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ORIGINAL ARTICLE

Morphological Parameters of *Jatropha curcas* L. seeds and their effect on Germination

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ABSTRACT
The present study examines the effects of the morphometric parameters of an energy crop, Jatropha curcas seeds, on their germination potential. These parameters include the weight, the height, the width, the thickness and the size of caruncle. The study was carried out on seeds of seven genotypes of Jatropha curcas L. The seeds were sown in a greenhouse and their germination was monitored. The results showed that the JAT 072 genotype has the highest weight, width and thickness values, i.e. 0.80 g, 11.56 mm and 9.35 mm respectively. The largest height was observed in the Laos genotype (19.20 mm), whereas the Senegal genotype had the lowest height (17.27 mm). Using different indices germination, we found that all genotypes germinated well but on different dates. The germination rate, however, varied according to genotypes. The Michoacán and Laos had the highest germination percentage (88.3 %), while the lowest one (16.88 %) was observed in the JCL Max. The maximum speed of germination was 25.15 and 30.6 observed in the Michoacán and Mali genotypes respectively, while the minimum speed was found in the Senegal genotype (4.38). The mean germination time of 6.92 was measured for the Michoacán genotype. The Laos genotype presented the highest mean daily germination and the germination value of 1.15 and 0.46 respectively. The study shows that there is a significant difference between the weight of germinated and non-germinated seeds in the case of JCL Max, Michoacán, Laos and Mali genotypes; the germinated seeds being heavier. However, there is no significant difference for the QVP 3014, JAT 072 and Senegal genotypes.
Keywords: *morphometric parameters, germination index, genotypes, Jatropha curcas L.*

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INTRODUCTION
Jatropha curcas L. (JatCur) receives a lot of attention as a source of renewable energy, last decades [1]. It is a perennial shrub or small tree belonging to the *Euphorbiaceae* family [2,3]. It is a semi-evergreen species shedding the leaves under stress conditions; normally grows up to 3-5 m in height, but it can reach 8-10 m under favorable conditions [4,5]. Its origin is from Central America but now it can be found throughout the tropical and subtropical region [6,7]. It grows in arid and semi-arid climates with rainfall ranging from 250 mm to 1200 mm [3,4,8] and at an altitude of up to 800 m where the average daytime temperature ranges from 20°C to 30°C [9,10]. JATCUR can live between 40 to 50 years and produces fruits for 25-35 years [5,6,11]. The cultivation of this plant can be performed either from seeds or from cuttings [12]. JATCUR has been recognized as a source of medium viscosity pure plant oil (PPO) that is easily converted to a good quality of biodiesel [5,13]. Both JATCUR pure plant oil and biodiesel have been tested successfully in stationary diesel engines [14,15]. The growing global biodiesel market has led investors and project developers to consider JATCUR biodiesel as a promising substitute for fossil fuels in order to reduce greenhouse gas emissions. JATCUR can also be used for the improvement of soil quality [6,16] and for the exploitation of marginal lands [17,18].

When planted as a hedge, *Jatropha* is not eaten by animals and, thus, is commonly used to protect food crops [4,19]. *Jatropha* oil also has the characteristic of inhibiting the corrosion of steel which can be used in industry [20].

Seed quality is an important aspect in the cultivation, agricultural production and industrial processing of JATCUR. The seed is formed of an oily endosperm containing the embryo provided with two flattened broad cotyledons. The seeds' weigh is composed of 53-62 % of dry fruit [21] and 15% of fresh fruit [22].

Germination is the first stage on a seeds development. It occurs only if external conditions such as humidity, temperature, access to oxygen, and conditions of storage are favorable. The germination's success also depends on internal factors such as maturity and seed's viability [9] and it includes four main phases [23]:

- The imbibition phase of a seed, results in a steady and significant increase of respiratory activity,
- The germination phase *sensu strict* is marked by stop of absorption of water and regular respiratory activity,
- The radical development is characterized by a resumption of water uptake and an increase respiratory activity,
- During the emergence stage where the stalk elongates until it pierces the peat and produces small leaves.

The correlation between germination and the seed physiological and biochemical characteristics have been widely investigated [24]. However, the correlation between seed morphometric parameters and seed germination success has not been well studied, especially in the case of JATCUR.

The main objective of this paper is to study the morphometric parameters (weight, height, width, thickness and caruncle size) of JATCUR seeds of different origins and to determine if these parameters have an effect on germination.

MATERIALS AND METHODS

Plant Material and measured morphometric parameters

Seven JATCUR genotypes collected from three continents were studied (Tab.1).

The seed's morphometric parameters studied are weight, height, width, thickness and size of the caruncle (Fig. 1). The measurements of seed morphometric parameters were carried out in order to identify the variability within each genotype and between genotypes. The measurements were performed on 77 seeds randomly selected from each genotype shown in Table 1.

Experimental setup

After measuring the morphometric parameters, the seeds were placed in small cups and soaked in tap water for 12 h. The seeds of each genotype were sown at a depth of 2 cm in a germination tray of 77 cells filled with commercial peat with caruncle downward. After sowing, the seeds were immediately irrigated with tap water. The cells were covered with newspapers to keep the peat moist. Thereafter, the irrigation was done twice per week directly on the newspapers in order to keep the substrate moisture. When the first seedlings emerged the newspapers were removed from the samples. The experiment was conducted in a greenhouse of the Faculty of Sciences Semlalia, Marrakech. The germination process was monitored every day for 4 months (February-May 2012). During this period, the recorded temperature ranged from a minimum of 16°C in February to a maximum of 41°C in May and the averaged greenhouse humidity was 61 %.

Germination monitoring

Daily counts of germinated samples were conducted in order to calculate appropriate indexes for germination description which allow for intra and inter genotype comparison. The average percentage of germination (PG) was calculated by dividing the number of germinated seeds by the total number of sown seeds per genotype. To quantify the germination, several indexes were used, namely: Speed of germination (SG), the mean of germination time (MGT), the mean daily germination (MDG), the peak value (PV) and the value of germination (VG) as defined in Table 2 [25,26].

Statistical Analysis

Statistical analysis was performed with the STATISTICA software version 6 [27], and statistical significance of differences between the parameter measured from each genotype were determined by means of Student t-test ($p<0.05$). The cluster analysis for grouping genotypes having similar morphometric parameters was conducted by hierarchical cluster analysis or HCA.

RESULTS AND DISCUSSION

Morphometric measurements on different genotypes

Weight

JAT072 had the highest weight value (0.80 g), followed by Laos genotype (0.79 g). The Senegal genotype, on the other hand, showed the lowest mean value (0.56 g) (Tab. 3). These results agree with those of Asim *et al*. [28] who reported a weight of 727 g for 1000 seeds, which an average of 0.727 g per seed of JATCUR. Another study made by FACT Foundation [21] showed that the range of weight of 550g to 800g for 1000 seeds, which average of 1400 seeds/kg. Zheng *et al*. and Shabanimofrad *et al*. [29,30] also gave the weight from 0.5 to 0.75 g per seed. The weight of *J. curcas* is bigger relative to the weight of seeds of castor with average value of 0.30 g, which is a species that belongs to the same family of *J. curcas* and used to extraction of vegetable oil [31].

Height

The highest value was observed in Laos genotype (19.20 mm), followed by JAT072 (18.88 mm) (Tab. 3), while the Senegal genotype had the lowest height (17.27 mm). These values are within the range of 11mm to 30 mm reported by Asim *et al*. [28] corresponding to an average height of 18 mm. Garnayak *et al*. [32] realized a study to determine the interaction between moisture and the physical properties of *Jatropha* seeds. This study gave similar results with our experiment, namely the height of the seeds was 18.65 mm to 19.21 mm according to moisture content. A similar data has been reported by Shabanimofrad *et al*. [30].

Width

The measurements of the width of the different genotypes showed that JAT072 had the highest value (11.56 mm) followed by Mali (11.44 mm). On the other hand, JCL Max, Senegal and Michoacán genotypes gave widths that were below the average value (Tab. 3). Asim *et al*. [28] found that the width of JATCUR seeds range from 7 mm to 11 mm with an average value of 10 mm. An experiment was conducted by Shabanimofrad *et al*. [30] on 48 Malaysian accessions, to assess morphological variation among JATCUR accessions. The results gave the width of 11.48 mm. Garnayak *et al*. [32] gives a range of 11.34 mm to 11.85 mm.

Thickness

Genotype JAT072 was the thickest (9.35 mm), followed by the Laos genotype (9.11 mm). The lowest value was found in Michoacán genotype. This genotype showed both the lowest width and thickness compared with other genotypes. The average value given by FACT Foundation and Agarwal [13,21] is 10 mm (Tab. 3). A range of 8.91 mm to 9.48 mm was reported by Garnayak *et al*.[32].

Caruncle

No significant differences in the size of the caruncle were found between the various genotypes. The minimum value (3.19 mm) was found in the Michoacán genotype and the maximum value of 4.37 mm was measured in the Senegal genotype, followed by genotype JCL Max with 4.26 mm. These values are above the widely accepted average of 3.91 mm (Tab. 3).

To determine the correlation between genotype studied based on morphometric parameters, we realized a bivariate correlation. The results given in table 3 showed that all genotypes exhibit a highly significant correlation at $p \leq 0.01$, depending of analyzed parameters (weight, height, width, thickness and dimension of the caruncle). The same results founded by Shabanimofrad *et al*. [30] between 48 accessions of JATCUR at $p \leq 0.01$.

To evaluate the relationships between genotypes based on their morphometric parameters the cluster analysis hierarchical cluster analysis or HCA was used which allows to group genotypes that have similar morphometric parameters. In the dendrogram, the 7 genotypes appeared to form 3 major clusters at similarity coefficient of 0.80 (Fig 2). Cluster **G1** contains only Michoacán which represents the lowest value of morphometric parameters, cluster **G3** composed of JAT072 and Laos which have the highest values, and cluster **G2** (JCL Max, Senegal, Mali, and QVP3014) which have values that are between those of G1 and G2. The same results were reported by Rao *et al*. and Shabanimofrad *et al*. [30,33] classified JATCUR according to origin using 11, 14 morphological characters respectively .

Germination genotypes

Global rate of germination.

The highest germination rate was found in the Michoacán and Laos genotypes (88.31%). The lowest percentages were recorded in the JCL Max and Senegal genotypes with 16.88% and 19.5% respectively. For all other genotypes, the percentage varied between 70.1% (JAT 072) and 58.4% (QVP3014) (Table 4).

Germination evolution in time

Evolution of germination presented in figure 3 shows that the Mali genotype initially had a high germination rate and then it remains constant until 09 May; after this date the seed germination restart and a significant number of seeds germinated. QVP3014 genotype followed with a lower number of germinated seeds than Mali genotype. The Michoacán, Laos and JCL Max genotypes had a number of germinated seeds significantly lower than Mali and QVP3014 until 09 May; after this date, the Michoacán

and Laos genotypes presented an increased rate of germination and reached the highest percentage. JCL Max germination remained low with a slight increase after the 16 May. The latest genotypes germinated were Senegal and JAT072 with a low percentage of germination. After 9 May, a germination increase was observed for the JAT072 genotype, while that of Senegal genotype remained low.
The lower number of germinated seeds during March and April was observed probably due to the lower temperatures occurred during this period. In May, the temperature increased resulting in an increased number of germinated seeds.

Germination index of JATCUR genotypes.

Germination index of different genotypes (Table 5) showed significant variations. The Mali and Michoacán genotypes presented the maximum values for the speed of germination (SG) which were up to 30.61 and 25.15 respectively, while the minimum speed of 4.38 was recorded for the Senegal genotype.
Michoacán genotype showed the highest values in terms of Mean Germination Time (MGT) (6.92) while the QVP3014 genotype showed the minimum MGT value (2.22). The highest Mean Daily Germination (MDG) value (1.15) was observed for the Laos genotype while the minimum MDG value (0.11) was recorded in the JCL Max genotype. The highest peak value of germination (0.40) was observed in the Laos genotype and the lowest value (0.04) recorded for the JCL Max genotype. On the other hand, the highest Germination Value (GV) (0.46) was recorded for Laos genotype and a minimum value (0.004) recorded for the JCL Max genotype.

Relationships between morphometric parameters of seeds and germination

The results presented in Table 6 showed that JCL Max, Michoacán, Laos and Mali genotypes have a significant weight difference between the germinated and non-germinated seeds, the germinated seeds having higher weights. The results also showed that there is no significant difference between the morphometric parameters of germinated and non-germinated seeds for genotypes QVP3014, JAT072 and Senegal.
Daghar *et al.* and Singh *et al*. [34,35] conducted a study on the classification and quality of *JATCUR*. seeds based on the weight of seeds. Their results showed that seeds that weigh more have a higher seed germination rate. These results correspond to those observed in the JCL Max, Michoacán, Laos and Mali genotypes.
To reveal the relationship between seed size and germination, He *et al*. [36] performed a study on a plant native to China's Qinghai-Tibet Plateau; *Ligularia virgaurea.* The results obtained showed that in comparison to small seeds, the large seeds have a higher germination percentage. In a similar study conducted by Hojjat [37], this time on 24 genotypes of lentils, the results show that seeds of a larger size have higher percentage of germination than smaller seeds. In our study, this is the case with the JCL Max, Michoacán, Laos and Mali genotypes.

CONCLUSION

After four months of the study aimed at evaluating the effects of morphometric parameters on germination in seven JATCUR genotypes, the results of the experiment show that:
The JAT 072 and Laos genotypes have the highest weight, height and thickness among the studied genotypes. The lowest morphometric values were observed in the Senegal genotype.
The Michoacán and Laos genotypes have a higher germination rate (88.38 %), while the lowest rates of 16.88% and 19.5% are present in the JCL Max and Senegal genotypes respectively.
Mali and Michoacán genotypes have maximum speed of germination of 30.61 and 25.15 respectively. The highest mean germination time (6.29) was observed in the Michoacán genotype. The Laos genotype presents the highest value for mean daily germination (1.15) and the highest germination value (0.46).
This study of the relationship between seeds' morphometric parameters and their germination shows that there is a significant difference between the weight of germinated and non-germinated seeds in the case of JCL Max, Michoacán, Laos and Mali genotypes; the germinated seeds being heavier. However, there is no significant difference between the morphometric parameters of germinated and non-germinated seeds for the QVP3014, JAT 072 and Senegal genotypes.

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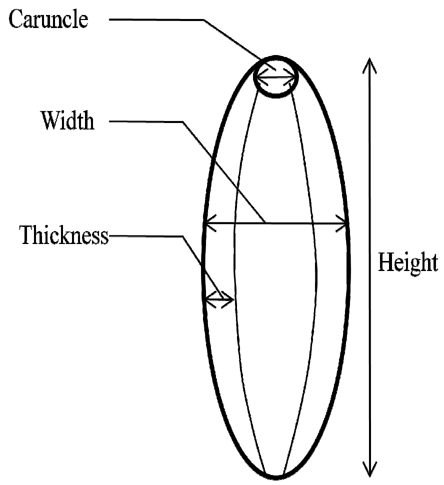

Figure1. Measured morphometric parameters of JATCUR seeds.

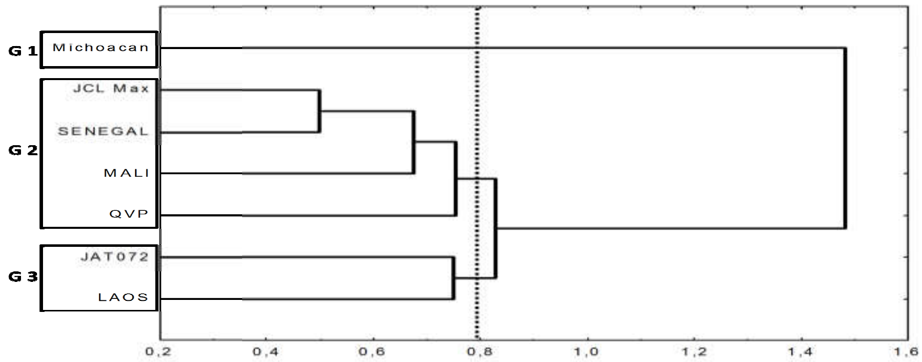

Figure 2. Clustering genotypes JATCUR according to their morphometric parameters.

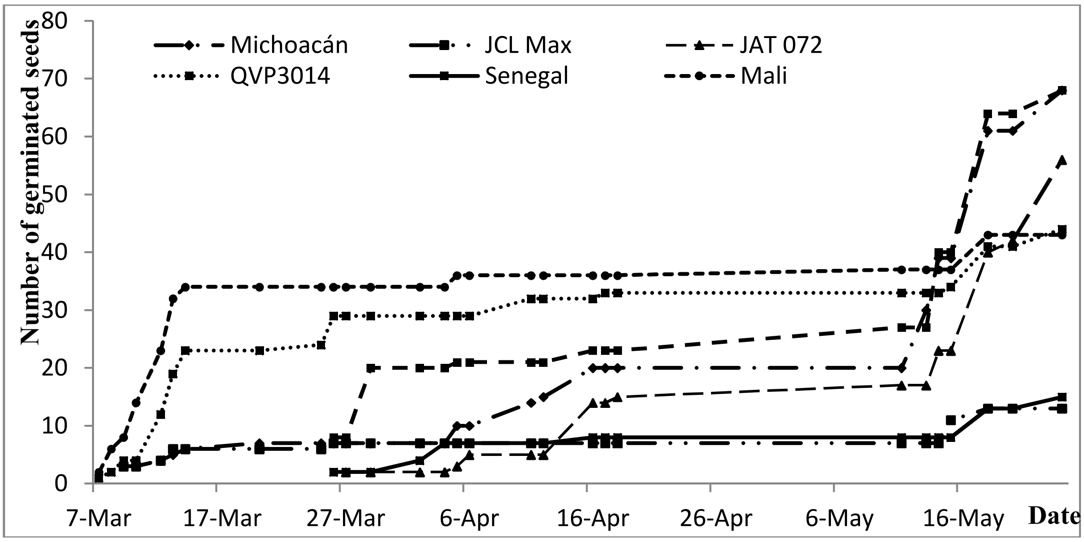

Figure 3: Evolution of germination in time

Table 1. JATCUR genotypes under investigation and their origins

	Genotypes	Origins
1.	**Michoacán**	Mexico
2.	**JCL Max 3.0**	India
3.	**JAT072**	Vietnam
4.	**QVP3014**	India
5.	**Mali**	Mali (grown in Morocco)
6.	**Laos**	Laos (grown in Morocco)
7.	**Senegal**	Senegal

Table 2. Germination parameters used[25,26]

Germination index	Formulas
Germination percentage (PG)	PG = germinated seeds/total seeds x 100
Speed of germination (SG)	SG = $n_1/d_1+n_2/d_2+n_3/d_3$ +………
Mean germination time (MGT)	(MGT) = n_1 x d_1 + n_2 x d_2 + n_3 x d_3 +…/ Total days of germination
Mean daily germination (MDG)	MDG = total number of germinated seeds / total number of days of germination
Peak value (PV)	PV= maximum germination/ Number of days
Germination value (GV)	GV= MGT /PV

Where: n = number of germinated seeds and d = number of days from sowing.

Table 3. Morphometric parameters of *Jatropha curcas* seeds.

	Michoacán	JclMax	JAT072	QVP	Mali	Sénégal	Laos
Weight (g)	0.66±0.08[a]	0.64±0.10[ab]	0.80±0.03[d]	0.74±0.10[c]	0.61±0.12[b]	0.56±0.09[e]	0.79±0.06[d]
Height (mm)	18.12±0.81[c]	17.66±0.95[ab]	18.88±0.47[d]	18.37±1.20[c]	17.74±1.43[bc]	17.28±0.80[a]	19.20±0.58[d]
Width (mm)	10.17±0.45[a]	11.02±0.48[b]	11.56±1.04[d]	11.18±0.47[bc]	11.44±0.55[cd]	11.03±0.48[b]	11.16±0.30[b]
Depth (mm)	8.29±0.39[a]	8.79±0.49[c]	9.35±0.42[e]	8.83±0.56[c]	8.62±0.44[bc]	8.52±0.55[b]	9.11±0.25[d]
Size of caruncle (mm)	3.19±0.51[a]	4.26±0.57[cd]	4.08±0.46[c]	4.14±0.43[c]	3.76±0.45[b]	4.37±0.46[d]	3.59±0.32[b]

Different letters indicate significant difference between genotypes at $p < 0.05$.

Table 4. Correlation coefficients among genotypes studied.

	Michoacán	JCL Max	Jat 072	QVP3014	Mali	Senegal
Michoacán	1.000					
JLC Max	0.996[a]	1.000				
Jat 072	0.998[a]	0.999[a]	1.000			
QVP3014	0.998[a]	0.999[a]	0.999[a]	1.000		
Mali	0.995[a]	0.999a	0.999[a]	0.998[a]	1.000	
Senegal	0.994[a]	0.999a	0.998[a]	0.999[a]	0.998[a]	1.000
Laos	0.999[a]	0.997[a]	0.999[a]	0.998[a]	0.997[a]	0.996[a]

a: correlation is significant at 0,01

Table 5. Number and percentage of germinated seeds genotypes JATCUR.

	Michoacán	JCL Max	JAT 072	QVP3014	Mali	Laos	Senegal
Number of sowing seeds	77	77	77	77	77	77	77
Number of germinated seed	68	13	54	45	46	68	15
%	88,3[a]	16,88[d]	70,1[c]	58,4[b]	59,74[b]	88,3[a]	19,5[d]

Different letters indicate significant difference between genotypes at $p < 0.05$.

Table 6: Germination index of genotypes

	Michoacán	JCL Max	JAT072	QVP3014	Mali	Laos	Senegal	Global mean
SG	25,15	6,16	16,11	24,98	30,61	23,65	4,38	18.72
MGT	6,92	4,28	5,13	2,22	3,11	5,63	3,52	4.40
MDG	0,92	0,11	0,98	0,54	0,98	1,15	0,26	0.71
PV	0,30	0,04	0,30	0,10	0,20	0,40	0,09	0.20
GV	0,28	0,004	0,30	0,06	0,20	0,46	0,02	0.19

SG: Speed of germination; MGT: the mean germination time; MDG: mean daily germination; PV: the peak value. GV: the germination value.

Table 7: Mean of morphometric parameters of seeds and Student's test for genotypes.

	Weight (g)		Height (mm)		Width (mm)		Thickness (mm)		Size of caruncle (mm)	
	Mean of germinated seeds	Mean of non germinated seeds	Mean of germinated seeds	Mean of non germinated seeds	Mean of germinated seeds	Mean of non germinated seeds	Mean of germinated seeds	Mean of non germinated seeds	Mean of germinated seeds	Mean of non germinated seeds
Michoacán	0.67	0.59	18.14	17.93	10.16	10.25	8.31	8.13	3.2	3.15
JCL Max	0.7	0.63	17.74	17.64	11.03	11.02	8.88	8.77	4.28	4.25
JAT072	0.8	0.8	18.84	18.97	11.42	11.9	9.38	9.29	4.09	4.05
QVP3014	0.76	0.72	18.53	18.17	11.16	11.2	8.85	8.8	4.14	4.13
Mali	0.65	0.57	17.83	17.62	11.48	11.38	8.76	8.44	3.82	3.69
Senegal	0.57	0.56	17.26	17.28	11.17	11	8.45	8.53	4.32	4.38
Laos	0.8	0.75	19.2	19.13	11.16	11.15	9.12	9.07	3.57	3.78
	Student's test of genotypes									
Michoacán	t	-2.787	-0.795		0.587		-1.349		-0.441	
	p	0.007	0.429		0.559		0.181		0.664	
JCL Max	t	-2.226	-0.356		-0.101		-0.692		-0.146	
	p	0.029	0.723		0.920		0.491		0.884	
Laos	t	-1.956	-0.358		-0.73		-0.585		1.792	
	p	0.054	0.722		0.942		0.560		0.077	
JAT072	t	-0.647	0.842		1.144		-0.789		-0.511	
	p	0.519	0.402		0.266		0.433		0.611	
QVP3014	t	-1.587	-1.323		-0.399		-0.413		-0.118	
	p	0.117	0.190		0.691		0.681		0.907	
Mali	t	-3.203	0.651		-0.804		-3.377		-1.5850	
	p	0.002	0.517		0.424		0.001		0.117	
Senegal	t	-0.217	0.084		-1.222		0.492		0.464	
	p	0.829	0.933		0.226		0.624		0.644	

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