

Full Length Research Paper

Germination and seed traits variations among West African provenances of *Moringa oleifera* Lam. (Burkina Faso)

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Moringa oleifera is a fruit species of economic interest to West African smallholder growers. To generate benefits to poor rural communities, improved knowledge is needed on plantation management and selection of the most reliable seed sources. The aim of the study was to investigate variation in seed traits and germination rates among 12 provenances of *M. oleifera* from West Africa in the women gardening center named "Amicale des Forestières du Burkina Faso (AMIFOB)" located at Ouagadougou, Burkina Faso (12°7'32"N, 01°40'24"W). The authors conducted an analysis of variance, a principal component analysis on seed traits variables (length, thickness and weight) and germination rates at 5 and 12 days after seed sowing, and finally ascending hierarchical classification based on similarity indices. The results showed significant variations ($P < 0.05$) among provenances in seed traits: Ouahigouya provenance in the Sahelian area of Burkina Faso had the largest and heaviest seeds. Germination rate was significantly different after 5 days for Ouahigouya, Ouagadougou, Koudougou, Dano and Tamale provenances ($P < 0.05$). Five days after seed sowing, the Sahelian provenance (Ouahigouya) recorded the greatest and fastest germination rate of 63%. Correlation analyses revealed no significant links between germination rate in 5 and 12 days after sowing and seed sizes. Seed traits and germination rates did not show clear cut distinct groups between Sahelian, Sudanian and sub Equatorial provenances. This research output provides an evidence of the genetic variability among *M. oleifera* provenances and hence the potential for future tree improvement programme.

Key words: Seed variability, agroclimatic zone, Burkina Faso.

INTRODUCTION

Moringa oleifera Lam. or 'drumstick tree' is a member of the *Moringaceae* family, which grows throughout the

tropics and is native to the sub-Himalayan tracts of north-western India, Pakistan, Bangladesh and Afghanistan

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(Makkar and Becker, 1997). The tree has a height of 7 to 12 m up to the crown. The leaves are twice or thrice pinnate and spirally arranged. The flowers are bisexual, white or cream colored with yellow stamens, and the fruit is a three sided or nearly cylindrical capsule. The seed is round, dark chestnut-colored, and with 3 white wings that facilitate spreading by the wind in natural conditions. *Moringa* can be propagated either by using seeds or cuttings (Morton, 1991). Fruits, seeds, leaves and flowers are consumed by humans as nutritious vegetables. *M. oleifera* leaves are known to be an excellent source of vitamins, minerals and protein and are used to fight against malnutrition and its associated diseases including blindness (Asante et al., 2014). In West Africa, leaves are commonly used to make sauces. It is grown in all types of soil, from acid to alkaline (Galdima et al., 2013) and can tolerate up to 6 months of dry season reasonably well. The tree grows at different altitudes from 0 to 1800 m.a.s.l. and rainfall between 500 and 1500 mm per year.

M. oleifera is planted and smallholder growers grow subsistence and cash crops in their rainfed, complex and resource deprived fields often combining the cultivation of the crops with scattered multipurpose trees and realizing a wide range of benefits (Mudyiwa et al., 2013). It is one of such multipurpose tree of global interest and is grown in combination with agricultural and horticultural crops by smallholder growers and this give growers a wide range of benefits (Mudyiwa et al., 2013). It is a suitable tree for traditional agroforestry in the home because of its versatility (Nduwayezu et al., 2007).

In Burkina Faso, the seeds sources for planting are coming from various agro ecological zones. For example, more than 80% of women growers used non certified seeds as plantation materials to produce *M. oleifera* leaves because they do not access the high cost of certified one. The demand of plantation material is most likely growing in the future due to increasing demand of the potential market. Better plantation and nursery management would generate benefits to poor rural communities, since villages and hamlets hold their own local nursery. However, before plantations can be recommended on a large-scale, improved knowledge is needed on plantation management and selection of the most reliable seed sources. There are noticeable differences among *M. oleifera* agroecological ecotypes concerning growth performances (Edward et al., 2014) which have considerable impact on the economic return. Differences among geographic sources in tree species are often substantial and economic improvement can be made by an appropriate provenance selection (Wright et al., 1976).

Altitudinal variations in seed and seedling characters of tree species have been reported by various studies (Gera et al., 2000). However, for the utilization of observed variation in species, it is a prerequisite to know the extent of variation and also whether it is due to genetic or environmental factors. Hence, information on variation in

the desirable parameters and their correlation is vital for any breeding programme (Fenner and Thompson, 2005).

Little is known about the role of *M. oleifera* seed size. Seed size has been regarded as an important plant property (Fenner, 1991). Considering the presence in various ranges of climate and soil conditions, and different agro ecological zones with different ecotypes may show contrasting performances (Aronson et al., 1993).

In the current study, the authors investigated the seed size among 12 provenances of *M. oleifera* from four agroecological zones of West Africa. They hypothesized that the seed size and germination rates correlated with geoclimatic data of seed collection sites.

MATERIALS AND METHODS

Study site

The study was conducted at the women gardening center named "Amicale des Forestières du Burkina Faso (AMIFOB)" located at Ouagadougou, Burkina Faso (12°7'32"N, 01°40'24"W). The rainfall is uni-modal with a mean annual rainfall of the last 15 years data from the nearest meteorological station in Ouagadougou, of 800 mm.year⁻¹, and the mean temperature of 28.5°C month⁻¹ (Figure 1). Soils are sandy clay to clay-sandy Ferruginous leached with very low nutrient content according to French soil classification (Pallo et al., 2009). The common natural vegetation found at Ouagadougou is described as semi-deciduous open woodland. Main genera include, *Eucalyptus*, *Azadirachta*, *Mangifera*, *Vitellaria*, *Lannea*, *Piliostigma*, *Acacia*, *Ziziphus*, *Tamarindus* and *Combretum*.

Seed sources, experimental design and establishment

The experiment included twelve *M. oleifera* provenances: 1 provenance from Segou (Mali), 1 from Niangon-Lokoua, Abidjan (Ivory Coast), 1 from Tamale (Ghana) and 9 from Burkina Faso (Ouahigouya, Dano, Gaoua, Ouagadougou, Fada N'Gourma, Dédougou, Bobo-Dioulasso, Koudougou, Centre National de Semences Forestières-CNSF) (Figure 2).

In this paper, provenances were referred to four climate areas according to their agro-ecological characteristics (Sahelian, sub Equatorial, Sudanian and north Sudanian) (Table 1). Seeds were collected in 2014 in plantation farmland from at least 12 mother trees per provenance.

Trial was planted in a randomized complete block design (RCBD) with three replications. Each plot represented a provenance planted at 5 x 6 rows in a contiguous arrangement of 20 x 20 cm (Figure 3). Plot measured 10 x 1 m and contained 30 trees and the distances between blocks were 2 m. Seed samples were pretreated with water for 24 h and sown in June 1st 2014 in a prepared and cleaned soil using hand hoes. Weeding was done twice during the rainy season. Watering was done once a day in the morning. The seedlings were grown without fertilizers and chemical control.

Data collection and analysis

To investigate the variability in seed parameters (length, thickness and weight), each provenance was represented by 240 randomly selected seeds, assessed in four replications of 60 seeds each. Each seed weight was determined by weighing three random samples of 60 seeds each. By the end of 2 weeks, the experiment

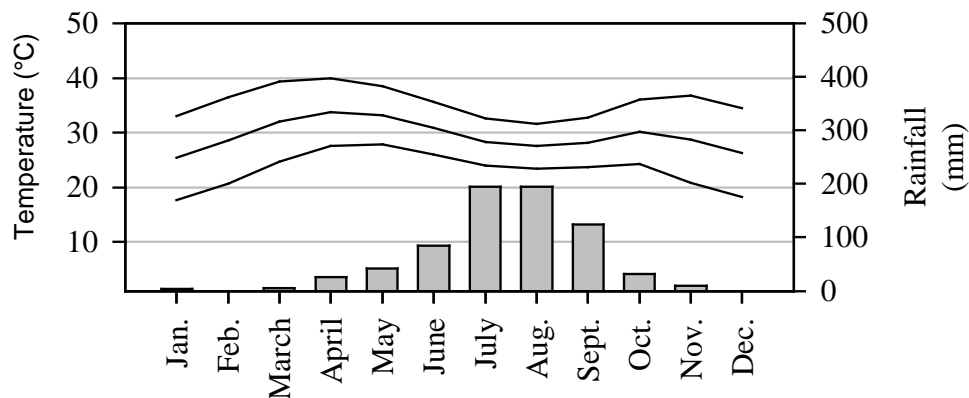


Figure 1. Monthly temperature (min., mean, max.) and precipitation level measured between 2000 and 2015 at Ouagadougou. Data were obtained from <http://www.infoclimat.fr/stations-meteo/climato-moyennes-records>.

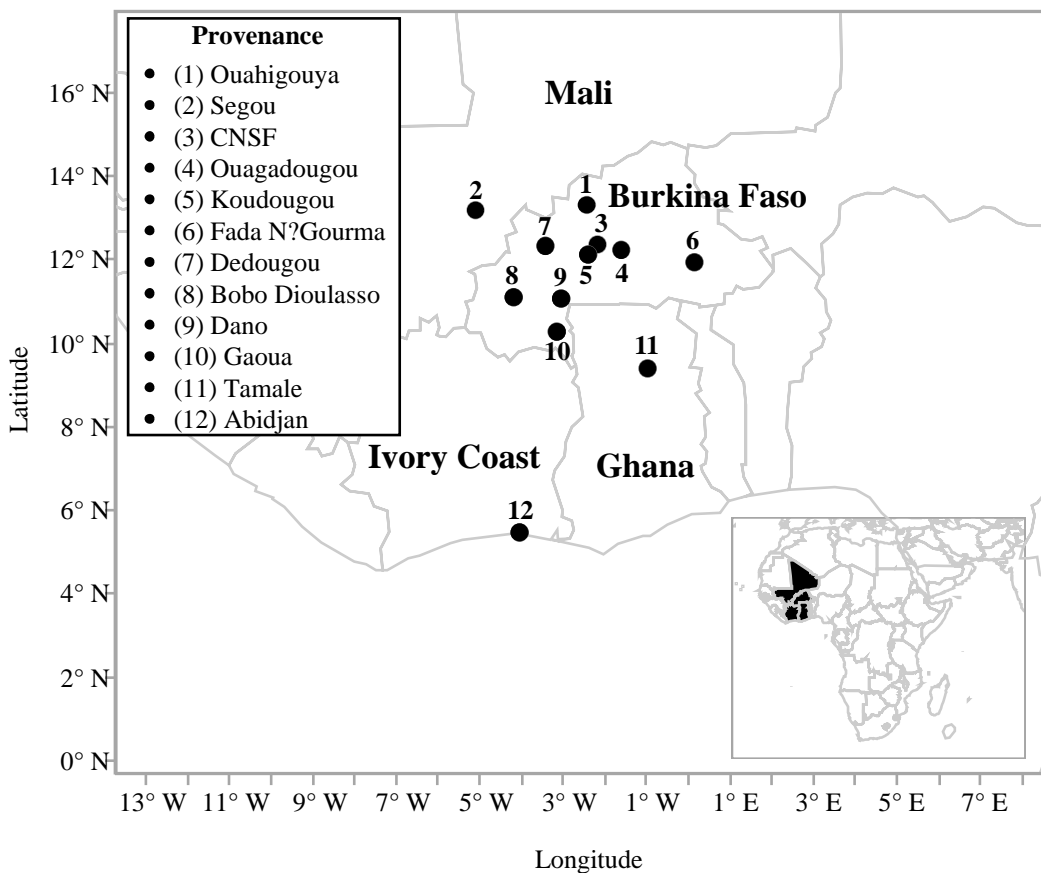


Figure 2. Provenances localizations.

had been measured twice at effective ages of 5 and 12 days since seed sowing, providing data for the assessment of germination rates of the twelve provenances. All statistical analysis were carried out using ANOVA performed with the MIXED procedure of JMP® Pro 11.1.1 (SAS Institute, Cary, NC, USA). Normality and homoscedasticity were graphically verified on residual plots of the

linear models (Quinn and Keough, 2002). When effects were significant, the Tukey-Kramer test was used for multiple mean comparisons. Statistical significance was fixed at 0.05, and only significant results are presented in graphs. A factorial analysis was conducted to determine the relationship of seeds, germination and their provenances of origin.

Table 1. Characteristics of the provinces, ecological zones and seed sources of *M. oleifera*.

Prov. code	Ecological Zone	Provenance	Country	Latitude	Longitude	Altitude (m)	Average rainfall (mm/year)	Mother trees number
1	Sahelian	Ouahigouya	Burkina Faso	13°30'4"N	2°24'31"W	306	500	24
2	North Sudanian	Segou	Mali	13°22'05"N	5°16'24"	294	500	35
3	North Sudanian	CNSF	Burkina Faso	12°30'07 N	2°07'34"W	304	800	35
4	North Sudanian	Ouagadougou	Burkina Faso	12°21'58"N	1°31'05"W	315	800	15
5	North Sudanian	Koudougou	Burkina Faso	12°15'04"N	2°22'28"W	308	800	12
6	North Sudanian	Fada N’Gourma	Burkina Faso	12°03'41"N	0°21'30"E	300	900	26
7	Sudanian	Dedougou	Burkina Faso	12°28'00"N	3°28'00"W	300	1100	16
8	Sudanian	Bobo Dioulasso	Burkina Faso	11°11'00"N	4°17'00"W	339	950	20
9	Sudanian	Dano	Burkina Faso	11°9'0"N	3°4'0"W	287	950	30
10	Sudanian	Gaoua	Burkina Faso	10°19'12"N	3°10'12"W	319	1000	17
11	Sudanian	Tamalé	Ghana	9°24'27"N	00°51'12"W	169	1100	15
12	Sub-Equatorial	Abidjan	Ivory Coast	5°18'28"N	4°6'19"W	73	2000	18



Figure 3. An experimental trial of *M. oleifera* provenance. Photo DAO MCE, Ouagadougou, July 2014.

RESULTS

Seed size traits

Analysis of variance showed highly significant differences

among *M. oleifera* provenances within ecological zones for seed length, thickness and seed weight ($P < 0.0001$) (Figure 4). Estimates of variance components indicated that the inter provenances effects varied from 42 to 45% of the total variation for all seed characters (Table 2). The

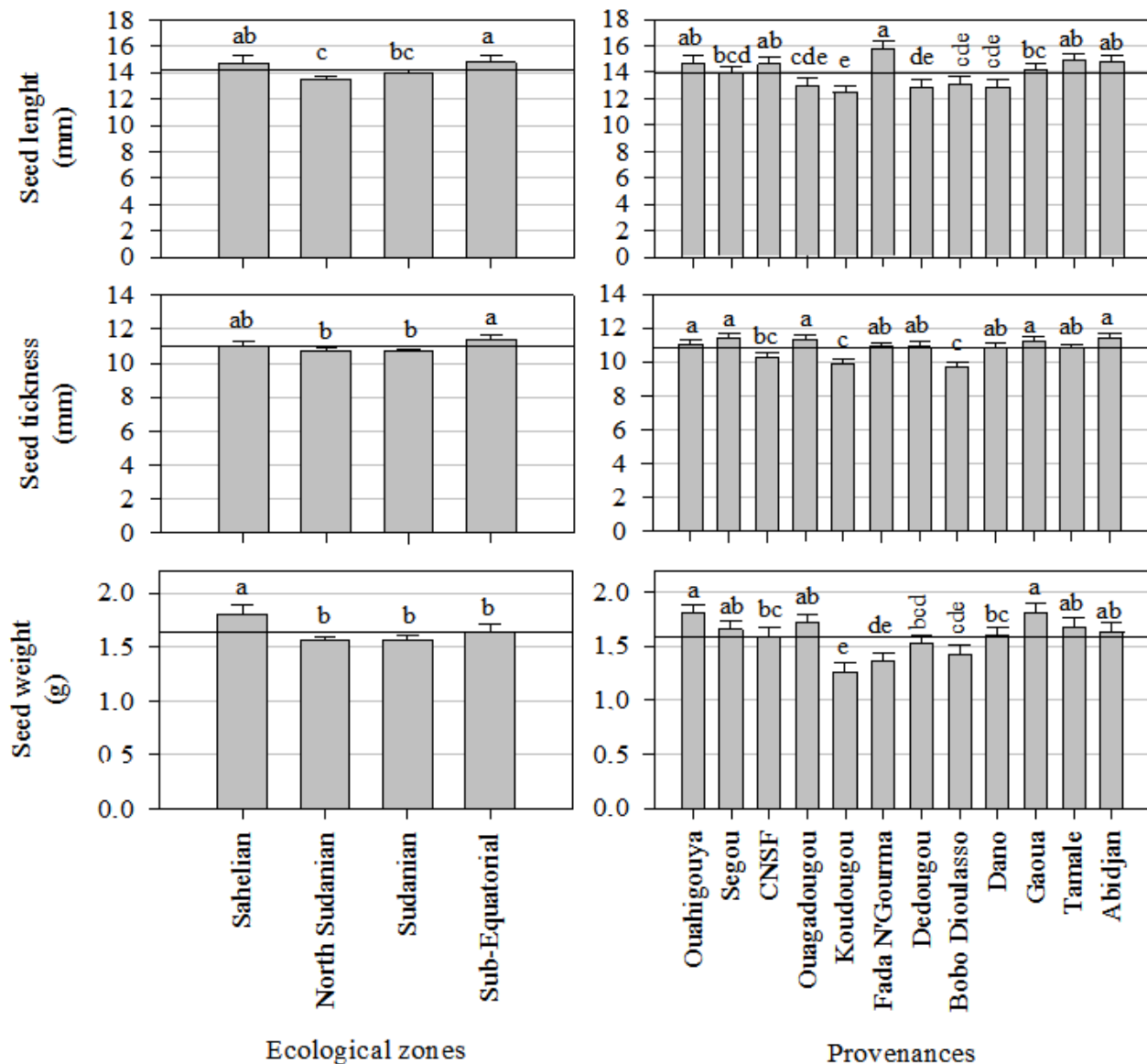


Figure 4. Seed characteristics of the 12 provenances of *M. oleifera*, least-square-means + 95% confidence intervals. Different letters indicate significant differences ($p < 0.05$, Tukey HSD test).

Table 2. Estimates of variance components, expressed as percentage of the total variation, and provenance heritability (h^2_p) within ecological zone for seed size traits of the 12 *M. oleifera* provenances.

Variables	Ecol. Zone	Prov[Ecol. Zone]	Residuals	h^2_p
Seed length	0	42.97***	57.03***	0.94
Seed thickness	0	43.75***	56.25***	0.94
Seed weight	0	45.20***	54.80***	0.94

*** $p < 0.001$.

provenance heritability for all seed characters was relatively high (0.94).

Maximum mean values for seed length (15.8 mm) were

found in Fada N'Gourma, for seed thickness (11 mm) in 5 provenances (Ouahigouya, Ségou, Koudougou, Gaoua and Abidjan) and for seed weight (1.8 g) in Ouahigouya

Table 3. Eigenvectors (W) and loadings (L) of the PC axis from PC analysis of the 12 *M. oleifera* provenances. Eigenvalues and their contribution to total variation are listed at the bottom of columns.

Variable	PCA 1		PCA 2		PCA 3	
	W	L	W	L	W	L
Latitude	0.506	0.900	0.257	0.330	0.007	0.008
Longitude	-0.019	-0.033	0.100	0.128	0.780	0.870
Altitude	0.534	0.949	0.118	0.152	-0.004	-0.005
Average rainfall	-0.476	-0.845	-0.338	-0.434	-0.016	-0.017
Germination rate	0.213	0.379	0.026	0.034	0.248	0.277
Seed length	-0.261	-0.464	0.295	0.380	0.478	0.533
Seed thickness	-0.291	-0.517	0.566	0.728	-0.067	-0.075
Seed weight	-0.186	-0.330	0.622	0.800	-0.311	-0.347
Eigen value	3.16		1.65		1.24	
Percent of total	39		21		16	

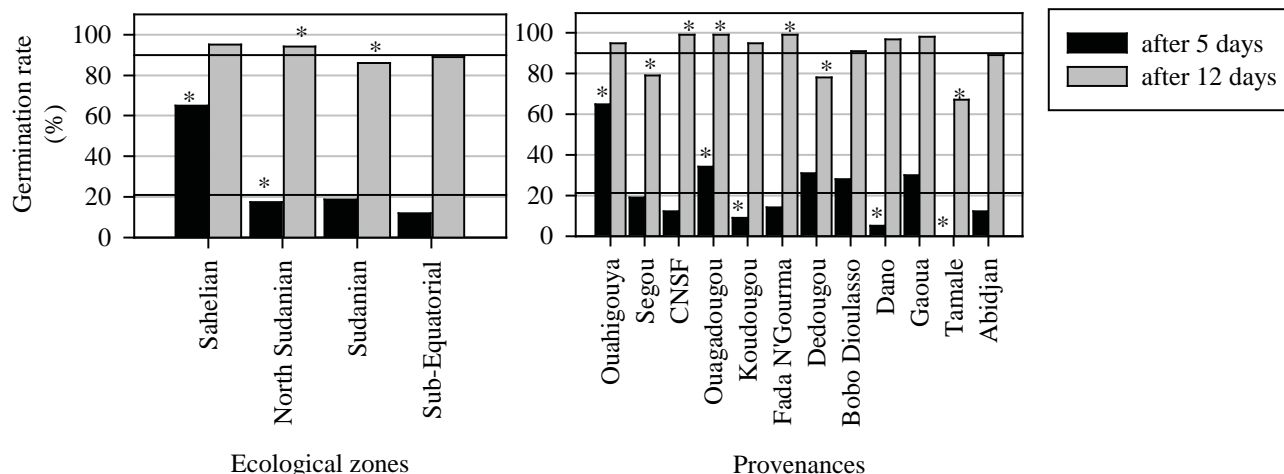


Figure 5. Cumulative germination rate (%) after 5 and 12 days of the 12 provenances of *M. oleifera*. * indicate significant differences ($p < 0.05$, ANOM method) with the means at the two evaluation days (5 and 12 days).

and Gaoua provenances (Table 3). Ranking of provenances based on mean seed length, thickness and weight indicated consistently lower values for Koudougou and Bobo Dioulasso (Table 3). Ouahigouya (Sahelian) and Gaoua (south Sudanian) were characterized by heaviest and largest seeds, while Koudougou (north Sudanian) and Bobo Dioulasso (south Sudanian) provenances were the shortest, tiniest and lightest seeds. The provenances were classified in descending order of magnitude by high, intermediate and low groups of seed characters as follows:

Seed length: Fada N'Gourma > Ouahigouya, CNSF, Abidjan, Gaoua, Ségou and Tamale > Ouagadougou, Dano, Koudougou and Bobo Dioulasso

Seed thickness: Ouahigouya, Gaoua, Ségou, Ouagadougou, Tamale > Fada N'Gourma, Dano, CNSF

and Tamale > Koudougou and Bobo Dioulasso
Seed weight: Ouahigouya, Gaoua > Ségou, Ouagadougou, Tamale, Abidjan, CNSF, Dano > Fada N'Gourma, Koudougou and Bobo Dioulasso.

Seed germination

Seeds started germinating 5 days after sowing and after day 12 no seeds germinated. The overall means of germination rate of *M. oleifera* seeds under experimental conditions were 21 and 90% after 5 and 12 days after sowing, respectively (Figure 5). The cumulative germination rate was significantly different after 5 days for Ouahigouya, Ouagadougou, Koudougou, Dano and Tamale ($P < 0.05$) (Figure 5). Five days after seed sowing, Ouahigouya provenance recorded the greatest

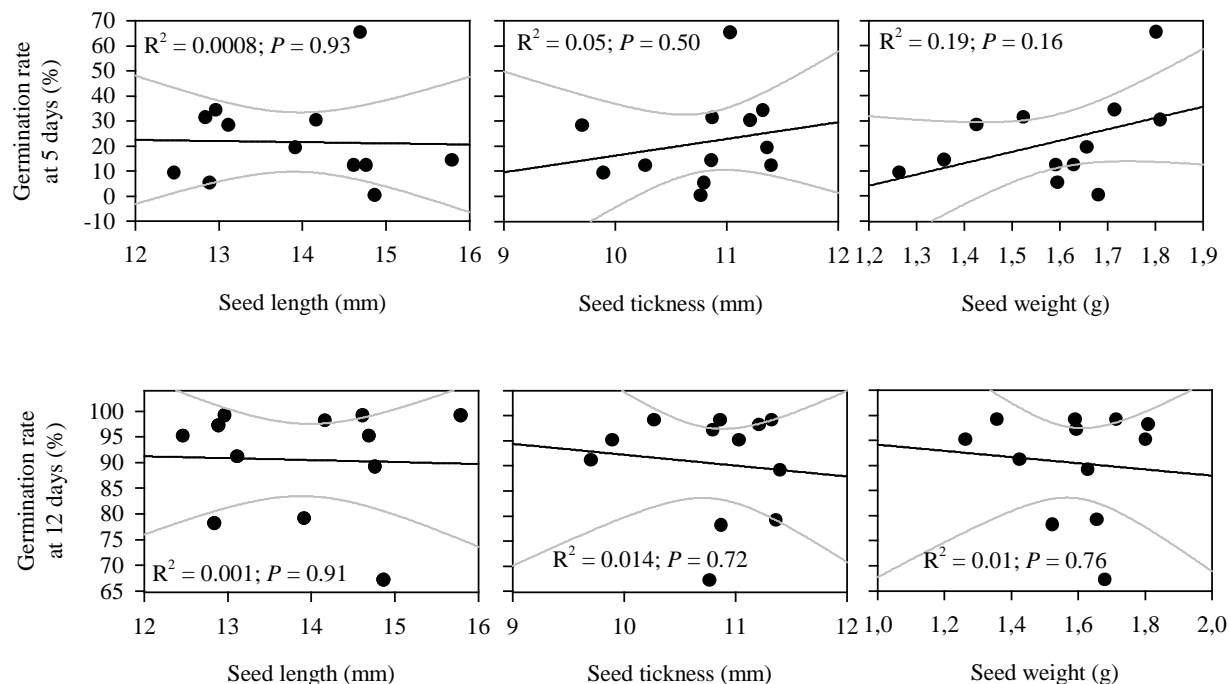


Figure 6. Linear regression between germination rate at 5 and 12 days and seed length, seed thickness and seed weight of the *M. oleifera*. R² is the regression correlation and P the prob. value of the ANOVA for the simple linear regression of Y on X.

and fastest germination rate of 63%.

The response of seeds showed great variability depending on provenance and ecological zone (Figure 5). Significant effects were found between ecological zones ($P < 0.05$). After 5 days, germination rate of the provenance in the Sahelian zone (Ouahigouya) was 3 times higher (more than 60%). Within the ecological zone, no significant effects were observed at both dates 5 and 12 days after sowing with Abidjan (sub Equatorial), Gaoua and Bobo Dioulasso (south sudanian) provenances (Figure 3). The highest final germination percentages of 100% were observed with provenances from the north Sudanian zone of Burkina Faso (CNSF, Ouagadougou and Fada N'Gourma). The final lowest germination rates were observed in Tamale (68%), Dedougou (78%) and Segou (80%) provenances (Figure 5).

Correlations of seed characteristics with germination rates

Correlation analyses revealed no significant links between germination rate in 5 and 12 days after sowing and seed length ($r^2 = 0.00$; $P = 0.93$ and $r^2 = 0.00$; $P = 0.91$ respectively in 5 and 12 days after seed sowing), seed thickness ($r^2 = 0.05$; $P = 0.50$ and $r^2 = 0.01$; $P = 0.72$, respectively in 5 and 12 days after seed sowing) and seed weight ($r^2 = 0.19$; $P = 0.16$ and $r^2 = 0.01$; $P =$

0.76, respectively in 5 and 12 days after seed sowing) (Figure 6).

Multivariate analysis of seed characters, germination and geoclimatic data of provenance origin

The principal component analysis (PCA) performed on the characteristics of seeds and twelve-day-old seedlings showed that the first three principal axes explained 77% of the observed variability (Axis 1: 40%, axis 2: 21% axis 3: 16%) (Table 3). The axis 1 variables were influenced by the average rain and latitude and altitude of the provenances origin and constituted the axis of geoclimatic characters of the provenance origin. The axis 2 explained the contribution to total variation of variables seed thickness and weight. This axis may be defined as the center of the seed characteristics. The axis 3 was mainly explained by the seed length, germination rate and longitude and was defined as the axis of the seed length (Figure 7).

By overlaying the correlation circles of variables and map of overall seed provenances with the main axes, it appears that provenances 11 (Tamale), and 12 (Abidjan) are highly correlated with average rainfall of the axis 1 and located at the negative side of the axis 1 (Figure 7). Axe 3, the provenance 12 and 11 and the variables seed thickness and weight were located on the positive side of the axis 3 while the provenance 6 and the variable seed length were on the opposite side. A hierarchical cluster

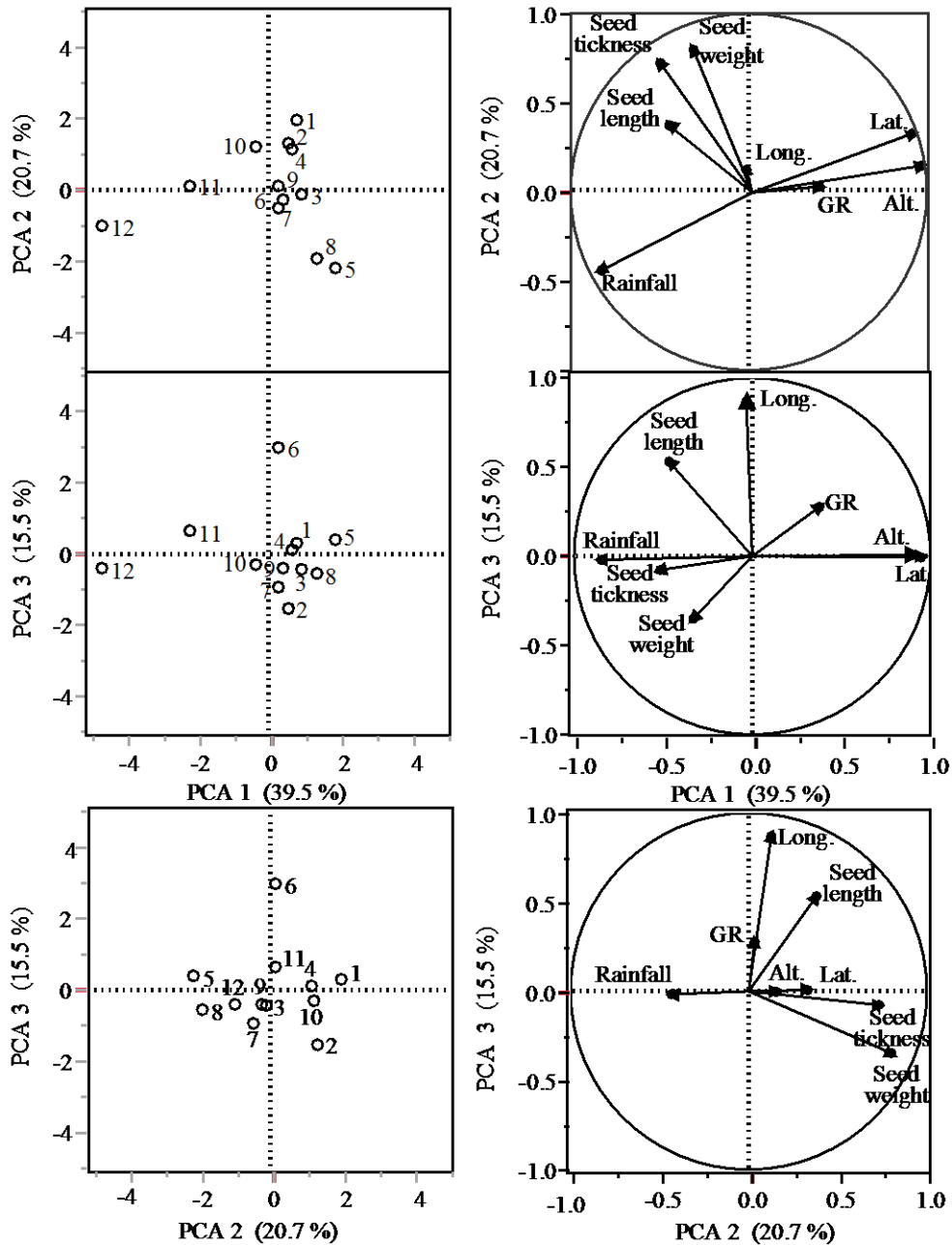


Figure 7. Principal component analysis of the 12 *M. oleifera* provenances based on a correlation matrix of association between seed characteristics and environmental variables. GR: final germination rate. [1] Ouahigouya, [2] Segou, [3] CNSF, [4] Ouagadougou, [5] Koudougou, [6] Fada, [7] Dedougou, [8] Bobo, [9] Dano, [10] Gaoua, [11] Tamale and [12] Abidjan.

analysis dendrogram showed no clear identified provenance groups (Figure 8).

DISCUSSION

This study was conducted in Ouagadougou on 12 provenances of *M. oleifera* from West African, and tested

the hypothesis that seed size and germination rates were correlated with geoclimatic data of seed collection sites. The results revealed clear differences among the 12 *M. oleifera* provenances in terms of seed weight, length and thickness. Seeds collected from Ouahigouya in the sahel zone were heavy and large with faster germination rate in the natural environmental conditions. In contrast, seeds collected in provenances located in more humid zones

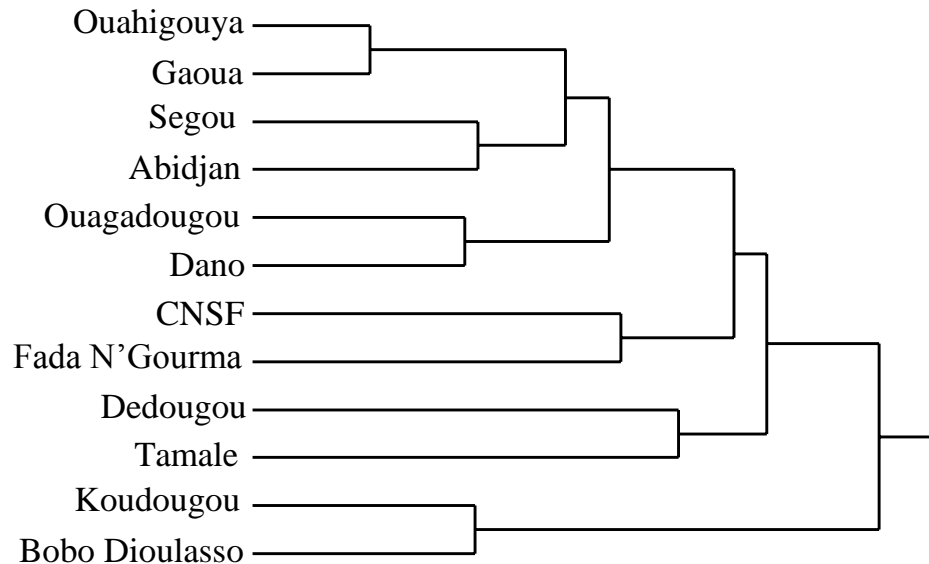


Figure 8. Hierarchical cluster dendrogram of the 12 *M. oleifera* provenances based on seed characteristic and final germination rate.

(north and south sudanian, sub equatorial) were small, less heavy and thin.

Seed size, is regarded as key factor of reproductive strategy because of its important role in establishment of the juvenile phase of tree life cycle. For example, large seed size has been documented to prolong dormancy during unfavorable light conditions, development of large amounts of photosynthetic tissue, allowing quick seedling growth and dispersal modes (Mamo et al., 2006). According to Roach et al. (1987), in very wet areas where rainfalls vary between 1000 and 1500 mm · year⁻¹, the seeds would be generally large. In this study, our results in contrary showed that the provenances seeds from wetter areas were smaller. The observed seed sizes differences are rather due to the effects of the genetics and would therefore not be environmentally based (Roach et al., 1987). Much of the total variation in seed traits are due to genetic influence as evidence from high heritability estimates (>0.90%). These genetic variations in seed traits have been reported for several tree species (Bonito et al., 2011; Khan et al., 2012; Ky-Dembele et al., 2014). Our findings in *M. oleifera* are in conformity with these research findings. Similar genetic variation in seed sizes has been reported for several tree species (Diallo et al., 2010; Ky-Dembele et al., 2014).

The present study revealed the existence of significant differences of germination rates between provenances 5 days after seeds sowing. Ouahigouya provenance from the dry agroclimatic area recorded the greatest and fastest germination rate of 63%. This variation among provenances at early stage of germination indicated that greater and heavy seeds of Ouahigouya provenance results in faster seedlings growth. Rapid germination and

subsequent seedling growth are, therefore, key phenotypes of vigorous seeds that are known to differ with genetic background (Betty et al., 2000). Thus, a vigorous seed must possess key trait of rapid germination to establish seedlings across a wide range of environments (Khan et al., 2012).

In the present study, the superior performance of Ouahigouya in seed size and germination rate suggests that conservation and management of this provenance as seed source could improve *M. oleifera* in the north Sudanian and Sahelian areas of Burkina Faso. In most plant species, seeds vary in their degree of germinability between and within populations and between and within individuals (Nyoka et al., 2015). Some of this variation can be of genetic origin, but much of it is known to be environmental, that is caused by the local conditions under which the seed matured (Mamo et al., 2006).

The study revealed no significant correlations between seed characters and germination capacity. The finding suggests that seed weight, length and thickness have little importance in predicting the germinability of *Moringa* seeds. This was already demonstrated by Fenner and Thompson (2005) and Kazmi et al. (2012). Thus, seed sizes are beneficial for the establishment of seedlings, but there appear to be no consistent correlation between seed and germination characteristics.

The hierarchical cluster analysis dendrogram revealed no clear difference between provenances. Thus, no groups of provenances were markedly discernable for seed characters, germination and geoclimatic data of provenance origin. This method of grouping of seed provenances indicated that distribution into different groups did not follow any pattern with regard to

agroclimatic seed sources.

Conclusion

This study is the first in West African to characterize *Moringa* seed sizes and germination rates. The results indicate that variability exist among provenances in seed sizes of *M. oleifera* from different agroclimatic zones of West Africa. Based on observed seed sizes and germination rates, the most prominent provenance is Ouahigouya. Seeds collected from this provenance are heavy and large with faster germination rate; thus this provenance might be considered for an eventual *M. oleifera* improvement program to enhance productivity in plantations in north Sudanian and Sahelian areas of Burkina Faso.

Conflict of Interests

The authors have not declared any conflict of interests.

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