Seed size polymorphism in *Khaya senegalensis* (Desr.) A. Juss.: Implications for seed propagation

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Seed size variation has implications for the success of seedling establishment, but the underlying mechanisms are yet to be fully explored in many species, including *Khaya senegalensis*. Moreover, seed size is measured in different ways (for example, mass or length), but the extent to which these different ways of measurement differ in predicting seedling growth parameters is unknown. In this study, how well seed mass and seed length predict seed food reserves was tested. Then, pot experiments were conducted to determine which of the two measures of seed size was a better predictor of seedling size and root biomass allocation. Also, effects of seed size variation and its relation to sowing depth on seedling parameters were investigated. Results showed that both seed mass and seed length significantly predicted the amount of seed food reserves, but seed mass explained a greater percentage of the variability in seed reserves than seed length (64.1% versus 19.3%) and as a result, seed mass also better predicted seedling size. However, both seed mass and seed length poorly predicted root length and root biomass allocation. Also, it was found that at all the tested sowing depths in this study, larger seeds produced larger and taller seedlings, but a combination of large seeds with 0 cm sowing depth yielded the largest and tallest seedlings. Root length decreased with sowing depth, regardless of seed size. Root mass fraction of seedlings from small seeds decreased with sowing depth, while those from large seeds were unaffected. It is recommended that to produce larger seedlings with a greater allocation to root biomass, large seeds in combination with superficial sowing depth should be used when nursing *K. senegalensis* seeds.

**Key words:** Seed size variation, sowing depth, seedling size, root biomass, *Khaya senegalensis*.

**INTRODUCTION**

Seed polymorphism is defined as “the production of two or more distinctly different types of seeds by a species” (Harper et al., 1970). Seed size polymorphism therefore refers to size variations in seeds produced by a species. A sizeable body of knowledge exists on this phenomenon (Poulin and Hamilton, 2000; Simons and Johnston, 2000; Einum and Fleming, 2002). In many species, seed size variation has important connection to the overall
biological fitness of parental species, by directly affecting the process of germination, seedling recruitment and competitive ability (Shaukat et al., 1999; Leishman et al., 2000; Coomes and Grubb, 2003; Souza and Fagundes, 2014).

According to Leishman et al. (2000), "seed size of a species represents the amount of maternal investment in an individual offspring, or how much 'packed lunch' an embryo is provided with to start its journey in life". But which is a better way to measure "packed lunch"; mass or length of 'lunch box'? This question represents an important challenge at the nursery when raising seedlings from species that vary in seed size.

*Khaya senegalensis* (Desr.) A. Juss., belonging to the family Meliaceae is a savanna tree species of enormous socio-economic importance, but has poor natural regeneration (Nikiema and Pasternak, 2008). Plantation development of this species is necessary for perpetual flow of benefits. It is recognized that production of good quality planting stock is a critical first step to successful plantation establishment. However, apart from variations in seed size among individuals within species resulting from differences in environment, seed size of *K. senegalensis* is also known to vary greatly among provenances (Ky-Dembele et al., 2014). In this species, seeds could be easily grouped into different size classes based on ocular estimates of seed length. Because seeds are winged as an adaptation to dispersal, variations in wing size may imply that for some seeds, not the entire length of the seed is filled with the endosperm. Also, variation in seed thickness makes it much more likely that great variability in seed mass may exist even among individuals that appear to have the same length. Therefore, knowledge of relationships between the various ways of measuring seed size (and between them and cotyledon mass, which is a measure of seed food reserves) is needed to be able to make right choices at the nursery. However, such data is lacking, particularly for this species.

Additionally, larger seedlings are required for higher establishment success in the savanna due to the frequent bush fires and the longer dry seasons in this environment (Fensham et al., 2003). This is important because planted seedlings of *K. senegalensis* are fairly susceptible to fires (Orwa et al., 2009) and are also known to suffer dry season drought stress (Arndt, 2015). Larger seedlings survive better because they have higher carbohydrate reserves (Westoby et al., 1996; Leishman et al., 2000), but the amount of carbohydrate reserves correlates with root mass fraction (RMF) and both traits are known to enhance drought survival (O’Brien et al., 2010) and post-fire re-sprout capacity (Hoffmann et al., 2004) among seedlings of savanna species. Therefore, to achieve higher seedling establishment success under harsh environmental conditions, larger seedlings or seedlings with a higher allocation to root biomass are needed. This may be accomplished by picking out and sowing large seeds and at the right sowing depths. Sowing depth is important because seeds sown deeper take a longer time to emerge, requiring much more energy to be expended. This could affect seedling size and competitive ability (Tripathi and Bajpai, 1985). Also, in containerized planting, deep sowing could obstruct root development.

Data on effect of seed size variation on seedling traits in this species are scarce (Ky-Dembele et al., 2014), but even more scarce are studies that have explored the interaction effects of seed size and sowing depth on seedling size and root biomass allocation. In this paper, findings on experiments in which the extent of the relationship of seed food reserves to seed mass and seed length are presented, and also, which measure of seed size better predicts seedling size and root biomass allocation was determined. The main and interaction effects of variations in seed size and sowing depth on seedling size and root biomass allocation was also determined.

**MATERIALS AND METHODS**

**Study site**

The experiments were carried out at the plant house of the Nyankpala Campus of the University for Development Studies, Tamale. The site is located within the Guinea savanna ecological zone in the Tolon district of Northern Region of Ghana. Geographically, the district lies between latitude 9° 25’N and longitude 0° 58’W. Average mid-day temperature at the plant house for the month of March, 2015 (when the experiments were conducted) was 29°C. The roof of the plant house reduces irradiance level by up to 40%.

**Seed collection and study approach**

In February, 2015, seeds of *K. senegalensis* were collected under 40 fruiting trees within the Tamale Metropolis located in the Guinea savanna ecological zone in Northern Ghana. Seeds gathered were put together in a 25 m² sack. The seeds were used in two separate experiments. The first experiment was conducted to determine the extent of the relationships of seed mass and seed length to seedling size and root biomass allocation. In this experiment, the extent of relationships of seed mass and seed length to cotyledon dry mass (a measure of seed food reserves) was also quantified with a view to establishing which of the two (that is, mass or length) better predicts amounts of seed reserves in *K. senegalensis*. The second experiment was carried out to establish main and interaction effects of seed mass with sowing depth on seedling size and root biomass allocation of seedlings. Prior to conducting the plant house experiments, some seeds were sampled for determination of seed reserves.

**Determination of seed reserves**

A total of 500 seeds were picked at random from a large seed pool. Fresh mass (g) and length (cm) were measured of each seed using an electronic scale and a ruler, respectively. Samples were then oven-dried at 70°C for 48 h after which seed coats were removed. The endosperm (cotyledon) were weighed to obtain cotyledon dry mass (which was used as a measure of seed food reserves).
Experiment I

Design, layout and data collection

Another 500 seeds were sampled and weighed. With the help of a divider and a ruler, lengths (cm) of the fresh seeds were taken along the long axis of each seed, making sure only cotyledon (endosperm) length was obtained. This was necessary because seeds of this species are winged. Seeds were then sown in rectangular seed boxes (with dimensions 50 cm x 15 cm x 10 cm) at 2.5 cm depth. At the start of the experiment, each seed box received 1000 ml of water per day given in a twice daily dose (morning and evening). This quantity was reduced to 500 ml after 2 days to avoid soil saturation. Emergence started 5 days after sowing and amount of water given was again increased to 1000 ml per day to cater for the increasing water demand. The position of each seed was marked. This was crucial because although each box contained 20 seeds, each seed was an experimental unit. Boxes only served as seed beds.

The number of days it took for each seed sample to emerge was recorded, noting samples that failed to emerge at the end of the experiment (that is, 90 days after planting). Seedling height of all samples was measured. They were then uprooted, tagged and oven-dried at 70°C for 48 h, and separated into root, stem and leaves and each part weighed separately. Taproot length was measured prior to oven-drying. Total seedling dry mass was calculated by summing up root, stem and leaf dry mass. Root mass fraction (RMF) was then determined by dividing root dry mass by total seedling dry mass.

Experiment II

Design, layout and data collection

For this experiment, 480 seeds were picked at random from the seed pool. Seed fresh mass was determined following same protocol as in experiment I. Seeds were then put into one of two size categories; large seeds (> 0.35 g) and small seeds (< 0.25 g). Seeds were sown in seed boxes (same dimensions as those used in experiment I) at three different depths; 0, 2.5 and 5.5 cm. Zero cm sowing depth meant placing the seed on the soil surface without covering with soil. Each size-depth treatment combination (total of 6) was assigned to a seed box in a completely randomized design (CRD) such that each box represented an experimental unit. Each treatment was replicated 4 times. Twenty seeds were sown in each box. Soils for this experiment were taken from top 10 cm in a mango plantation of the Faculty of Renewable Natural Resources, Nyankpala. No fertilizers were added. Watering regime was same as in experiment I. The experiment ended 65 days after sowing. 7 seedlings were randomly sampled from each box and height of each sample was measured with a ruler. The samples were uprooted and their taproot lengths were measured. They were then separated into roots, stems and leaves and oven-dried at 70°C for 48 h. Dry weight of roots, stems and leaves were measured with an electronic scale. Total seedling dry mass and root mass fraction were determined following same protocol as in experiment I.

Data analysis

Data from the 500 seeds used for the determination of seed reserves were combined with the 500 seeds from experiment I and explored for descriptive statistics (n = 1000 seeds). Means and standard deviations were then used to determine coefficients of variation for seed mass and seed length. To determine the better predictor of seed food reserves, separate linear regression analyses were conducted with seed mass and seed length as predictors and cotyledon dry mass as the dependent variable. Also the measure of seed size which better predicts seedling size and root biomass allocation was determined by subjecting each seedling trait measured in experiment I to linear regression analysis. Pearson’s correlation coefficient was used as a measure of strength of the relationships between each seedling trait and the predictor (that is, either seed mass or seed length). Two regression equations, one for each predictor, were also derived for each measured seedling parameter.

To determine main and interaction effects of seed size and sowing depth on seedling size and root biomass allocation, a multivariate analysis of variance (MANOVA) was performed on seedling height, seedling total dry matter and RMF. Our choice test statistic was Roy’s Largest Root as that proves more powerful with smaller sample sizes (Olson, 1974, cited in Field, 2009). A MANOVA was chosen over multiple ANOVAs due to the possibility of relationships existing among the dependent variables (that is, seedling dry mass, seedling height, root length and RMF), but more importantly to control familywise error rates (Field, 2009). Where a significant interaction effect of seed size and sowing depth was found, adjustment for multiple comparisons was done using SIDAK. All analyses were done on SPSS version 22.0 (IBM Corp., 2013).

RESULTS

Is there evidence for seed size variation?

Seed mass varied from 0.1 to 2.8 g (range = 2.70 g). The mean seed mass was 1.117 ± 0.026 g. The coefficient of variation for seed mass was 76.4%. Seed length varied from 0.15 to 2.8 cm (range = 2.65 cm). Mean seed length was 1.156 ± 0.0584 cm. The coefficient of variation for seed length was also found to be very high (75.8%).

Which predicts seed food reserves better: seed mass or seed length?

Seed mass and seed length were significantly correlated (r = 0.482, p < 0.001). Therefore, both produced regression models that significantly (F1 = 888.499, p < 0.001 and F1 = 120.500, p < 0.001, respectively) predicted cotyledon dry mass (seed food reserves). However, the amount of variation in cotyledon dry mass explained by seed mass was higher (64.1%) than variation explained by seed length (19.3%) (Figure 1A and B). The resulting regression equations are Y = 0.28 + 0.613X and Y = 0.070 + 0.02X for seed mass and seed length, respectively.

Which better predicts seedling size and root biomass allocation; seed mass or seed length?

Results of the linear regressions conducted on data from experiment I revealed significant correlations between seed mass and seedling dry mass (Figure 2A), and between seed mass and seedling height (Figure 2C). However, correlations between seed mass and both root length and RMF were not significant (Figure 2E and G,
Figure 1. Relationships of cotyledon dry mass (seed reserves) with seed mass (A) and seed length (B). (N = 500 seeds).

Table 1. Regression equations for measured seedling parameters with seed mass and seed length used as predictors in the model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Regression equation</th>
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<tr>
<td></td>
<td>Seed mass (g)</td>
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<tr>
<td>Plant dry weight (g)</td>
<td>Y = 0.228 + 0.620X (**)</td>
</tr>
<tr>
<td>Seedling height (cm)</td>
<td>Y = 13.305 + 7.076X (*)</td>
</tr>
<tr>
<td>Root length (cm)</td>
<td>Y = 7.069 + 0.752X (ns)</td>
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<tr>
<td>RMF (gg(^{-1}))</td>
<td>Y = 0.150 + 0.009X (ns)</td>
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** p < 0.001; * p ≤ 0.05 > 0.001; ns = no significant difference.

respectively). Seed length on the other hand, correlated significantly with seedling height and RMF (Figure 2D and 2H, respectively), but did not correlate significantly with seedling dry mass and root length (Figure 2B and 2F, respectively). Seed mass as a predictor produced a regression model that predicted seedling dry mass and height significantly (F\(_1\) = 12.710, p < 0.001 and F\(_1\) = 5.046, p = 0.025, respectively), but did not significantly (F\(_1\) = 0.246, p = 0.620 and F\(_1\) = 0.019, p = 0.889, respectively) predict root length and RMF. Seed length on the other hand produced a regression model that significantly (F\(_1\) = 5.946, p = 0.015) predicted seedling height, but regression models for seedling dry mass, root length and RMF were not significantly (F\(_1\) = 0.177, p = 0.674; F\(_1\) = 0.066, p = 0.798 and F\(_1\) = 3.233, p = 0.073, respectively) predicted by seed length. The resulting regression equations are presented in Table 1.

What are the effects of seed size and sowing depth on seedling size and root biomass allocation?

Results of the MANOVA revealed significant (V = 1.247, F\(_4, 16\) = 4.987, p = 0.008, \(\eta^2 = 0.55\)) interaction effects of sowing depth and seed size on mean seedling height, total dry mass, root length and RMF. Separate univariate ANOVAs on the outcome variables revealed significant main effects of seed size (F\(_1\) = 47.99, p < 0.001, \(\eta^2 = 0.727\)) and sowing depth (F\(_2\) = 9.355, p = 0.002, \(\eta^2 = 0.51\)) on seedling dry mass. The general pattern revealed was that regardless of seed size, seedling dry mass decreased with sowing depth and large seeds consistently produced larger seedlings regardless of planting depth (thus, no interaction effect of seed size × sowing depth was detected with the F-test). However, pairwise comparisons (with SIDAK adjustment) showed that a combination of large seeds with 0 cm sowing depth yielded the highest dry matter (Figure 3A). Just as in the case of seedling dry mass, it was found that although the F-test did not produce a significant (F\(_1\) = 2.835, p = 0.08) seed size × sowing depth interaction effect on seedling height, pairwise comparisons showed that large seeds were significantly (p = 0.02) taller than those from small seeds at 0 cm sowing depth, but this effect of seed size was not found at higher sowing depths (Figure 3B).

Root mass fraction (RMF) was significantly (F\(_2\) = 6.27,
$p = 0.009$, $\eta^2 = 0.41$) affected by the interaction of seed size and planting depth. Pairwise comparisons revealed that whereas seedlings from large seeds were not significantly affected by planting depth, seedlings from small seeds had lower RMF at higher sowing depths, such that the two seed size categories differed significantly at 5.5 cm sowing depth. For small seeds, RMF at 0 and 2.5 cm sowing depths were similar but both were significantly ($p = 0.001$ and $p = 0.002$, respectively) higher than 5.5 cm sowing depth (Figure 3C). Root length was significantly ($F_2 = 22.797$, $p = < 0.001$, $\eta^2 = 0.717$) affected by sowing depth. The effect size of sowing depth on root length was very high. Seed size effect on root length was not significant ($F_1 = 1.278$, $p = 0.27$, $\eta^2 =$

**Figure 2.** Relationships of measured seedling parameters to seed mass and seed length. $N = 500$ seeds. The extent of correlations between seed and seedling parameters is shown with $r$ and its $p$ value.
Figure 3. Estimated marginal means of (A) seedling dry matter, (B) seedling height, (C) RMF and (D) root length of the two seed size classes at different sowing depths. Open bars represent seedlings from large seeds and grey-filled bars are seedlings from small seeds. Letters indicate significant (different letters) or non-significant (same letters) differences at 0.05 level of significance. Error bars are standard errors from table of estimated marginal means after pairwise comparisons. Adjustment for multiple comparisons was done using SIDAK.

0.06). Pairwise comparisons revealed that root length was highest at 0 cm sowing depth and lowest at 5.5 cm sowing regardless of seed size (Figure 3D).

DISCUSSION

Plantation development is increasingly becoming relevant as natural forests begin to succumb to anthropogenic pressure. For many species, raising good quality planting stock from seeds is a critical first step. Seed size is clearly important, but important questions remain unanswered about the extent of seed size variation and its exact effects in many species. These questions were investigated in *K. senegalensis* and a very high size variability among seeds was found. This was the case whether seed size was measured either in mass or in length. Due to both genetic variability and differences in site resources and/or conditions, individuals of the same species could vary greatly in sizes of seeds produced (Leishman et al., 2000; Halpern, 2005). Seeds used in our experiments came from many individuals which may also belong to different provenances. This may explain the high variability in seed size observed in this study. This does not represent a limitation in methodology because seeds used in large scale nursery operations are often collected from many individual trees. Moreover, between-provenance variability in both seed length and seed mass has already been demonstrated in this species (Ky-Dembele et al., 2014).

Also, it was found that both seed length and seed mass significantly predicted seed reserves, but seed mass was a better predictor than seed length because it explained a greater percentage of the variability in seed reserves than seed length. Thus, there were many seeds of same length that had different amounts of seed reserves than there were seeds of same mass that had varying amounts of seed reserves. Therefore, seed mass also predicted seedling height and dry matter yield better than seed length, although both did not predict root length and root biomass allocation very well. These findings are consistent with the expectation as it is known in many species that the amount of seed reserves determines seedling size (Westoby et al., 1996; Leishman et al.,
The implication of this finding is that it is better for seeds to be selected for sowing based on seed mass rather than seed length, in spite of the fact that it may be easier to pick out seeds based on length as length appears to be more easily estimated by ocular means than seed length, which has to be measured. This is important because the amount of seed reserves determines success of planted seedlings via its influences on seedling size (Westoby et al., 1996; Coomes and Grubb, 2003).

The second experiment revealed that larger seeds produced larger and taller seedlings than smaller ones, but there was a decreasing pattern of seedling size and height with sowing depth such that differences in height between the two seed size categories existed at the highest sowing depth (5.5 cm). This may be because more reserves (energy) was needed to emerge from deeper layers, consistent with findings in other species (Tripathi and Bajpai, 1985; Schmidt, 2000). Additionally, it was found that both RMF and root length did not depend on seed size, but both decreased with sowing depth, possibly due to physical limitation of container. It was also observed that RMF of seedlings from smaller seeds suffered the adverse effect of deep sowing, but seedlings from larger seeds were not affected. The deeper a seed is sown in a container the less space the roots have to extend into deeper layers because of the physical limitation imposed by the bottom of the container.

Conclusion

Seed size variation has important implications for seedling success. The study investigated the extent of this phenomenon in K. senegalensis and explored its influences and underlying mechanism on seedling traits that are crucial for field survival. Seeds of this species vary greatly in terms of both length and mass, but it is better to measure size as mass rather than length of seed, as mass predicts seed reserve better than length. Also, findings revealed that the size of sown seed determines seedling dry mass and height, with larger seeds producing larger and taller seedlings than smaller ones. However, while seed size does not determine how much biomass is allocated to roots or how deeply rooted the seedlings are, sowing depth determines both the size of the seedling obtained and how deeply rooted the seedlings are in the container. The deeper the sowing depth, the shorter the roots of resulting seedlings. Root mass fraction also decreases with sowing depth when seedlings are small.

It is suggested that a combination of large seedlings with shallow sowing depth (shown in this study as > 0.35 g and 0 cm, respectively) yields the largest seedlings with the highest allocation to root biomass and therefore recommended for use when raising K. senegalensis seeds in containers.

Conflict of interest

Authors have not declared any conflict of interest.

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