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Full Length Research Paper

Phenology, productivity, and chemical characterization of *Jatropha curcas* L. as tool for selecting non-toxic elite germplasm

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A phenological, physical and chemical descriptive study was carried out on six germplasms of *Jatropha curcas* L. collected from different areas of Mexico. From these six germplasms, elite germplasms that are better adaptable, with high seed, oil and protein yield, and low levels of phorbol esters were identified. The thermal constant (2570 ± 9) and seed weight (0.695 ± 0.065 g) were similar in all the six germplasms. The average plant height was 1.47 ± 0.25 m after one year of evaluation. Variation in the number of inflorescences (0 to 22) and seed yield (27.35 ± 22.65 g) per plant, and fruit per cluster (2.9 ± 2.2) was considerable. Kernel oil and crude protein contents were 57 ± 3% and 26 ± 2%, respectively. Germplasms India and Isla (Veracruz) had the highest levels of phorbol esters (5.982 and 2.070 mg·g^{-1}, respectively). The reproductive stage and phorbol esters content were critical in selecting elite germplasms. Based on our results, we were able to select three elite germplasms.

Key words: *Jatropha curcas,* elite germplasm, accumulated degree days (ADD), yield, oil, protein, phorbol esters.

INTRODUCTION

Jatropha curcas L. (Jatropha), a plant native to Mexico and Central America, belongs to the Euphorbiaceae family. It is a shrub/small tree that grows in low fertility soils and shows resistance to drought, pests and diseases (Brittaine and Lutaladio, 2010). *J. curcas* adapts to almost all tropical and subtropical areas with a broad genetic variability which is the basis for differences in phenology, morphology, productivity and seed composition (Ginwal et al., 2005; Mishra, 2009; Pecina-Quintero et al., 2011). Seeds of Jatropha growing in different areas vary in protein, oil and phorbol ester contents, but, the basis of these differences has not been determined (Ginwal et al., 2005; Makkar et al., 1997; Rathree, 2004; Martinez-Herrera et al., 2006; Machado and Suarez, 2009). Jatropha kernels contain 19 to 30 % protein and 42 to 60% oil, which make it suitable for biodiesel, soap and glycerin production (Heller, 1996; De Oliverira et al., 2009; Nazir et al., 2009; Singh and Pahdi, 1998).

Apart from the commonly toxic wild seeds of Jatropha found in Africa, Asia and America, non-toxic *J. curcas* wild seeds have been found in Mexico. These seeds have low (< 1.78 mg·g^{-1}) or non-detectable levels of phorbol esters, and are used for human consumption in Veracruz, Morelos and Quintana Roo States (Martinez-Herrera et al., 2006; Makkar et al., 1998). In Coatzacoalcos (Veracruz) seeds contained 3.85 mg·g^{-1} of phorbol esters (Martinez-Herrera et al., 2006). Generally, studies related to characterization and plant phenological

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development in Mexican *J. curcas* germplasm are limited, and the adaptability and productivity of this species in conditions different from their origin remain unknown (Sandoval, 2010).

The objectives of this study were to determine the biological cycle of six provenances (germplasms) of *J. curcas,* grown under the environmental conditions in Sinaloa, Mexico; germplasms with higher yield were to be selected and characterized according to physical and chemical properties of their seeds. Elite non-toxic germplasms that showed superior traits would be identified as potentially suitable for agro-industrial use and would then be included in a breeding program.

MATERIALS AND METHODS

Seeds of four Mexican and one Indian germplasms of Jatropha pre-cultivated in Sinaloa were selected; Sinaloa (SI), Morelos (MR), Puebla (PB), Papantla Veracruz (PP) and India (IN). A sixth wild germplasm was selected from Isla, Veracruz (IS). From each of the six germplasms, a random sample of 100 seeds were collected and disinfested by immersion in NaOCl (5%) for 5 min. Seeds were sown in plastic bags containing substrate formed by soil + SOGEMIX® moss (80:20), and were maintained in a shade house for three months. Thirty two healthy plants of 30 ± 5 cm height from each germplasm were selected for transplanting in an experimental field in the Agronomy School of the Autonomous University of Sinaloa (N 24° 37'5", W 107° 26' 35.3"). The climate is sub-humid receiving rainfall during summer. The soil was sandy clay with pH 7.92, 1% organic matter and traces of NO_3, and potassium (K) were 1.56, and 442 ppm ($mg \cdot L^{-1}$), respectively. Each germplasm was sown in two randomly assigned rows with 16 plants per row at a distance of 3 × 2 m, and 30 cm deep. Plants received an initial dose of 500 mL CIANEEM (0.75% azadirachtin active ingredient) to control pests, 60 g NPK (17-17-17), 500 g compost and 1.5 L water. Plants were sown in a completely randomized design.

Determination of accumulated degree days in plant phenological stages

Maximum and minimum temperatures were recorded daily from April, 2009 to April, 2010 using an automatic environmental station [ADCON (administrative control) Telemetry, Klosterneuburg, Austria]. At the beginning of each phenological stage, the thermal requirement in accumulated degree days was calculated using the formula:

$$ADD = \Sigma \text{ (Maximum temperature + Minimum temperature)}/2 - 18.6$$

where 18.6°C was the base temperature (Wassner, 2007). For each of the germplasms, height, basal diameter, number of branches, flowering and fruiting period (inflorescences, cluster and seeds per plant) were recorded in five randomly selected plants.

Physical characteristics of seeds

Nine months after transplanting (mat), mature fruits were collected and depulped to obtain seed yield per plant. A random sample of 20 seeds per germplasm was used to determine the weight, length and diameter. The seeds were cracked, the shell manually separated and kernel weight was determined. Weight of the shell was obtained by the difference between weight of complete seed and kernel weight.

Chemical composition

The kernels were ground and then the proximate (fat, protein, raw fiber, moisture and ash) and mineral analyses were conducted according to the Association of Official Analytical Chemists (AOAC) procedures (1998). Carbohydrates were determined by difference (100 - ∑ proximal compounds obtained).

Phorbol esters content

Phorbol esters content (EF) was determined according to the methods described by Makkar et al. (2007). Briefly, ground kernel (2 g) of each germplasm was extracted with methanol in three stages. The phorbol esters content was determined by HPLC (Varian Prostar 335), equipped with a manual injector (Rheodyne 7725), solvent pump system, diode array detector (Varian Prostar 330), integrity data system version 6 (Varian Prostar Work Station) and an analytical reverse-phase C_{18} column (Agilent, endcapped 5 µm) 250 × 4.6 mm i. d. The mobile phase consisted of a gradient of acidified water, acetonitrile and tetrahidrofurane at 1.3 $mL \cdot min^{-1}$ flow rate. The signal of phorbol esters appeared at 22 to 26 min and was reported as equivalents of the standard 12-miristate 13-acetate of phorbol (Sigma) with 29.4 min retention time.

Statistical analysis

Statistical analysis was done using the statistical package MINITAB version 15 (Minitab Inc, EUA). All data were subjected to one-way analysis of variance. Multiple mean comparisons were conducted by the Tukey test and the association between parameters measured through correlation analysis.

RESULTS

Phenological stages and thermal requirement

Plant phenological stages of the six *J. curcas* germplasms were recorded during the one year growth period. It was possible to observe the initial stages of shoot formation, flowering, fruiting and fruit maturity. The accumulated degree days (ADD) at the beginning of each phenological stage in each germplasm are shown in Table 1. The thermal constant of 2570 ± 9 ADD was similar for all the germplasms.

Plant phenological stages and yield

Germplasm IS was the tallest (height, 116.4 cm) and germplasms PP and PB had the least growth parameter (height, 80.2 and 82.4 cm, respectively) at 9 months after transplanting (Table 2). Germplasm MR showed more branches per plant (4.8). Germplasms PB and MR had the highest number of inflorescences (8.4 and 8.2) and fruit (35.6 and 29.8) per plant, respectively. In contrast, germplasm IN showed the lowest number of inflorescences per plant (2.0). The analysis of variance in the number of fruit per plant required a log transformation to stabilize the variance. Germplasms PB, MR and SN

Table 1. Thermal requirement (accumulated degree days (°C)) in different phenological stages of six *J. curcas* germplasms grown in Sinaloa.

Phenological stage	Germplasms					
	PP	MR	PB	IN	SN	IS
Seedling emergence (initial)	72.7	72.7	50.8	50.8	50.8	50.8
Seedling emergence (50%)	127.4	127.4	127.4	89.7	221.6	127.4
Seedling emergence (full)	221.6	304.8	358.7	186.5	393.2	358.7
Branching (initial)	1653.1	1653.1	1653.1	1653.1	1653.1	1653.1
Flowering (initial)	2334.4	2248.6	2235	2422.2	2235	2248.6
Fruiting (initial)	2500.1	2479.3	2478.8	2525.5	2478.8	2480.1
Maturing (initial)	2566.5	2561.7	2561.7	2579.0	2561.7	2561.7

PP. Papantla, Ver; MR. Morelos; PB. Puebla; IN. India; SN. Sinaloa; IS. Isla, Ver. Data obtained during the first year of growth.

Table 2. Plant growth, development and productive stages in six *J. curcas* germplasms from different origins grown in Sinaloa.

Variable	Germplasms					
	PP	MR	PB	IN	SN	IS
Plant height (cm)	80.2	86.8	82.4	92.2	106	116.4
Plant diameter (cm)	47.2	34	43.6	44.8	48	45
Branches·plant^{-1}	2.8	4.8	3.4	4.0	3.6	4.0
Inflorescens·plant^{-1}	1 - 6	1 - 20	3 - 22	0 - 4	1 - 17	2 - 8
Cluster·plant^{-1}	3.6	8.2	8.4	1.4	5.8	4
Fruit·cluster^{-1}	2.9	3.3	5.1	0.7	4.1	2.1
Seeds·plant^{-1} (g)	22.3	47.5	50	4.7	39	17.4

PP. Papantla, Ver; MR. Morelos; PB. Puebla; IN. India; SN. Sinaloa; IS. Isla, Ver. Data obtained during the first year of growth (n = 5).

produced the most seeds (50, 47.5 and 39 g per plant, respectively), and IN the least number of seeds (4.7 g) per plant.

Correlation analysis revealed a positive association between height and basal diameter with the branching number (r = 0.631, 0.539, P < 0.0001), respectively. Production of seed was highly correlated with the number of inflorescences (r = 0.935, P = 0.0001) and with fruits per plant (r = 0.991, P < 0.0001) and moderately correlated with branches per plant (r = 0.495, P < 0.005). Germplasms PB, MR and SN showed differences with PP, IS and IN in the number of inflorescences, cluster, fruit·cluster^{-1} and seeds·plant^{-1} (Table 3).

Seed physical parameters

Physical parameters measured on seeds are shown in Table 4. The average seed length was less than 2 cm and diameter ranged between 0.9 to 1.15 cm; however, a significant difference (P < 0.0001) was observed between the maximum and the minimum length of seeds from germplasm Isla (1.94 cm) and Papantla (1.82 cm), respectively.

Seed weight and kernel weight were positively correlated (r = 0.959, P < 0.0001). Kernel weight was also positively correlated with seed diameter (r = 0.706, P < 0.0001).

Chemical characteristics

Oil content in the six germplasms ranged between 54 and 61% (dm), whereas protein content ranged between 24 to 28%. Seeds of germplasm Morelos showed the highest oil content (60.1%), significantly different (P < 0.0001) from other germplasms (Table 5). Protein content in germplasm PB (27.3%) was similar to germplasm SN (26.1%) and both were statistically different from the remaining germplasms (p < 0.05). Fiber and mineral contents were similar in the six germplasms tested. However, mineral content in the germplasm IN was the lowest (Table 6).

Toxicity

Phorbol esters content in the kernels ranged from non-

Table 3. Correlation analysis between plant phenological stages and seed yield of six *J. curcas* germplasms from different origin grown in Sinaloa.

Parameter	Height	Diameter	Branches	Inflorescences	Fruits
Diameter	0.564 0.001				
Branches	0.631 0.000	0.539 0.002			
Inflorescences	0.430 0.018	0.352 0.057	0.534 0.002		
Fruits	0.310 0.096	0.278 0.137	0.492 0.006	0.951 0.000	
Seed yield	0.296 0.113	0.278 0.137	0.495 0.005	0.936 0.000	0.991 0.000

Data obtained during the first year of growth (n = 5).

Table 4. Physical characteristics in seeds of six *J. curcas* germplasms from different origin grown in Sinaloa.

Germplasm	Length* mm	Diameter* mm	Weight* (g)	Kernel (g)	Shell (g)
PP	18.20^{c}	9.77^{cd}	0.66^{bc}	0.42^{cd}	0.24^{b}
MR	18.84^{ab}	9.95^{bc}	0.69^{b}	0.44^{bc}	0.25^{b}
PB	18.58^{bc}	9.85^{c}	0.64^{c}	0.40^{d}	0.24^{b}
IN	18.23^{c}	11.20^{a}	0.76^{a}	0.48^{a}	0.28^{a}
SN	18.43^{bc}	9.45^{d}	0.63^{c}	0.39^{d}	0.24^{b}
IS	19.37^{a}	10.31^{b}	0.76^{a}	0.46^{ab}	0.30^{a}

PP. Papantla, Ver; MR. Morelos; PB. Puebla; IN. India; SN. Sinaloa; IS. Isla, Ver. *Data from complete seeds. Means with different letters in each column are statistically different (Tukey, $p < 0.05$).

Table 5. Chemical and toxicological composition in seeds of six *J. curcas* germplasms from different origin grown in Sinaloa.

Variable	Germplasm					
	PP	MR	PB	IN	SN	IS
Dry mass (%)	96.09^{a}	96.03^{ab}	95.74^{b}	95.76^{b}	95.77^{b}	95.83^{b}
Lipids (%)	56.02^{b}	60.13^{a}	55.69^{b}	54.79^{b}	54.09^{b}	54.42^{b}
Protein (%)	24.08^{c}	24.21^{c}	27.30^{a}	24.13^{c}	26.08^{ab}	25.34^{bc}
Fiber (%)	6.56^{a}	4.83^{a}	4.75^{a}	4.63^{a}	4.70^{a}	4.31^{a}
Ash (%)	4.34^{c}	4.68^{bc}	4.78^{abc}	4.98^{ab}	5.14^{ab}	5.18^{a}
Carbohydrates (%)	5.08^{ab}	2.18^{b}	3.21^{ab}	7.23^{a}	5.75^{ab}	6.57^{a}
Phorbol esters ($mg \cdot g^{-1}$)	0.014^{c}	0.003^{c}	ND	5.98^{a}	ND	2.07^{b}

PP. Papantla, Ver; MR. Morelos; PB. Puebla; IN. India; SN. Sinaloa; IS. Isla, Ver. Means with different letters in each row are statistically different (Tukey, $p < 0.05$).

detectable to 5.98 $mg \cdot g^{-1}$ (Table 5). Germplasms IS and IN showed the highest concentrations of phorbol esters (2.07 and 5.98 $mg \cdot g^{-1}$, respectively), compared with germplasms PB and SN that showed undetectable levels.

Table 6. Mineral composition in seeds of six *J. curcas* germplasms from different origin grown in Sinaloa.

Variable	Germplasm					
	PP	MR	PB	IN	SN	IS
Calcium (%)	0.52^{b}	0.59^{ab}	0.54^{b}	0.27^{c}	0.58^{ab}	0.63^{a}
Potassium (%)	0.64^{a}	0.69^{a}	0.72^{a}	0.28^{b}	0.67^{a}	0.73^{a}
Magnesium (%)	0.56^{b}	0.70^{a}	0.65^{ab}	0.31^{c}	0.63^{ab}	0.68^{ab}
Sodium (mg·L^{-1})	801.84^{a}	1150.78^{a}	1002.66^{a}	528.43^{a}	1046.61^{a}	933.38^{a}
Zinc (mg·L^{-1})	49.18^{a}	50.64^{a}	53.40^{a}	26.84^{b}	48.58^{a}	55.20^{a}
Iron (mg·L^{-1})	49.95^{a}	41.29^{a}	37.35^{a}	20.44^{a}	31.83^{a}	36.34^{a}
Manganese (mg·L^{-1})	27.86^{b}	32.78^{ab}	36.08^{a}	9.57^{c}	32.59^{ab}	33.13^{ab}
Copper (mg·L^{-1})	15.64^{bc}	16.12^{b}	16.82^{b}	9.47^{d}	14.06^{c}	18.85^{a}

PP. Papantla, Ver; MR. Morelos; PB. Puebla; IN. India; SN. Sinaloa; IS. Isla, Ver. Means with different letter in a row are statistically different ($p < 0.05$).

DISCUSSION

All six *J. curcas* germplasms, grown in the environmental conditions of Sinaloa showed differences in their phenological development. This suggests a possible influence of the genetic diversity, although this latter has not been confirmed. Environmental conditions were not suitable for the development of the Indian germplasm since that showed faster emergence (in plastic bags) but lower flowering, fruiting and seed yield (Tables 1 and 2).

Plants height was similar to that reported by Ratree (2004), 77 to 110 cm, at 8 months after the transplantation of ten collections from different zones in Thailand. In contrast, Ginwal et al. (2005) reported significant differences in plant height (53 cm, 6 mas) in one out of ten Indian germplasms tested. They assumed that these differences were due to the environmental conditions from the sites where the seeds were collected.

The factors influencing the productivity have been reported by Rao et al. (2008), who found significant differences in the number of branches among 29 Indian germplasms (4 to 12 branches/tree, 34 mas), similar to our results considering the evaluation period of one year without prunning. Jongschaap et al. (2007) showed that flowering and fruiting in *J. curcas* occur homogeneously two years after planting and is stabilized after 5 years from planting. Germplasm IS had more inflorescences than germplasm PP, but the number of fruits was the reverse. This indicates that germplasm PP has better fruit setting, maybe due to the presence of more female flowers since no abortion was observed (Raju and Ezradanam, 2002). Seed yield was higher in germplasms MR, PB and SN (39 to 50 g·plant^{-1}) than in some germplasms reported by Rao et al. (2008) in a 34 months old *J. curcas* crop (36 to 264 g·plant^{-1}) and Ratree (2004) in a two year old crop (2 to 660 g·plant^{-1}). In addition, the seed yield in our experiment was higher than theirs, from plants sown in July (29 g·plant^{-1}), that coincided with the transplanting date in our experiment.

Seeds from India and Isla showed higher seed weight, diameter and kernel weight that can be attributed to bigger canopy and less production of seeds per plant, which caused less competition for photoassimilate compounds among fruit. However, Sirisomboon et al. (2007) and Makkar (2008) reported differences in size and weight between toxic (0.8 g) and non-toxic (0.7 g) *J. curcas* seeds in wild collected seeds.

Lipid content was higher in the germplasm MR and protein content was higher in the germplasms PB and SN. A high oil and protein content was detected in *J. curcas* from the central region of Mexico. However, it is important to compare these results with those of wild seeds and those obtained from seeds grown in other locations to observe their sustainable performance.

The protein level in the germplasms from Papantla and Morelos (24%) was lower than that reported by Martinez-Herrera et al. (2006) in wild seeds (31 and 32%), respectively. That could be due to the environmental and edaphological conditions in the region where the plant were grown, because the nutritional and mineral content in seeds depends on the soil nutrient availability and the absorption ability of the plant (Salisbury and Ross, 1994).

Differences in the phorbol esters content among the six germplasms were due to genetic characteristics and are not due to the environmental conditions since all six germplasms were grown on the same environmental conditions and in the same soil characteristics. In India, the low genetic diversity on *J. curcas* populations has not been associated with the geographic origin (Sudheer-Pamidimarri et al., 2009); whereas, in Mexico a genetic study to test that hypothesis is still needed. In previous studies, Makkar et al. (1997) and Martinez-Herrera et al. (2006) did not detect phorbol esters in wild samples of *J. curcas* from Papantla, Veracruz and Morelos; but in this study low levels of phorbol esters were found. Differences in the detection of phorbol esters in this study, could be attributed to the implemented modifications to the extraction method based on Makkar et al. (2007), such modifications could have increased the ability of the sensors to detect those compounds.

The concentration of phorbols esters in kernels to be considered as toxic remains unspecified; but, the amount of 1.78 $mg \cdot g^{-1}$ phorbol esters in flour is considered toxic according to the study of Aregheore et al. (2003). In that case, seeds from India and Isla germplasms could be considered toxic. The relationship between seed weight and toxicity are in agreement with the study of Makkar (2008), who reported a higher weight and size in toxic seed in comparison with the non-toxic seed, 0.8 and 0.73 g, respectively.

The phorbol esters content in seeds from Isla and India means limited use as food (without previous detoxification), since toxic effects at low concentrations have been reported in animals. For example, Makkar et al. (1998) reported adverse effects on *Cyprinus carpio* L. when supplemented with a diet containing 15 ppm (15 $\mu g \cdot g^{-1}$) of phorbol esters isolated from *J. curcas,* while Aregheore et al. (2003) detected feed and growth reduction in rats fed with phorbol esters at a concentration of 0.13 $mg \cdot g^{-1}$.

Results indicated that germplasms from Puebla, Morelos and Sinaloa have agronomical and chemical potential to be grown and propagated; since they showed a good phenological development and the highest values of yield, seed, oil and protein contents. Furthermore, the propagation of non-toxic *J. curcas* will allow the use of oil extraction by products as feed supplements without the detoxification procedure, further reducing the production costs. However, it is necessary to conduct additional tests, since *J. curcas* still is a non-domesticated plant, which requires selection and breeding programs.

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