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Impact of climate on seed morphology and plant growth of *Caesalpinia bonduc* L. in West Africa

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Key words: Sudanian and Guinean climate zones, Hierarchical classification, Canonical discriminant analysis, Morphotypes.

Abstract

Caesalpinia bonduc L. is an important medicinal plant threatened by overexploitation. In the present study, the impact of climate on seed morphology, germination capacity, seedling and plant growth of *C. bonduc* were evaluated. A total of 2000 seeds were collected in Sudanian and Guinean climate zones of Africa and their length, width, thickness, weight and color were recorded. A hierarchical classification and canonical discriminant analysis were applied to the above traits of seeds from the different climatic zones. An analysis of variance with repeated measures was applied to seeds morphotypes identified by the hierarchical classification to test for the effect of these morphotypes on seed germination, seedling and plant growth. Hierarchical classification helped to identify four seed morphotypes. Canonical discriminant analysis performed on these morphotypes revealed highly significant differences. Morphotypes 1 and 3 comprised green seeds mainly from Sudanian zone while morphotypes 2 and 4 gathered grey seeds mainly from Guinean zone. Morphotype 3 had the longest seeds while the shortest seeds were from morphotype 1. The heaviest seeds were found in morphotype 4 whereas the lightest ones were from morphotype 1. Seeds of morphotype 4 were the thickest and widest, while the slimmest and most narrow ones were grouped in morphotype 1. Moprhotype 3, consisting of large green seeds mainly from Sudanian zone, was superior in terms of seedling and plant growth among all morphotypes and should be the best choice for planting purposes of the species.

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Introduction

***C**aesalpinia bonduc* L. is an important medicinal plant widely distributed throughout the tropical and subtropical regions of the world (Anonymous, 1956; Kapoor, 1990). Different parts of the plant are used to treat several diseases (Jain *et al.*, 1992; Nandkarni, 1976; Jethmalani *et al.*, 1966), e.g. to ease childbirth, to treat burns and also for cultural practices like games, weddings and the Fâ ritual in Benin (Assogbadjo *et al.*, 2011). Different parts of the plant have been found to have a variety of pharmacological activities (Simin *et al.*, 2001; Kannur *et al.*, 2006; Datté *et al.*, 2004; Rastogi *et al.*, 1996). The roots of the plant are more intensively used than the leaves and seed, which cause a stress to exploited populations; moreover, there is low genetic diversity within the species which accentuates a need for conservation (Assogbadjo *et al.*, 2012).

The medicinal and sociocultural importance of the species had led to an overexploitation making it a rare and endangered species (Harden, 2002), and in Benin *C. bonduc* has been reported to be extinct in the wild, although it can be found in home gardens from the Guinean zone to the Sudanian zone (Adomou, 2005; Assogbadjo *et al.*, 2012).

Few researches have addressed the conservation and domestication of *C. bonduc* in Benin. The existing results were only related to the contribution of ethnic migrations on the propagation and persistence of the species and its morphological variability between climate zones (Assogbadjo et al 2012); the ethnic differences in use value and use patterns of the species (Assogbadjo et al 2011); and the germination technique of the seeds of the species (Hessou, 2009).

Little is known about the morphological variation among seeds of *C. bonduc* in different climate zones although the local environment is known to shape morphological trait of seed (Salazar and Quesada, 1987; Assogbadjo *et al.*, 2005; 2006). Moreover, the link between seed morphology, seed germination, and seedling and plant growth of *C. bonduc* were not yet established while many studies revealed a link between seed morphology, germination and plant growth (Assogbadjo *et al.*, 2006 Fandohan *et al.*, 2010; Padonou *et al.*, 2013, 2014). Thus the present study aims to contribute to the conservation and domestication of *C. bonduc* by (i) assessing the level of natural variation in seed morphology related to the climatic zones in order to identify *C. bonduc* morphotypes based on seed characteristics (ii) assessing the germination and plant growth ability of the identified morphotypes in order to determine the suitable morphotype and climate zone for propagation of *C. bonduc*.

Materials and methods

Study area

Seeds were collected in the Sudanian and Guinean climatic zones of Benin (Fig. 1).

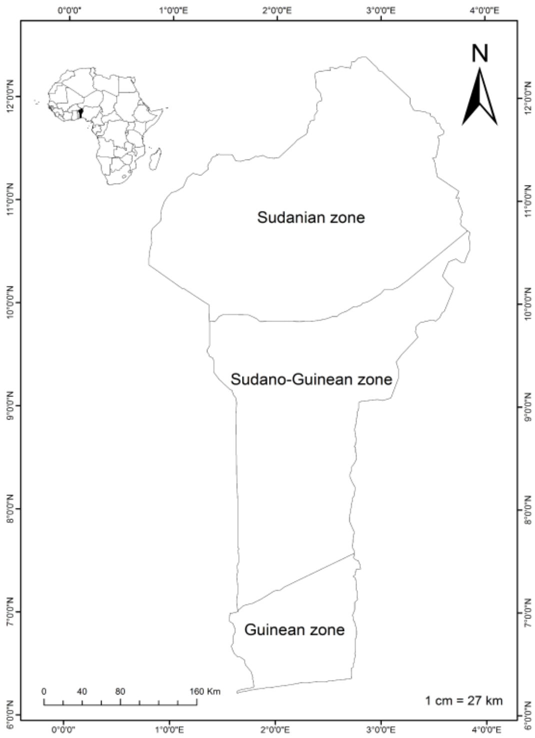

Figure 1. Climate zones of Benin

The Sudanian zone is located between 9°45' and 12°25' N, while the Guinean zone is located between 6°25' and 7°30' N. The mean annual rainfall in the Sudanian zone is often less than 1000 mm and the relative humidity vary from 18 to 99% (highest in August). The temperature varies from 24 to 31°C. The Sudanian zone has hydromorphic, well-drained soils and lithosols. The vegetation in this zone is composed mainly of savannas with trees of smaller size. In the Guinean zone, the rainfall is bimodal with a mean annual rainfall of 1200 mm. The mean annual temperature varies between 25 and 29°C and the relative humidity ranges from 69 to 97%. The soils are either deep ferralitic, or rich in clay, humus and minerals. The vegetation in this zone is mainly dense semi-deciduous forest.

Identification of C. bonduc *morphotypes and differences between the climate zones according to seed morphology*

A total of 100 mature trees of *C. bonduc*, located at least 100 m from each other, in order to avoid narrowing-down the genetic base due to relatedness or inbreeding, were sampled in each climatic zone, as recommended by Turnbull (1975). A total of 1000 seeds were collected at random in each climatic zone. The collected seeds were kept under ambient temperature conditions for 10 days prior to sowing. The length, width, and thickness of each seed were measured using electronic calipers during these 10 days. The weight of each seed was measured using electronic balance with 0.0001 g sensitivity. The color of the dry seeds was determined using the standard color chart published by the Royal Horticulture Society (1966). Color was coded by 1 if green and 0 if grey. Seed were subjected to a viability test using the flotation method in which those seeds that floated on water after 24 hours of soaking were considered to be non-viable and were discarded.

The seed length, width, thickness, weight and color data were subjected to Ascending Hierarchical Classification (AHC) using Ward agglomerative method and Euclidian distance using SAS 9.2 statistical software (SAS, 2008). Mahalanobis distance was used to test the distance between pairs of the morphotypes. Canonical discriminant analysis was performed on the morphotypes identified from the AHC in order to validate and test the differences between the morphotypes. The assumptions of the canonical discriminant analysis were met (the within-group covariance structure was homogeneous for all morphotypes and data within the morphotypes had multivariate normal distributions). The morphotypes were also described according to their differences, using canonical discriminant axes defined by seed morphology. The same analysis was also performed to test and describe the differences between the climate zones according to seed morphology.

Germination, seedling and plant growth of C. bonduc *seeds according to the morphotypes*

For germination tests on *C. bonduc* seeds and measurement on seedling and plant growth, a nursery experiment was carried out in January 2013 at the University of Abomey-Calavi, Benin (6°45'N; 2°35' E in the Guinean climatic zone). Four morphotypes of seeds were identified from the hierarchical classification. For each morphotype, 90 seeds were sown; one in each pot (5.5 cm × 18 cm) made from a polythene bag and filled with forest soil. The seeds were sown at an equal depth and the pots were watered equally twice daily (morning and evening) throughout the duration of the experiment. The experimental units were arranged in a randomized complete block design in a nursery with three replicates of 30 pots per each of the four morphotypes. The experimental units were kept in a weaning shed to reduce the rate of evaporation. The number of seeds of each morphotype that germinated was recorded daily over a 30 days period. At the end of this period collar diameter, stem height and number of leaves were measured weekly on five seedlings selected at random for each morphotype and from each replicate block for 45 days.

The five seedlings measured per morphotype and replicate were planted in the field following the same design and measured during 180 days. The collar diameter, stem height and the number of leaves were measured monthly on two plant select at random for each morphotype per replicate block for 180 days.

The germination rate of each morphotype was calculated each day over 30 days and the data were used to test the effects of time and morphotype on the germination of *C. bonduc* seeds using a mixed model ANOVA with repeated measures in SAS software (SAS, 2008). In this model, the factor "block" was considered to be random, whereas the factor "morphotype" was considered to be fixed. No data transformation on germination percentages was needed because normality and homoscedasticity assumptions were met using the Ryan-Joiner test of normality, and the Levene test for homogeneity of variances (Glèlè Kakaï *et al.*, 2006). The effects of morphotype on seedling and plant growth were assessed using the same statistical methods.

Results

Identification of C. bonduc morphotypes

Four morphotypes were identified from the hierarchical classification accounting for 58% of the information. The results of canonical discriminant analysis performed on the morphotypes of *C. bonduc* seeds showed that Mahalanobis distances between pairs of the four identified morphotypes were all highly significant ($p \leq 0.001$).

Table 1. Standardized canonical coefficients (SCC) and correlation coefficients between canonical axes (Can 1, Can 2) and the morphological traits of *C. bonduc* seed according to the morphotypes.

Variable	Can 1		Can 2	
	SCC	r^z	SCC	r^z
Length	0.13	0.06	0.79	0.90
Width	-0.21	-0.09	0.56	0.89
Thickness	-0.09	-0.16	0.28	0.71
Weight	-0.23	-0.20	0.34	0.85
Colour	4.50	0.99	0.12	0.07

[z]Simple correlation between each morphological traits and its respective canonical axes

The morphotypes identified were thus highly significantly different according to morphological traits of *C. bonduc* seed. Other results from canonical discriminant analysis performed on individuals of the four morphotypes revealed that the first two axes were highly significant ($P \leq 0.001$) and explained 80% of the variations between morphotypes (Fig. 2). The standardized canonical coefficients and the correlation coefficients between the two canonical axes and the morphological traits of *C. bonduc* seed (Table 1) indicated that the first axis (Can 1) described 91% of the variation discriminated between morphotypes according to seed color. The second axis (Can 2) describing 9% of the variation discriminated between morphotypes according to the length, width, thickness and weight of the seed. On this axis, heavy seeds were often long, wide and thick.

Seeds from morphotypes 1 and 3 were green, while seeds from morphotypes 2 and 4 were grey. The heavy, long, wide and thick seeds were from morphotype 3 and 4 while the small seeds were from morphotypes 1 and 2. A more detailed description of each morphotype is provided in Table 2. A deep analysis of our data indicated that each morphotype identified, was composed of seeds from the two climate zones. However, seeds of morphotype 1 and 3 came mainly from the Sudanian zone (56% and 63%, respectively), while morphotype 2 and 4 seeds were mainly from the Guinean zone (58% and 88% respectively). Morphotype 3 had the longest seeds (mean length 19.29 mm), while the shortest seed were from morphotype 1 (mean length 17.65 mm). The heaviest seeds were found in morphotype 4 (mean weight 2.85 g), whereas the lightest ones were from morphotype 1 (mean weight 1.95 g). With regard to seed thickness, seed of morphotype 4 were the thickest (mean thickness 15.27 mm), while morphotype 1 seed were the thinnest (mean thickness 13.46 mm).

Table 2. Mean values and standard deviations of the morphometric traits of four morphotypes of *C. bonduc* seed.

Traits	Morphotype 1		Morphotype 2		Morphotype 3		Morphotype 4		Guinean zone		Sudanian zone	
	m	s	m	s	m	s	m	s	m	s	m	s
Guinean zone (%)	44	-	58	-	37	-	88	-	-	-	-	-
Sudanian zone (%)	56	-	42	-	63	-	12	-	-	-	-	-
Length (mm)	17.7	0.76	17.89	0.75	19.29	0.6	19.28	0.45	18.3	0.92	18.4	1.09
Width (mm)	15.8	0.99	16.52	1.14	18.04	0.7	18.64	0.65	16.9	1.47	16.9	1.32
Thickness (mm)	13.5	0.79	14.05	0.8	14.75	0.9	15.27	0.75	14.2	1	14.1	1.03
Weight (g)	1.95	0.26	2.2	0.33	2.52	0.3	2.85	0.17	2.29	0.45	2.24	0.34
Colour	Green		Grey		Green		Grey		Grey		Green	

All values are means +/- SD (n = 2000)

Differences between the climate zones according to seed morphology

The results of canonical discriminant analysis performed on the seed morphology of *C. bonduc* according to the climatic zones showed that Mahalanobis distance between the two climate zones was highly significant (p ≤ 0.001). Seed morphology of *C. bonduc* was thus highly significantly different according the climate zones.

Table 3. Standardized canonical coefficients (SCC) and correlation coefficients between canonical axes (Can 1) and the morphological traits of *C. bonduc* seed according to climate zones.

Variable	Can 1	
	SCC	r^z
Length	0.39	0.24
Width	-0.01	-0.03
Thickness	0.13	-0.09
Weight	-0.49	-0.25
Colour	0.88	0.94

^{z}Simple correlation between each morphological traits and its respective canonical axes

Other results from canonical discriminant analysis performed on individuals of the seeds revealed that the first axe was highly significant (P ≤ 0.001) and explained 100% of the variations between the climate zones (Fig. 2). The standardized canonical coefficients and the correlation coefficients between the canonical axe and the morphological traits of *C. bonduc* seed (Table 3) indicated that the first axis (Can 1) describing discriminated between morphotypes according to seed color. Seeds from the Guinean zone were mostly grey while those from Sudanian zone were mostly green (Table 2). Seeds length varied from 18.38 mm in Sudanian zone to 18.26 mm in Guinean zone. The mean width of the seeds was 16.91 mm in Guinean zone and 16.88 mm in Sudanian zone. The mean tick of seeds from Guinean zone was 14.18 mm while it was 14.13 mm in Sudanian zone. The weight of the seeds varied from 2.29 g in Guinean zone to 2.24 g in Sudanian zone.

Germination ability of C. bonduc seeds according to morphotype

The germination ability of each morphotype varied in time between sowing and 30 days after sowing (Table 4).

Table 4. ANOVA with repeated measures related to the germination ability of the four morphotypes of *C. bonduc* seed

Source	DF	Type III SS	Mean Square	F-value
Time (T)	5	1952.31	390.46	3400.81***
Block (B)	2	74.23	37.11	45.55ns
T x B	10	23.82	2.38	20.75ns
Morphotype (M)	3	20.79	6.93	8.51***
T x M	15	22.65	1.51	13.15***
B x M	6	44.28	7.38	9.06ns
T x B x M	30	24.77	0.83	7.19***

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

Table 5. ANOVA with repeated measures related to seedling growth on collar diameters, heights and number of leaves in four morphotypes of *C. bonduc* seed.

		Collar diameter		Height		Number of leaves	
Source	DF	Mean Square	F-value	Mean Square	F-value	Mean Square	F-value
Time (T)	8	3.23	58.48***	278.69	555.15***	24.00	75.16***
Block (B)	2	2.50	2.14ns	1.38	1.24ns	6.17	0.57ns
T x B	16	0.04	0.78ns	0.63	1.27ns	0.31	0.98ns
Morphotype (M)	3	2.66	2.27ns	9.41	8.41**	0.38	0.04ns
T x M	24	0.06	1.13ns	0.61	1.23ns	0.16	0.51ns
B x M	6	0.10	0.09ns	4.30	3.85*	14.16	1.30ns
T x B x M	48	0.03	0.60ns	0.63	1.27ns	0.28	0.89ns

DF, degree of freedom; F-value, Fisher value, ns, non-significant at P ≥ 0.05; *, significant at P ≤ 0.05; **, significant at P ≤ 0.01; ***, significant at P ≤ 0.001.

The blocking factor and the two ways interactions with the block were non-significant, indicating homogeneity of the environmental characteristics between blocks. Germination of the seeds started 5 days after sowing and reached their maximum in 20 days (Fig. 2). The germination rate varied significantly in time, between morphotypes, between morphotypes in time and between block and morphotypes in time. The percentage of germination varied from 80% (morphotype 2 and 4) to 88% (morphotype 1) after 30 days.

C. bonduc seedling growth according to morphotype

No difference was observed between morphotypes in number of leaves and collar diameter, while the growth in height differed between morphotypes (Table 5). The blocking factor and all its interactions were non-significant indicating homogeneity of the environmental characteristics between blocks. Morphotypes seedling growth in terms of collar diameter, height and number of leaves varied between the end of germination (30 days after sowing) and 45 days later (Fig. 4).

Plant growth of C. bonduc according to the morphotypes

There are significant differences in plant growth between morphtypes (Table 6).

Table 6. ANOVA with repeated measures related to plant growth on collar diameters, stem heights and number of leaves in the four morphotypes of *C. bonduc* seed.

		Collar diameter		Height		Number of leaves	
Source	**DF**	**Mean Square**	**F-value**	**Mean Square**	**F-value**	**Mean Square**	**F-value**
Time (T)	5	156.63	42.28***	2504.47	30.68***	343.61	18.78***
Block (B)	2	6.38	12.19*	20.04	4.83*	9.36	0.62ns
T x B	10	0.70	0.19ns	25.46	0.31ns	10.30	0.56ns
Morphotype (M)	3	22.52	42.98***	1124.23	24.70***	34.35	8.34**
T x M	15	1.74	0.47ns	197.55	2.42**	34.35	1.88*
B x M	6	1.95	3.74ns	112.18	2.46ns	50.97	3.40*
T x B x M	30	0.70	0.19ns	64.95	0.80ns	14.89	0.81ns

DF, degree of freedom; F-value, Fisher value; ns: non-significant at $P \geq 0.05$; *, significant at $P \leq 0.05$; **, significant at $P \leq 0.01$; ***, significant at $P \leq 0.001$.

Plants of morphotype 3 have the highest values of collar diameter and height. The highest values of the number of leaves were observed with morphotype 4, followed by morphtype 3 (Figure 5). The blocking factor and all its interactions were non-significant, indicating homogeneity of the environmental characteristics between blocks. Growth in terms of collar diameter, height and number of leaves varied between the end of seedling period and six months later for all four morphotypes (Figure 5).

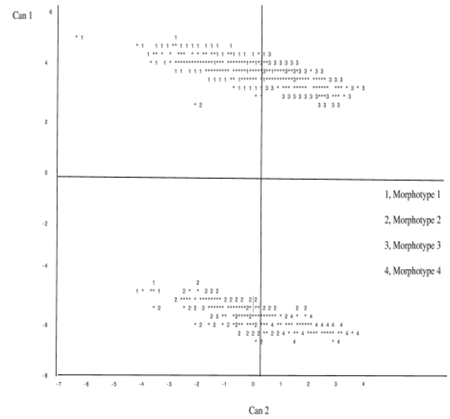

Figure 2. Projection of the four morphotypes of *C. bonduc* seed on the canonical axes defined by seed morphology. Can 1 discriminated between morphotypes according to seed color. Can 2 discriminated between morphotypes according to the length, width, thickness and weight of the seed

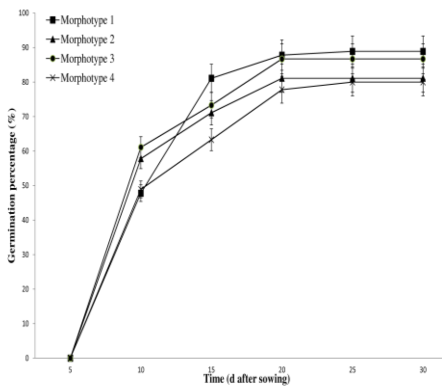

Figure 3. Cumulative germination rates of *C. bonduc* seed according to morphotype.

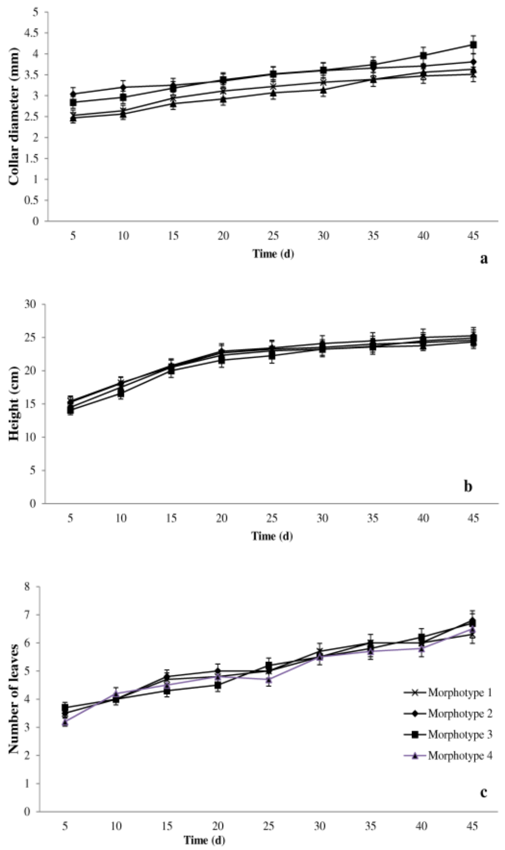

Figure 4. Trends in seedling traits over time (in days) following germination in *C. bonduc* based on collar diameter (Panel a), height (Panel b) and leaves (Panel c) according to seed morphotype.

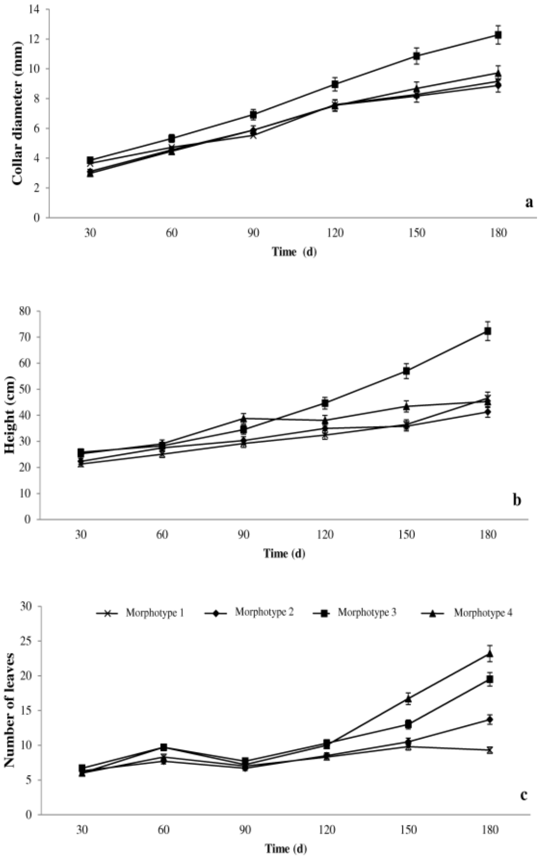

Figure 5. Trends in plant traits over time (in days) following seedling growth in *C. bonduc* based on collar diameter (Panel a), height (Panel b) and leaves (Panel c) according to seed morphotype.

Discussion

Morphological variation in C. bonduc seed

C. bonduc showed variation concerning morphological seed traits. Four morphotypes of seed were significantly distinct according to some of their morphological traits. Seed of morphotypes 3 and 4 were heaviest, longest, widest and thickest, in contrast to morphotypes 1 and 2 which were smaller. This differentiation confirmed with canonical discriminate analysis confirmed the statement that tropical plant species often have important intraspecific variation of seed traits (Foster, 1986; Khan *et al.*, 1999, 2002; Khan and Uma, 2001; Khan, 2004). The results corroborated with recent studies on other species such as *Jatropha curcas* (Ginwal *et al.*, 2005; Padonou *et al.*, 2014), *Afzelia africana* (Padonou *et al.*, 2013) *Tamarindus indica* (Fandohan, 2010) and *Prunus jenkinsii* (Upadhaya, 2007). Indeed, seeds from Guinean zone were mostly grey and presented more compact morphological traits (shortest, thickest, widest, and heaviest) than those from Sudanian climatic zone. However, none of the four morphotypes had characteristics which fitted perfectly those of Guinean or Sudanian seed traits. Thus the overall environmental factors cannot explain the substantial morphological variation found within populations (Assogbadjo *et al.*, 2012). These results are consistent with a recent study in Benin on the morphological and genetic diversity of *C. bonduc* trees, which reported that the species is characterized by a high morphological variation among individuals of the same populations, while a much lower degree of such variation were observed among the populations and climatic zones (Assogbadjo *et al.*, 2012). According to Mathur *et al.* (1984), the morphological difference between seeds could be of genetic origin, due to the adaptive strategies of species to their environment. But Assogbadjo *et al.*

(2012) revealed that *C. Bonduc* is characterized by a very low genetic diversity within populations. The phenotypic plasticity of the species in response to micro habitat factors, and the difference of age of targeted individuals can explain the observed differences of seed morphology (Heaton *et al.*, 1999; Assogbadjo *et al.*, 2005; Assogbadjo *et al.*, 2012).

Seed germination, seedling and plant growth in C. bonduc seed

This difference in germinataion rate could be explained by morphology, which is an important factor influencing the germination rate. Many reports existed on the conflicting relationships between the variation in seed weight and germination behaviour. For instance, large seeds may germinate at higher percentages than small seeds (Tripathi and Khan, 1990; Bhuyan and Khan, 2000; Khan and Uma, 2001), and small seeds may germinate at higher percentages than large seeds (Marshell, 1986), or germination may be independent of seed size (Gross and Kromer, 1986; Perez-Garcia *et al.*, 1995). Since morphotype 1 is the lightest and morphotype 4 is the heaviest, we could conclude that our data revealed an independent relationship between germination and seed mass. Beside the main factor which discriminated these two groups, and could explain the difference observed, is the color of the coat. Indeed morphotypes 1 and 3 were green while the two others had a grey coat. Similar results were obtained by Mavi (2010) who revealed that the seed coat color of Crimson sweet seeds could explain the differences of seed quality. Moreover, the grey coat color was mainly characteristic of seeds from Guinean climatic zone while green coat indicated seeds collected from Sudanian. But these hypotheses need additional research to test. On one hand, for Crimson sweet seeds, the seeds color (brown) was coupled with high seeds mass, to explain good germination rate. But in our case, seed size do not influence germination percentage.

As far as seedling and plant growth concern, seeds of morphotype 3 confirmed their vigour and quality. Indeed, these seeds germinated maximally and had the greatest collar diameter of seedling and the greatest collar diameter, height and number of leaves of plant. We could suggest that seed mass could explain observed performance of morphotype 3. Many authors reported that heavier seeds possess large reserves of nutritive substances available, which confer an advantage to their seedlings for survival and growth (Tripathi and Khan, 1990; Ke and Werger, 1999; Khan *et al.*, 1999, 2002; Khan and Uma, 2001). This advantage is a good start which remains during plant growth phase. Nevertheless bad performance of seeds of morphotype 4, which were heavy seeds too, supposed that good germination percentage of *C. bonduc* seeds, influenced juvenile and plant growth phase later.

Conclusion

The study revealed that considerable variation exists in *C. bonduc* populations with respect to seed morphology. Except seed color which varied from grey in Guinean zone to green in Sudanian zone, there were no significant differences between the two climatic zones on the others traits. Seed germination was not significantly related to seed morphology. Nevertheless, seedling and plant growth were influenced by seed morphology. Moprhotype 3, which consists of large green seeds mostly from the Sudanian zone is superior in terms of seedling and plant and should be the best choice for planting purposes.

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