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GERMINATION OF SEEDS FROM EARLIER FRUITS OF BITTER AND SWEET AFRICAN BUSH MANGO TREES

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ABSTRACT

Plant species are basic component of agro-biodiversity and a complex situation created by their own ability to disperse; and the rapid changes of land use and climate is endangering their efficient conservation and use. In order to increase knowledge of bitter and sweet African bush mango trees (ABMTs) (*Irvingia* spp.: Irvingiaceae) and support small-scale farmers in establishing uniform plantations, germinability of earlier fruited trees was assessed. Germination rate and speed, from both systems were analysed in order to differentiate bitter and sweet bush mango trees and identify types of seeds suitable to overcome the climatic hindrances for uniform plantations establishment in the Dahomey Gap, a drier eco-region where savannah reach the sea coast including Benin and Togo. Fresh seeds of both bitter and sweet fruited trees, showed the highest growth performance (98 - 100%). Seed germination speed significantly depended on the drying level and the germination system (sunshine versus covered condition). The speed was higher for fresh seeds in closed condition, confirming bush mango seeds as typically recalcitrant, but not strictly photoblastic. Results also demonstrated that bush mango seeds do not require specific treatments for optimising germination. Germination did not depend on mango tree type (bitter or sweet) and fresh seeds were the best material for establishing viable and uniform plantations.

Key Words: Benin, Dahomey gap, *Irvingia*

RÉSUMÉ

Les plantes sont une composante essentielle de l'agrobiodiversité; leur habileté à se propager crée une situation complexe et le changement rapide des systèmes d'utilisation des terres menace leur conservation et utilisation. Afin d'améliorer l'état de nos connaissances sur les types amer et sucré de manguiers sauvages d'Afrique (*Irvingia* spp.: Irvingiaceae) et aider les petits paysans à disposer de plantations uniformes, la germination des fruits précoces de Irvingia a été évaluée en systèmes ouvert et fermé. Le taux et la vitesse de germination dans ces deux milieux ont été analysés afin de différencier les arbres producteurs de fruits versus sucrés et d'identifier les types de semences qui sont appropriés pour réduire les contraintes climatiques pour l'établissement des plantations uniformes dans le Dahomey-Gap, une écorégion sèche où la savane atteint la côte, beaucoup plus au Benin et au Togo. Seul le niveau de séchage influence significativement le taux de germination. Les graines fraiches des deux types d'arbres ont le taux de germination le plus élevé (98 - 100 %). La vitesse de germination dépend à la fois du taux de séchage et du système de germination. La vitesse est plus élevée pour les graines fraiches en système d'ombrage, confirmant les graines de mangues sauvages comme étant typiquement récalcitrantes, mais non pas photoblastique strictes. Les résultats ont aussi montré que les graines de mangues sauvages ne nécessitent aucun traitement spécifique pour optimiser leur germination. Enfin l'étude démontre que la germination ne dépend pas du type d'arbre de manguier (arbres à fruits amers de ceux à fruits sucrés) et que les graines fraiches sont les meilleurs matériels pour l'établissement des plantations viables et uniformes.

Mots Clés: Benin, Dahomey gap, *Irvingia*

INTRODUCTION

Plant species are basic component of agro-biodiversity and a complex situation created by their own ability to disperse; and the rapid changes of land use and climate is endangering their efficient conservation and use (Trakhtenbrot and Perry, 2005; Fuller and Allaby 2010). The over-exploitation of plant non-timber forest products (NTFPs), in natural ecosystems, is causing their scarcity and growing economic value in sub-Saharan Africa (Ticktin, 2004). This is extremely risky for seed crop species, since humans limit their (non-timber products) dispersal and evolution in an increasingly severe physical environment. In a global context of faster growing human population, handling valuable tree species reproductive biology and adaptation capacity for establishing promising domestication and large plantation programmes (Simons and Leakey, 2004; Leakey *et al*., 2005) is essential for planning and responding to future increasing demands, as well as for maintaining the complex co-evolution in which human and plant species are engaged (Trakhtenbrot and Perry, 2005; Tchoundjeu *et al*., 2006; Scheldeman *et al*., 2007). This is important in transforming landscape, by establishing agricultural systems that can mitigate and adapt to climate change in the benefit of future generations livelihood improvement.

Tropical trees germinability revealed their recalcitrance, high sensitivity to conservation and significant differences regarding rate and speed of germination between and within species under different environments (Lindgren and Wei, 1994; Kyereh*et al*., 2001). In addition, light intensity and temperature are key influential factors for tropical trees germination (de Souza and Valio, 2001). Furthermore, fruit maturation is an important physiological parameter that might determine extents of germination in any plant species. Even though farmers in SSA successfully regenerate plant biodiversity, the heterogeneity of traditional agroforestry systems demonstrates lack of handling of fruits tree species' reproductive biology.

In lowlands of West and Central Africa, African bush mangoes trees are the top priority food tree species abundant in traditional land use systems (Shiembo *et al*., 1996; Tchoundjeu *et al*., 2006). They are the most economically important trees among the seven species of the Irvingiaceae family that occur in Africa, and are under two decades-long domestication programme led by the World Agroforestry Centre. We can clearly distinguish sweet and bitter fruited bush mango trees. Okafor (1975) presented those two types as varieties of a unique species *Irvingia gabonensis*: *I. gabonensis* var *gabonensis and I. gabonensis* var excels Okafor, respectively. However, they were raised up to species level: *I. gabonensis* and *I. wombolu*, respectively (Harris, 1996). Consequently, the taxonomic status within bush mango trees remains a big issue to be address (National Research Council, 2006). The mesocarp of the sweet bush mangoes are edible; while the endocarp of both bitter and sweet fruits are important part of African communities' diets and is marketed all over the world (Lowe *et al*., 2000; Tabuna, 2000; Ekpe*et al*. 2008).

The Dahomey Gap (the broad savannah inside the West African forest block) is characterised by increasing water scarcity with high temperature and bush mango trees widely found with a very high variation in the spatial density pattern (Vihotogbé *et al*., Unpublished). Farmers in the region are challenged with: (i) optimising earlier seeds germination, (ii) obtaining drought-resistant samplings to face short rain seasons and increasingly longer dry seasons, and (iii) establishing uniform and viable plantations. Germinability of bush mango trees has intensively been investigated in the eastern part of their distribution range (Omokaro *et al*., 1999; Nya *et al*., 2000; Nzekwe *et al*., 2002; Mbakwe, 2004; Dolor, 2011). Even though Ladipo *et al.* (1996) identified easy-to-crack (or self-cracked) seed, fresh seeds displayed low germinability (Nya *et al*., 2000) due to the long dormancy induced by the highly fibrous seeds (Lesley and Brown, 2004). In general, these studies lack a comparative basis between bitter and sweet fruited trees, while seed dormancy and germinability have strong genetic basis (Fuller and Allaby, 2010).

In the Dahomey Gap where little research is undertaken on bush mango trees, preservation of spontaneous trees and transplantation of spontaneous seedlings remain important strategies for enriching traditional agroforestry systems. As a solution for intensive cultivation of bush mango trees, direct sawing of 2 - 3 seeds in 5 - 10 cm depth holes is adopted. This technique guarantees high seed germination, though with evidence of waste of seeds. Hypothesizing earlier fruits to have low viability, locals establish plantations in July, while the small rainy season (September - October) is not sufficient for guaranteeing viable plantations throughout the long November - March dry period. Therefore, non-uniform plantations are common, adversely affecting small scale farmers' motivation for intensive bush trees' cultivation. Thus, interventions to optimise early fruit germination and early establishment of seedlings is necessary. The objective of this study was to assess differences in germinability between bitter and sweet trees and to hypothesize possible taxonomic implications.

MATERIALS AND METHODS

Study area. This study was conducted in the Guinean-Congolian rainforest in West Africa. The Dahomey Gap refers to the mosaic of savannah, drier type of lowland, fields and fallows found from Badagry (Nigeria) through Benin and Togo to Accra in Ghana (Sowunmi, 2007). This low rainfall and high temperature area splits the West African Forest into Upper and Lower Guinean blocks (Salzmann and Hoelzmann, 2005). Among the tropical rainforest trees that survive this climatic anomaly, bush mango trees are the most economically important of the six Irvingiaceae species occurring in Africa (Asaah *et al*., 2003). The study covered the South of Benin and Togo, the two countries that mostly contribute to the Dahomey Gap. In this area, bush mango trees are found in natural forests, forest gardens (in the Volta Forest Region, South-western Togo) and most abundantly in intensive cultivation systems: home gardens, orchards, agroforestry parks all over in the Dahomey Gap (Vihotogbé *et al*., 2014). In general, the peak of fruit production in this region occurs in March and June, for the bitter and the sweet fruited trees, respectively (Vihotogbé *et al*., 2014). Earlier fruits fall in January - February and are suspected to have low germination rate by local farmers who practice intensive cultivation.

Sampling design and germination experimentation. Five provenances from different ecological areas were tested. The provenances were defined based on their geographic origin, type of bush mango trees, current domestication process and type of agroforestry systems from which the trees were sampled (Fig. 1, Table 1). Seven to eleven healthy trees that fruited earlier (in February - March) were randomly sampled in each provenance. For each provenance, seven hundred and twenty healthy and mature fruits of 148 ± 51 g that had freely fallen down were collected under the sample trees. The fruit mesocarp was removed and the seeds sun-dried outside in open air. The weather parameters recorded by the Beninese Institute for Agronomic Research indicated 9.7 mm, 35.3 ± 2.16°C and 66 ± 5.2% for total rainfall, mean temperature and moisture, respectively.

Three replications (each made of a cluster of 30 seeds) of each provenance were sown fresh (just after removal of mesocarp) and after seven, fourteen and twenty-one days of sun-drying (Fig. 2). The three replications for all provenances at each drying level were sown in a completely randomised design with regard to each germination condition (sunshine and covered). In total, three thousand and six hundred seeds were germinated in polybags filled with compost, in closed/covered and sun-shine germination conditions. The germination condition totally exposed the polybags to sunshine, while in the closed/covered one, were covered with vegetable mats, preventing them from intensive light of February - March 2011. However, this covering system did not prevent the experiment from accessing rainfall.

Seeds were sown in 1 cm soil depth in the polybags watered with 261.7 mm of water (12 mm every two days until the fortieth day in addition to the 9.7 mm rain fall during the essay).

Daily observations were made to register the total number of seeds that germinated within each replication (cluster of 30 seeds) up to forty days after sowing (number of day after which no new germination was obtained). Here, we considered germination to be the complete emergence of the seedling, since this is an indicator of successful plantation establishment.

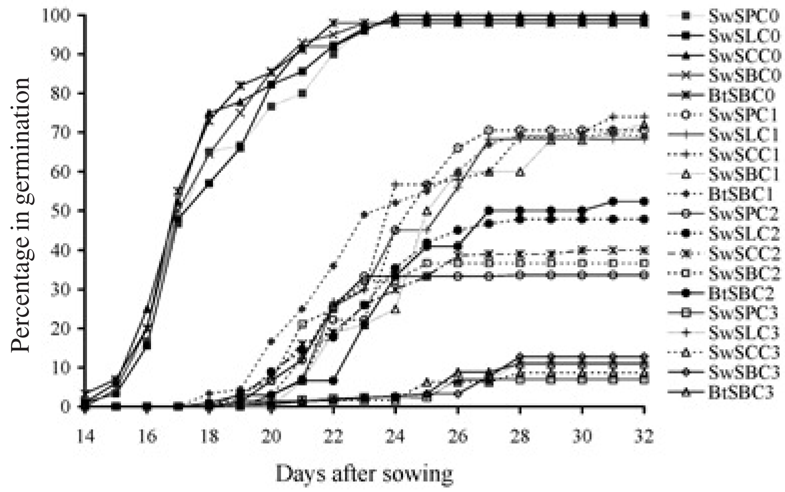

Figure 1. Germination rate of bush mango seeds in sun-shining germination condition in Benin. SwS = seeds of sweet bush mangoes; Bts = seeds of bitter bush mangoes; P= from Pobè; L = from Lomé; C = from Couffo; B = from Badou; O = germinated in sun-shining condition; second C = covered condition; 0 = fresh; 1, 2, 3 = dried for 1, 2, 3 weeks.

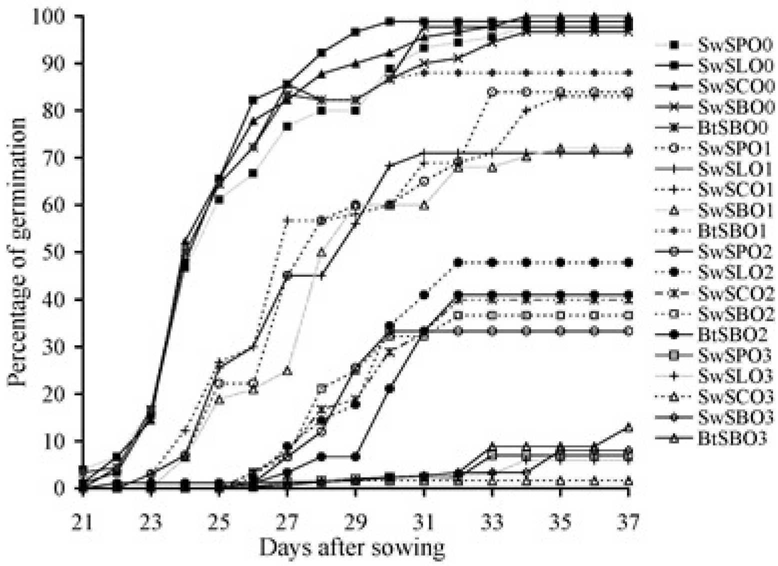

Figure 2. Germination rate of African bush mango trees in close germination condition in Benin. SwS = seeds of sweet bush mangoes; Bts = seeds of bitter bush mangoes; P= from Pobè; L = from Lomé; C = from Couffo; B = from Badou; O = germinated in sun-shining condition; second C = covered condition; 0 = fresh; 1, 2, 3 = dried for 1, 2, 3 weeks.

Data analysis. The following computations were made:

(i) The germination rate (GR_r) which is the final germination percentage until the fortieth day after sowing:

$$GR_r = N_{gr}\, x \frac{100}{Nt} \qquad (1)$$

Where:

N_{gr} = total number of seed that germinated in each replication

N_t = 30 (number of seeds in the replication);

(ii) Germination speed at different germination rates up to the highest one reached in the replication: GS_i:

$$GS_i = \frac{NG_i}{ND_i} \quad (2)$$

Where:

NG_i = number of seed corresponding to the i[th] considered germination rate;

ND_i = number of days required for the i[th] germination percent to be reached in the replication. In this study, the germination rates considered

Where:

T = 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100%.

For each cluster of the three replications representing each combination of the different levels of the four factors studied (type of bush mango trees, drying level, provenance and germination condition), we calculated:

(iii) Mean germination rate (mGR_{rj}) which is the average of the GR_r values for the three replications at the j[th] combination of the different levels of the three factors:

$$mGR_{rj} = \frac{1}{3}\sum_{z=1}^{3} GR_{rjz} \quad (3)$$

Where:

GR_{rjz} = GR_r value of the z^{th} (z = 1...3) replication in the j^{th} combination the levels of factors.

(iv) Mean germination speeds at the ten potential germination rates considered (mGS_{ij}), that is, the average of the GS_i values considering the three replications at the j^{th} combination of the different levels of the three factors:

$$mGS_{ij} = \frac{1}{3}\sum_{z=1}^{3} GS_{ijz} \quad (4)$$

Where:

TABLE1. Characteristics of mango provenances tested in the Dahomey Gap in Benin

Type of ABMTs	Geographic areas and corresponding GPS position around which sampling was done	Description of the sampled provenances
Sweet trees	Couffo: South West BeninLong = 1.77106Lat = 6.75011	P1 = Only cultivated trees(in orchards and agroforestry parks) selected throughout decades in mass selection process for endocarp commercialization
	Pobè: South East BeninLong = 2.68083Lat = 6.7896	P2 = Spontaneous trees from thrown seeds after the mesocarp consumption on fields and home gardens.
	Lomé: South TogoLong = 0.91072Lat = 6.53256	P3 = Only cultivated trees on farm without selection process
	Badou: South West Togo in the Volta Forest RegionLong = 0.56461Lat = 7.60055	P4 = only spontaneous trees in forest gardens without any selection process
Bitter trees	Badou: South West Togo in the Volta Forest RegionLong = 0.57450; Lat = 7.45222	P5 = Only native trees sampled natural forest

GS_{ijz} germination speed at the i^{th} germination rate in the z^{th} ($z = 1...3$) replication of the j^{th} combination the levels of factors. The germination speed indices, GS_i and GS_{ijz}, depict the dynamic aspect in the germination process and as such, are useful for identifying both categories of seeds based on means of the germination speeds as well as uniformity of the germination inside each provenance.

The influence of the main effects, i.e. the type of bush mango tree, provenance, drying level and germination condition on the germination rate, as well as on the germination speed were assessed using Analysis of Variance (ANOVA) in a Generalised Linear Model carried out in Statistica 6 (StatSoft, 2001). Using PAST package (Hammer *et al.*, 2001), a Principal Component Analysis (PCA) was carried out on the correlation matrix of the speed at the 10 considered germination rates for categorising seeds and identifying the type of seed that could meet small farmers' expectations in the Dahomey Gap. The trend in the germination (integrating the beginning and total days necessary to accomplish their germination process) of the different categories of seed was compared by plotting the first two axes of the PCA.

RESULTS

Only the drying level showed a strong significant ($P<0.0001$) influence on the germination rate. Fresh seeds had the highest percentages of germination (98 – 100%). This steadily decreased when the drying duration increases: (82, 49 and 11% for 7 days, 14-15 days and 21 days of drying, respectively; Figs. 1 and 2).

Germination speeds significantly depended on the drying level ($P<0.0001$) and on the germination condition ($P= 0.001$). In the closed condition, germination started earlier (13-14 days after sowing) with higher speeds (mean= 1.7± 1.24 seeds day^{-1}; max = 4.5 seeds day^{-1}), than in the sun-shining condition (mean = 1.32 ± 0.93 seeds day^{-1}; max = 3.5 seeds day^{-1}) for which the earlier germination occurred 21 days after sowing. In addition, the highest speed was found in fresh seeds (2.9 ± 1.02; max = 4.5) and this decreased with the increase of the drying duration (1.8 ± 0.62, 1.1 ± 0.3, 0.23 ± 0.08 for 7; 14 and 21 days,

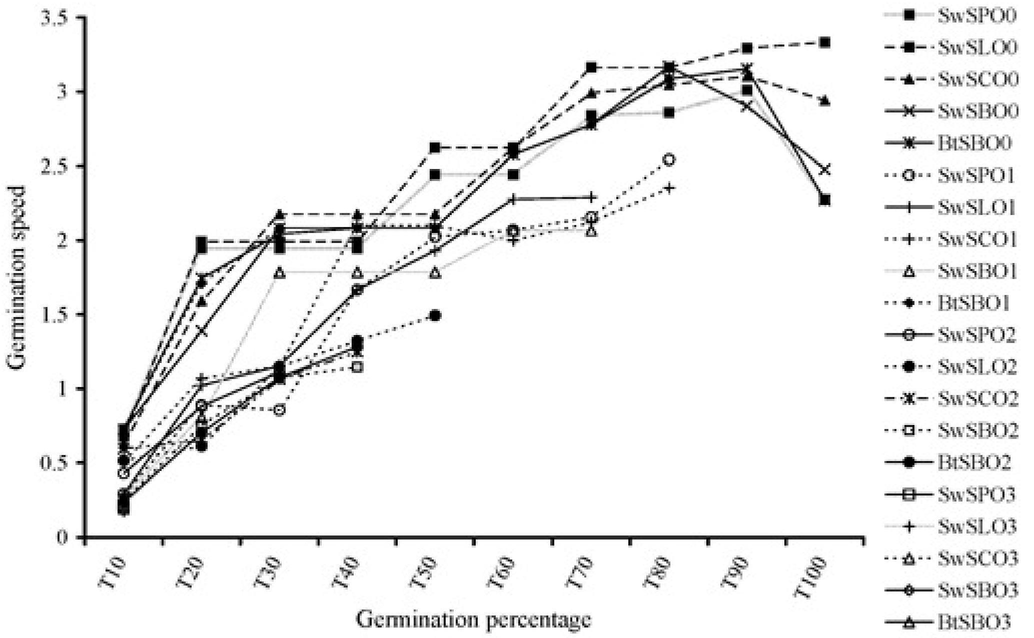

Figure 3. Germination speed of bush mango seeds at different percentages of germination in the sun-shining condition in Benin. SwS = seeds of sweet bush mangoes; Bts = seeds of bitter bush mangoes; P= from Pobè; L = from Lomé; C = from Couffo; B = from Badou; O = germinated in sun-shining condition; second C = covered condition; 0 = fresh; 1, 2, 3 = dried for 1, 2, 3 weeks.

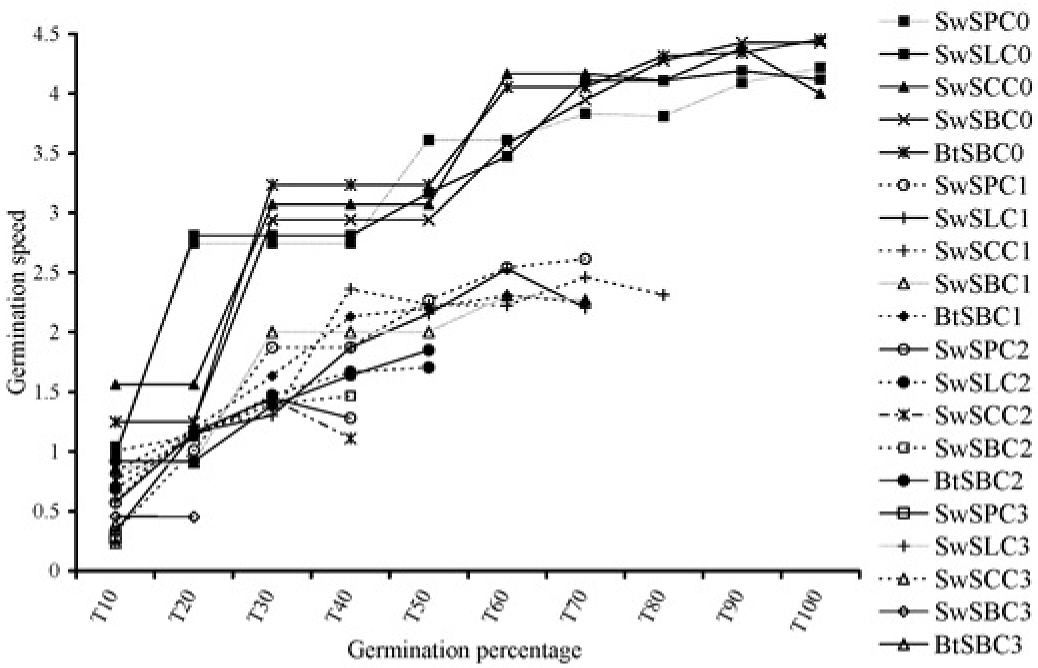

Figure 4. Speed of germination of bush mango seeds at different percentages of germination in the close condition in Benin. SwS = seeds of sweet bush mangoes; Bts = seeds of bitter bush mangoes; P= from Pobè; L = from Lomé; C = from Couffo; B = from Badou; O = germinated in sun-shining condition; second C = covered condition; 0 = fresh; 1, 2, 3 = dried for 1, 2, 3 weeks.

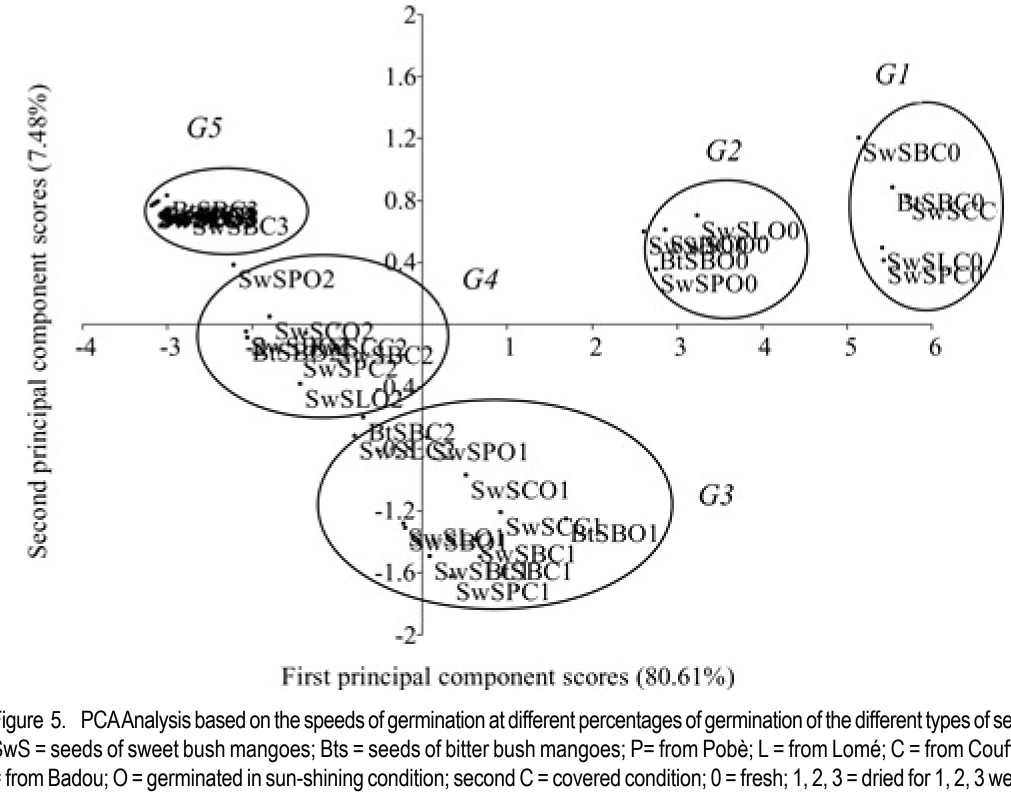

Figure 5. PCA Analysis based on the speeds of germination at different percentages of germination of the different types of seeds: SwS = seeds of sweet bush mangoes; Bts = seeds of bitter bush mangoes; P= from Pobè; L = from Lomé; C = from Couffo; B = from Badou; O = germinated in sun-shining condition; second C = covered condition; 0 = fresh; 1, 2, 3 = dried for 1, 2, 3 weeks.

respectively). No significance effect was found for provenance and type of bush mango trees (Figs. 3 and 4).

The first two axis of the PCA accounted for about 88% of the variations of the germination speeds of the different categories of seeds through the 10 different germination rates considered (Fig. 5). The first axis of the PCA (80%) presented the highest and positive correlations with all percentages of germination (Fig. 5). Using the position of the different types of seed on this first PCA, we defined groups of seeds characterised by the quick start as well as the speed of their germination. The general trend showed that the position of the types of seed of the first PCA decreased with the drying duration. Thus, 5 groups were clearly defined in Figure 5. The first group (cluster G1: made up of all fresh seeds of bitter and sweet trees in the close germination system) started their germination very quickly and maintained higher germination speed (3.5 - 4.5 seed per day) to quickly reach the maximum of germination (95 - 100% of percentage). The second group (cluster G2) was composed of all fresh seeds of bitter and sweet trees, germinated in the sun-shine germination system. This group started germination and completed it with high speed; but, lower than the first group (2.5 - 3.5 seed per day to reach 90 - 100% of percentage). The other three groups (clusters G3, G4 and G5) started germination late and completed with lower germination speeds. The three weeks dried seeds of every provenance represented the group with worst germination speed (Fig. 5). Thus, fresh seeds, regardless of germination condition, type of bush mango trees and the provenance, had the highest positions on the first axe indicating that they germinated faster than any other category of seeds.

DISCUSSION

This study highlighted no valuable discriminative power of both the germination rate and speed to distinguish provenances and types of bush mango trees (Table 1). Therefore, in addition to the morphological high similarity of bitter and sweet fruited trees (Harris, 1996), similarity in their germinability strengthens the uncertainty regarding their species integrity (National Research Council, 2006). Because morphological data available (Harris, 1996; Vihotogbé *et al*., 2013) did not cover the total life cycle of bush mango trees, we suggest a broader and detailed vegetative evaluation that includes seedling characterisation, as well as evaluation of earlier adaptation of seedlings/samplings in different ecological conditions in order to strengthen conclusions regarding the taxonomic distinction of bitter and sweet trees.

Local assumption on the questionability of the viability for earlier bush mango seeds was not confirmed in this study. The low germination percentages obtained by locals using earlier fresh seeds also appeared in the results of Ewedjê *et al*. (2007), who germinated seeds collected during the pick fruiting time. The sowing depth of more than 1 cm might presumably be excessive, jeopardising the completion of the germination process in both situations. Indeed, direct sowing in heavy clayey soils, common over the entire distribution range of bush mango trees, by farmers might not guarantee high percentage of germination. As a solution to this, Nzekwe *et al*. (2002) recommended a mixture of soil and sawdust to predict high percentage of germination (80%) and vigorous seedling. Therefore, sowing fresh seeds in less depth than 1 cm hole guarantees better performances (germination rate of 98 - 100%) for any provenance and for both bitter and sweet trees. Thus, our result demonstrated that no particular treatment of bush mango seeds; drying plus rehydration or scarification or particular chemical-based pre-treatment (Omokaro *et al*., 1999; Nya *et al*., 2006) was necessary to optimise the germination rate of bush mango trees. We, therefore, conclude that these usual dormancy suppression techniques often applied to recalcitrant seeds are not always necessary, most importantly when they cannot be promoted in low income Sub-Saharan African farmers' condition. In the case of bush mango trees, just compost or forest soil (affordable by local nurserymen) holds sufficient water to allow the highest germination rate of fresh seeds with healthy and vigorous seedlings for both bitter and sweet trees under the close germination condition. In contrast, attempting to dry these high oil content seeds (up to 70%; Joseph, 1995) in a tropical high moisture environmental condition exposes them to rapid moulds colonisation, jeopardising the viability of seeds after few days of conservation under ambient conditions (Lowe *et al*., 2000). Thus, Okafor (1999) already observed less germinability for pick time fruits (80%) after two drying days. Consequently, our results evidenced the recalcitrance of bush mango seeds as suggested for many tropical trees species (Kyereh *et al*., 2001; Nya *et al*., 2006).

High temperature, intensive light and fluctuations of moisture for breaking pioneer recalcitrant tropical seeds dormancy (Kyereh *et al*., 2001), was proven less efficient than steady maintenance of low temperature, low light and of humid substrate, regarding the speed of germination. Both bitter and sweet trees are non-strict photoblastic taxa and, therefore, seedlings and samplings might germinate faster under deeper shade conditions. This confirms the easy recolonisation of bush mango trees in low land natural forests (Van Dijk, 1996), as well as their abundance in human made agrosystems even in the Dahomey Gap (Shiembo *et al*., 1996). Therefore, climate change plays fewer roles in the low regeneration numerous valuable fruit tree species in natural stands, over their entire distribution range, that land use change and seed or other body parts collection for marketing. Indeed, the low densities of bush mango trees in natural areas are typically human driven consequence of the intensive collection of fruits for marketing

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