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Natural variation in fruit characteristics and seed germination of *Jatropha curcas* in Benin, West Africa

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SUMMARY
An investigation was conducted in the ten phytodistricts of Benin, West Africa, in order to assess the level of morphological variation in *Jatropha curcas* seed and their germination potential. Hierarchical classification of the morphological traits of seeds identified five morphotypes, using 54% of the overall morphological information. Canonical discriminant analysis performed on the five morphotypes revealed highly significant differences. Morphotype 1 included seeds from the phytodistricts of Côtier, Pobè, The Ouémé Valley, and Plateau. Morphotype 2 and Morphotype 5 seeds were from the phytodistricts of Bassila, Zou, and Borgou-Sud; while Morphotype 3 and Morphotype 4 seeds were from the phytodistricts of Borgou-Nord, the Atacora Chain, and Mekrou-Pendjari. Significant morphological variation existed within the seeds as a consequence of genetic make-up and/or environmental effects. Seed from Mekrou-Pendjari and the Atacora Chain were black, smooth, light (0.67 g seed^{-1}), and small (1.76 cm-long and 1.15 cm-wide). Seed from Plateau, Côtier, The Ouémé Valley, and Pobè zones were brown, rough, heavy (0.84 g seed^{-1}), and large (1.97cm-long, 1.2 cm-wide). All seed germination started 4 d after sowing and ended between day-7 and day-8. Seed germination timing varied significantly with morphotype.

Attempts are now being made to promote the widespread cultivation of plants or crops previously grown only in localised areas. Species such as *Jatropha curcas* L., which can be grown for processing into biodiesel, have captured the attention of researchers in temperate and tropical zones. *J. curcas* is well-adapted to semi-arid marginal areas. The oil from *J. curcas* can be processed and used as a substitute for diesel fuel, while growing the plant can control soil erosion (Heller, 1996; Foidl and Elder, 1997; Subramanian *et al.,* 2005). The seeds, leaves, and bark of *J. curcas* are also used in traditional medicine and for veterinary purposes (Assogbadjo *et al.*, 2009). *J. curcas* can be propagated by cuttings and through seed. If propagated by seed, the plant develops a single tap-root structure (Padonou, 2009). However, low seed viability limits the efficiency of propagation by seed (Gadekar, 2006). When using cuttings, no tap-root will develop and the root system will develop into a dense and superficial carpet of adventitious roots, suitable for preventing soil erosion and for accumulating sediment, but vulnerable to landslides and uprooting by wind (Padonou, 2009).

Such a multiple-use crop requires genetic improvements to promote cultivation for oil production, the prevention of erosion, as well as medical and veterinary purposes. Currently, crop improvement in *J. curcas* has been limited (Ginwal *et al.*, 2005). *J. curcas* has high economic importance in Benin. Indeed, *J. curcas* plants were exported from Benin to France in the 1940s for the production of household soap and were widely used as hedges in the North of Benin in the 1990s (Global Facilitation Unit for Underutilized Species, 2007). However, few scientific data are available on the natural variation in seed morphology and seed germination in this species in Benin (Assogbadjo *et al.*, 2008; 2009; Padonou, 2009) although *J. curcas* has recently been considered by the Benin Government as a priority tree species to be developed in agroforestry systems for bio-fuel production. In addition, significant genetic variation exists in seed morphology and in the oil content of *Jatropha* seed. This is important for tree improvement programmes. Unfortunately, little work has been done on germplasm conservation (Kumar and Sharmar, 2008). Kaushik *et al.* (2007) reported some variation in seed traits and oil contents in 24 accessions of *J. curcas* collected in Haryana State, India. Consequently, one important question to be addressed was the possible link between seed morphology and environmental factors (or phytodistricts) in Benin.

Since no information was currently available, the first objective of the present study was to assess the level of natural variation in seed morphology in order to identify morphotypes of *J. curcas* based on seed characteristics. If the morphological variation in seeds could be correlated with their provenance and/or linked to their germination parameters, it would be possible to predict which seed provenances were most suitable for large-scale propagation. The second objective of this study was to compare the rates of germination and the germination parameters of the different morphotypes of *J. curcas* seed, our hypothesis being that there was a strong correlation between the morphological variation in seed and their germination ability.

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TABLE I
Characteristics of the ten phytogeographical districts of Benin

PChor‡	Phytogeographic district	R¶	Rainfall (mm)	Major soil type	Major plant formation
GCR	Côtier	Bi	900–1,300	Sandy + hydromorphic & allomorphic soils	Coastal forest and derived thickets, mangrove
GCR	Pobè	Bi	1,200–1,300	Ferralitic soils without concretions	Semi-deciduous forest
GCR	Plateau	Bi	900–1,100	Ferralitic soils without concretions	Semi-deciduous forest
GCR	Vallée de l'Ouémé	Bi	1,100–1,300	Hydromorphic soils	Swamp and semi-deciduous forest
SGR	Bassila	TUn	1,100–1,300	Ferralitic soils with concretions and breastplates	Semi-deciduous forest, woodland, and riparian forest
SGR	Zou	TUn	1,100–1,200	Ferruginous soils on crystalline rocks	Dry forest, woodland, and riparian forest
SGR	Borgou-Sud	TUn	1,100–1,200	Ferruginous soils on crystalline rocks	Dry forest, woodland, and riparian forest
SR	Borgou-Nord	Un	1,000–1,200	Ferruginous soils on crystalline rocks	Dry forest, woodland, and riparian forest
SR	Chaîne de l'Atacora	Un	1,000–1,200	Poorly evolved & mineral soils	Riparian forest, dry forest, and woodland
SR	Mékrou-Pendjari	Un	950–1,000	Ferruginous soils with concretions on sedimentary rocks	Tree and shrub savannahs, dry forest and riparian forest

‡PChor, phytochorological zones based on the composition in distribution range types; GCR, Guineo-Congolian region; SGR, Sudano-Guinean transition zone; SR, Sudanian region.
¶R, rainfall regime; Bi, bimodal (two rainy seasons); TUn, tendency to unimodal; Un, unimodal (one rainy season).

MATERIALS AND METHODS

Seeds of *J. curcas* were collected from each of the ten phytogeographical districts (phytodistricts) of Benin, (Figure 1). Ten trees, spaced at least 100 m from each other to avoid narrowing the genetic base due to relatedness or inbreeding, were selected and sampled in plantations in each of the ten phytodistricts, as recommended by Turnbull (1975). Mature black fruit ($n = 10$) were collected directly from each tree. A total of 100 seeds were therefore collected at random in each phytodistrict.

The colours of the dried seeds of *J. curcas* were determined using a standard colour chart (Royal Horticultural Society, 1966). Seeds were coded 1 if black, or 0 if brown. The length, width, and thickness of each seed were measured using electronic calipers. Seed texture was determined by touch, and coded 1 if rough, or 0 if smooth. The weight of each seed was measured using an electronic balance with a sensitivity of 0.0001 g.

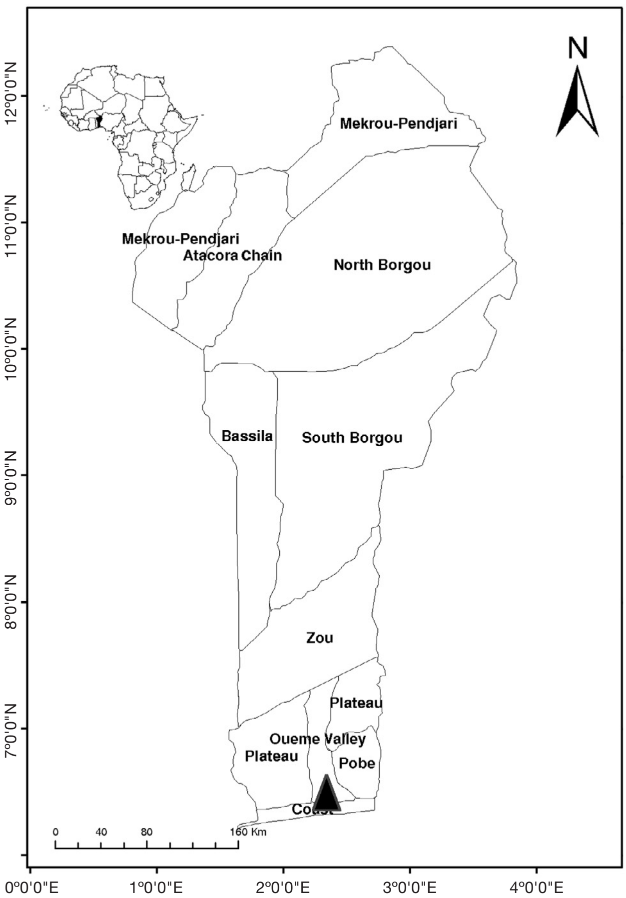

FIG. 1
The ten phytogeographical districts (phytodistricts) of Benin, West Africa. Inset shows the location of Benin. The black triangle marks the location of the township of Abomey-Calavi.

Seed length, width, thickness, weight, colour, and texture data were subjected to an Ascending Hierarchical Classification (AHC) using SAS statistical software (SAS, 2003). This enabled classification of the seeds based on similar morphological traits. Canonical discriminant analysis was then performed on the morphotypes identified from the AHC in order to validate and test the differences between morphotypes. The clusters (considered to be morphotypes) of *J. curcas* seed were also defined by their differences, using discriminant axes defined by the seed traits being measured. The same analysis was also performed to test and describe the differences between the ten phytodistricts according to the morphological parameters of the *J. curcas* seed.

Five morphotypes were identified from the AHC. Five seeds of each morphotype were sown in a single pot made from a polythene bag measuring 5.5 cm × 18 cm and filled with forest soil. Ten pots were used for each morphotype. The experimental units (all pots) were arranged in a randomised block design with three replicates. For each morphotype, the number of seeds that germinated in all ten pots was recorded each day over a 10 d period. The nursery experiment was carried out at the University of Abomey-Calavi, located between 6°21' – 6°42'N and 2°13' – 2°25'E in the Côtier phytodistrict, in March 2009 (Figure 1).

The germination percentage of each morphotype of *J. curcas* seed was calculated each day over 10 d and used to measure the effect of time and morphotype on the rate of germination of *J. curcas* seed. The statistical test used was analysis of variance on repeated measures (Crowder and Hand, 1990) available in SAS statistical software (SAS, 2003), using the mixed model. In this model, the factor "block" was considered to be random, whereas the factor "morphotype" was considered to be fixed. No data transformation was applied to the germination percentages because normality and homoscedasticity were checked without transformation using the Ryan-Joiner test of normality and the Levene

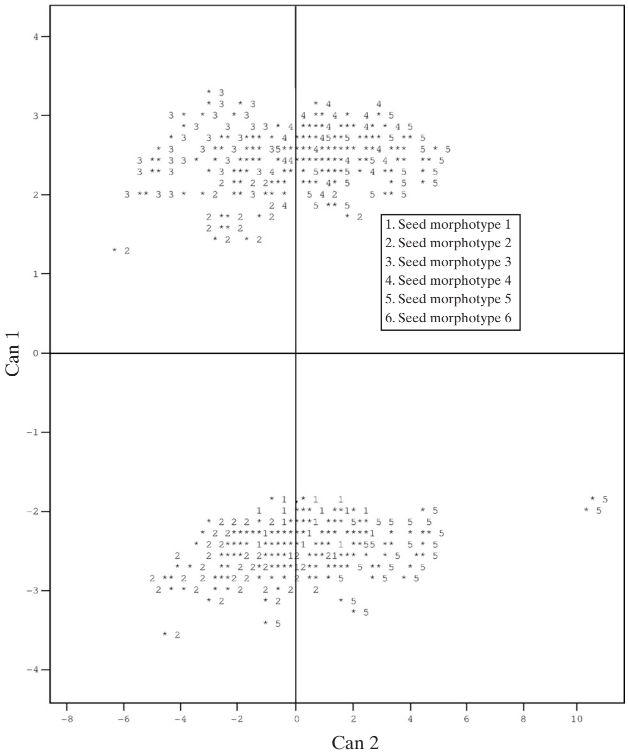

Fig. 2
Projection of the five morphotypes of *J. curcas* seed on the canonical axes defined by morphological traits. Can 1 discriminated on seed colour, texture and weight. Can 2 discriminated on seed size.

test for homogeneity of variances (Glèlè Kakaï *et al.*, 2006).

RESULTS

Identification of J. curcas *morphotypes*

Five clusters (morphotypes) were identifed from the AHC using 54.2% of the information recorded on all seeds. The results of canonical discriminant analysis performed on the five morphotypes of *J. curcas* seed showed that the Mahalanobis distances between pairs of the five clusters identified were all highly significant ($P \leq 0.001$). The morphotypes identified from the AHC were therefore different according to the morphological traits of *J. curcas* seed. Morphotype 1 was derived from the Côtier, Pobè, The Ouémé Valley, and Plateau phytodistricts. Morphotypes 2 and 5 were from the Bassila, Zou, and Borgou-Sud phytodistricts; whereas Morphotypes 3 and 4 were from Borgou-Nord, the Atacora Chain, and Mekrou-Pendjari phytodistricts.

Other results from the canonical discriminant analysis performed on individuals of the five seed morphotypes revealed that the first two axes were significant ($P \leq 0.05$) and explained 61.6% of the variation seen in the morphotypes. The coefficient of correlation between the two canonical axes (Can) and the morphological traits of *J. curcas* seed indicated that the first axis (Can 1) discriminated the morphotypes according to seed colour, texture, and weight. On this axis, heavy seeds were also often black and smooth. The second axis (Can 2) discriminated the five morphotypes according to the size of the seed. It showed that long seeds were also wide and thick.

Seeds from Morphotype 3 and Morphotype 4 were black, light, and smooth, while seeds from Morphotype 1 were brown, heavy, and rough (Figure 2). Most seeds from Morphotype 2 and Morphotype 5 were brown, heavy, and rough, but some were dark, light, and smooth. The Can 2 axis (Figure 2) discriminated the five morphotypes according to the size of their seed. For example, Morphotype 2 and Morphotype 3 differed from Morphotype 4 and Morphotype 5 according to the length, width, and thickness of their seed. Seeds from Morphotype 2 and Morphotype 3 were the smallest. A more detailed description of each morphotype is provided in Table II, from which we observed that Morphotype 5 had the longest seeds (mean = 1.91 cm), while the shortest seeds were from Morphotype 3 (mean = 1.77 cm). The widest seeds were found in Morphotype 1 and Morphotype 4 (mean = 1.2 cm), whereas the thinnest seeds were from Morphotype 3 (mean = 1.08 cm). With regard to seed weight, those of Morphotype 1 were the heaviest (mean = 0.80 g), while the lightest seed were grouped in Morphotype 2 (mean = 0.66 g).

On the basis of the ten phytodistricts, we noticed that seeds collected from Mekrou-Pendjari or the Atacora Chain were black, smooth, lightweight (mean = 0.67 g), short (mean = 1.76 cm), and thin (mean = 1.15 cm), while seeds from Plateau, Côtier, The Ouémé Valley, and Pobè were brown, rough, heavy (mean = 0.84 g), long and wide (mean length = 1.92 cm; mean width = 1.2 cm). Seeds from Zou, Borgou-Sud, and Bassila were black, smooth, and heavy, but some had the opposite features (Table III).

Germination ability of J. curcas *seed according to morphotype*

The germination ability of each seed morphotype varied over time between sowing and 10 d after sowing (Table IV). The germination percentages of seeds also varied throughout the 10 d. The blocking factor and all its interactions were non-significant, indicating homogeneity of the environmental characteristics within and between the blocks. Morphotypes 1, 2, and 3 showed a rapid increase in germination percentage from 4 d to 10 d after sowing (Figure 3). Morphotypes 4 and 5 showed less variation in germination percentage over time.

Table II
Mean values and standard deviations of traits in the five morphotypes of J. curcas *seed*

Seed trait	Morphotype 1	Morphotype 2	Morphotype 3	Morphotype 4	Morphotype 5
Length (cm)	1. 87 ± 0.09[†]	1.80 ± 0.12	1.77 ± 0.10	1.85 ± 0.10	1.91 ± 0.09
Width (cm)	1. 20 ± 0.01	1.11 ± 0.06	1.09 ± 0.03	1.20 ± 0.01	1.18 ± 0.07
Thickness (cm)	0.73 ± 0.05	0.71 ± 0.07	0.69 ± 0.05	0.73 ± 0.07	0.98 ± 0.07
Weight (g)	0.80 ± 0.07	0.66 ± 0.16	0.72 ± 0.09	0.71 ± 0.09	0.74 ± 0.11
Colour	Brown	Black/Brown	Black	Black	Black/Brown
Texture	Rough	Smooth/Rough	Smooth	Smooth	Smooth/Rough

[†]All values are means (n = 1,000) ± SD.

TABLE III
Morphometric traits of J. curcas *seed from the ten phytodistricts of Benin*

PChor‡	Phytodistrict	Length (cm)	Width (cm)	Thickness (cm)	Weight (g)	Seed colour	Seed texture
RGC	Côtier	1.85 ± 0.10†	1.2 ± 0.00	0.76 ± 0.08	0.77 ± 0.10	Brown	Rough
RGC	Pobè	1.86 ± 0.07	1.18 ± 0.03	0.71 ± 0.06	0.83 ± 0.09	Brown	Rough
RGC	Plateau	1.85 ± 0.11	1.16 ± 0.06	0.77 ± 0.10	0.76 ± 0.11	Brown	Rough
RGC	Ouémé Valley	1.97 ± 0.05	1.2 ± 0.00	0.76 ± 0.05	0.84 ± 0.12	Brown	Rough
SGR	Bassila	1.85 ± 0.08	1.16 ± 0.05	0.77 ± 0.12	0.72 ± 0.12	Black/Brown	Smooth/Rough
SGR	Zou	1.87 ± 0.10	1.16 ± 0.07	0.78 ± 0.13	0.75 ± 0.09	Black/Brown	Smooth/Rough
SGR	Borgou-Sud	1.81 ± 0.13	1.16 ± 0.05	0.76 ± 0.10	0.74 ± 0.10	Black/Brown	Smooth/Rough
RS	Borgou-Nord	1.83 ± 0.08	1.15 ± 0.05	0.73 ± 0.00	0.70 ± 0.08	Black	Smooth
RS	Atacora Chain	1.77 ± 0.10	1.15 ± 0.06	0.74 ± 0.10	0.69 ± 0.10	Black	Smooth
RS	Mékrou-Pendjari	1.80 ± 0.12	1.16 ± 0.06	0.72 ± 0.08	0.66 ± 0.11	Black	Smooth

‡PChor, phytochorological zones based on the composition and the types of distribution of species; RGC, Guineo-Congolian zone; SGR, Sudano-Guinean transition zone; RS, Sudanian zone.
†All values are means (n = 1,000) ± SD.

DISCUSSION

Morphological variation in J. curcas *seed*

This study on the morphological characteristics of *J. curcas* seed identified five morphotypes in Benin. These morphotypes were significantly different according to their morphological traits, as revealed by canonical discriminant analysis. This morphological variation in *J. curcas* seed was similar to that observed by Ginwal *et al.* (2005) in India. Some studies dealing with different plant species have reported that morphological characteristics vary with climatic region and ecological gradient. Maranz and Wiesman (2003) showed a significant relationship between trait values (e.g., fruit size and shape, pulp sweetness, and kernel content of the species) and abiotic variables (e.g., temperature and rainfall) in sub-Saharan Africa (North of the Equator) for shea butter trees (*Vitellaria paradoxa*). Soloviev *et al.* (2004) also reported a significant influence of the climatic zones of Senegal on fruit pulp production in *Balanites aegyptiaca* and *Tamarindus indica.* Therefore, the phenotypic differences between morphotypes of *J. curcas* seed could also be explained by environmental factors. In fact, apart from the ages and genotypes of the trees, the soil and climate where they grew were important factors that affected the morphological traits of the seed and fruit (Salazar and Quesada, 1987; Assogbadjo *et al.*, 2005; 2006).

In this study, we noticed that part of the morphological variation in *J. curcas* seed could be explained by the phytodistrict in which the seeds were collected. Nevertheless, an important overlap between the provenance of *J. curcas* seed in Benin has been observed, indicating that factors other than environment affect seed morphology. The morphological differences between seeds could also be of genetic origin, resulting from adaptation of the species to diverse environmental conditions (Mathur *et al.*, 1984). This genetic variation, if it exists, could be an important source for varietal selection.

TABLE IV
Analysis of variance on repeated measures related to the germination ability of the five morphotypes of J. curcas *seed*

Source	DF‡	Type III SS¶	Mean Square	*F*-Value
Time (T)	9	55.58	6.18	1,615.66***
Block (B)	2	0.00	0.01	0.16ns
T x B	18	0.01	0.01	0.17ns
Morphotype (M)	4	3.11	0.78	83.40***
T x M	36	2.34	0.06	17.00***
B x M	8	0.01	0.01	0.07ns
T x B x M	72	0.03	0.01	0.12ns

‡DF, degrees of freedom.
¶Type III SS, Type III Sum of Squares; *F*-value, Fisher value.
ns, non-significant at $P \geq 0.05$; ***, significant at $P \leq 0.001$.

Seed germination

Rai and Tripathi (1982) reported the positive influence of a large seed size and seed reserves on the establishment and early growth of seedlings. In that respect, one can expect some variation in the rates of seed germination among the five morphotypes identified in *J. curcas*. Indeed, phenotypic variation is generally assumed to reflect the inherent genotypic variation among and within groups of plants growing under the same environmental conditions. In our study, the germinating ability of *J. curcas* seed showed significant variation between morphotypes. The lowest rate of germination at the end of the experiment was 50%, recorded for Morphotype 4, while the highest rate of germination (89%) was recorded for Morphotype 2. This could reflect genetic variation between these morphotypes. The differences observed in seed germination rate could be considered genetic, because environmental variation at the experimental site was negligible, and the experimental design reduced any residual variation that could persist on site. It has been reported that genotype has a strong influence on seed vigour (Schmidt, 2000). Since the five morphotypes consisted of seeds from different sites, the *J. curcas* populations we sampled would have restricted gene flow, which may lead to discontinuous variation in seed germination characteristics, which are genetically controlled (Whittington, 1973).

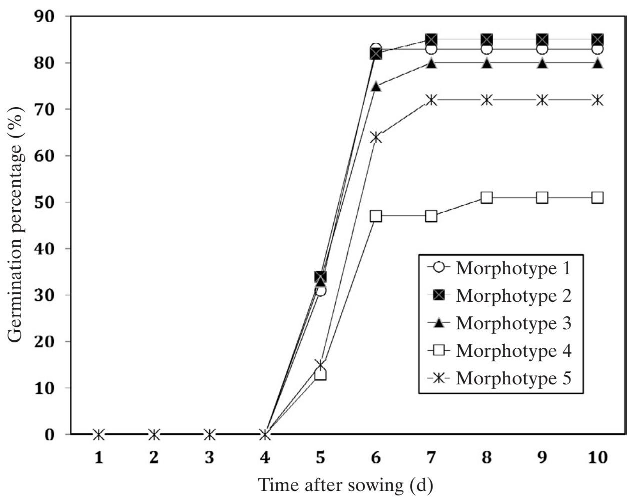

FIG. 3
Trends in the rates of germination of *J. curcas* seed according to morphotype.

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