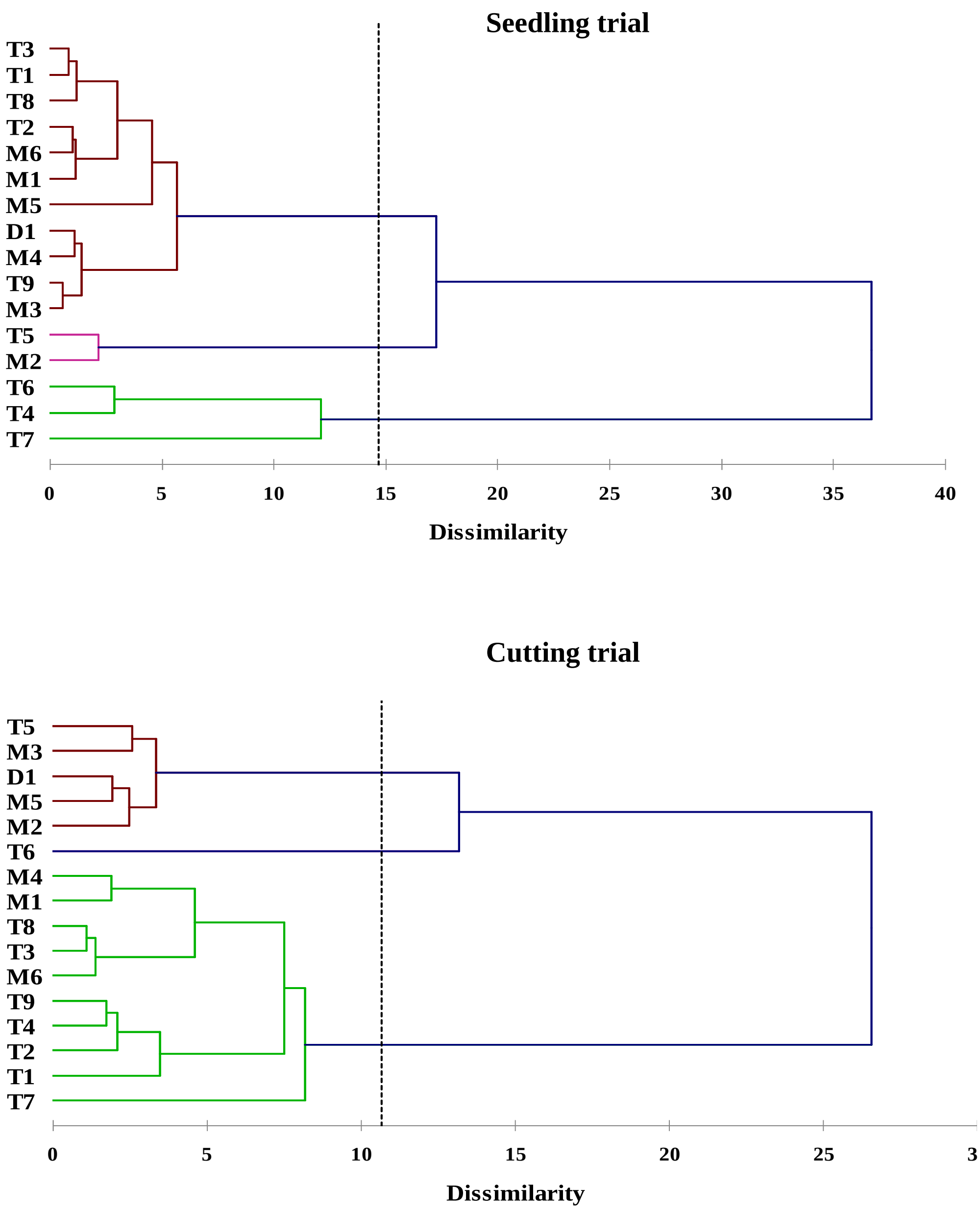

Figure 3 Grouping of 16 jatropha accessions based on their agronomic characters from seedling and cutting trials.

Grouping of jatropha accessions by using multivariate techniques is useful for germplasm collection and utilization. Since the techniques classified accessions based on phenotypic dissimilarity, certain distinct accessions from the same country may not be included in the same cluster in both trials. Although seedling and cutting trial assigned different members into each cluster, dissimilarity index value was considered small. It revealed that these jatropha accessions are narrow in phenotypic (and presumably genetic) variation.

Conclusions

The present study showed that there is low genetic variability among the 16 jatropha accessions with respect to morphological characters. India, Myanmar and Thailand accessions showed very similar genetic variation in both planting materials (seedling vs cutting). This study showed high effect of planting materials and environment, especially temperature and rainfall in identifying the better genotypes of jatropha. Although it is a long duration crop, more trials should be conducted in order for the breeders to choose the right parental genotypes carrying the desirable characters.

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