

Response of seedling growth attributes to seed size variation of *Albizia lebeck*

ABSTRACT

Aims: To investigate the influence of seed size variation on seedling growth attributes of *Albizia lebeck* in the nursery.

Place and Duration of Study: The experiment was conducted in greenhouse, nursery or where at Malawi College of Forestry and Wildlife nursery between March and September 2014.

Methodology: Seeds were categorized into four groups in regard to their length and weight: large (≥ 0.80 cm) and (≥ 0.20 g); medium (0.51-0.79cm) and (0.11-0.19g); small (≤ 0.50 cm) and (≤ 0.10 g) and a mixture of large, medium and small seeds (acted as a control). The treatments were completely randomized into four replications. A total of 800 seeds were used with each treatment having 50 seeds. In order to improve germination, of seeds were nicked/ nipped with mechanical nicking using secateurs, was applied. sown into (describe where and the potting media) and their root collar diameter, height, dry weight and survival assessed. Root collar diameter and height were assessed at x intervals, whereas survival and dry weights were determined at the end of the experiment (Just an example of how you can become more clearer)

Results: There were significantly ($P < 0.001$) differences in seedling growth attributes and survival percentage between seed size categories, with large seeds having the highest seedling growth attributes values and percentage survival percentage while small seeds had the lowest values. This was attributed to differences in the food reserves. The differences on growth attributes between mean values of large and small seeds; large and medium; large and control, ranged from 99% to 197%; 34% to 95%; and 41% to 107% respectively. This Results suggests the confirms merits of grading seeds and selecting large seeds to enhance transplant quality. There were no significant ($P > 0.05$) differences between small seeds and control on dry weight and survival percentage. Even though control had slightly higher dry weight (5.9g) and survival percentage (53%) compared to 5.1g (dry weight) and 46% (survival percentage) of small seeds. There was a strong positive significant ($P = 0.05$) relationship ($r = 0.80$) between seed size and

Comment [N1]: Include Authority for the species. The title can be sharpened eg. Effects of seed size on seedling survival and biomass production as "attributes" is rather vague.

Comment [M2]: State the attributes that were investigated

Comment [M3]: You lose nothing by omitting this noisy control as the three treatments are more defined

Comment [M4]: State all parameters that were significantly impacted by seed weight and end with ($P < 0.05$).

Comment [M5]: Specify the attributes i.e whatever was measured.

Comment [M6]: Did you measure the food reserves? If not this is not your result?

Comment [M7]: Its better to rank and use the absolute values e.g The ranking of seedling height was Large seedlings (x cm) > medium seed seedlings (y cm) > small seed seedlings (z cm) a trend that was also found in seedling root collar diameter dryweights and survival.

Comment [M8]: I suspected so because of the noise inherent in the control. Consider discarding that control as its not effective and has little practical value..

30 seedling growth attributes. This indicate that seed size could be used as parameter for predicting seedling
31 growth rates in the nursery.

32 **Conclusion:** It is recommended to use large sized seeds for production of high quality transplants of
33 *Albizialebbeck* in the nursery.

34 **Key words:** shoot length; root collar diameter; rootlength; leaf area; number of leaves; dry weight; survival.

35

36 1. INTRODUCTION

37 Seed plays an important role in transfer of genetic characters and improvement of qualitative and
38 quantitative traits of production. One of the most important aspects in maximizing crop yield is planting
39 high quality seed [1]. Seed size is an important parameter of seed quality that influences the germination,
40 growth and biomass of the nursery seedlings and that trend leads to the future crop [2].

41

42 The influence of seed size on seedling emergence and growth in various tree species have been studied
43 by many researchers [1, 2, 3 – 9] but there is no consistent trend between seed size and seedling growth
44 and survival??. For example, However, these results varied widely between species. Most researchers
45 have reported that large seeds of larger size produced more vigorous vigour seedlings when compared to
46 that of than small seeds in *Mammea suriga* and in *Azaliaquanzensis*. [2, 9], because of higher food
47 reserves stored in large seed [1]. On the contrary, er ones. Higher seedling growth attributes with
48 increased seed size were observed in *Mammea suriga*[2] and in *Azalia quanzensis*[9]. These results
49 showed that the higher seedling growth attributes that occurred in larger seeds is due to the larger food
50 reserves in these seeds [1]. In contrast, there were no significant differences in seedling growth of *Bauhinia*
51 *thonningii* due to seed size [8].

52

53 *Albizialebbeck* L. Benth. is an important multipurpose tree species belonging to the family of
54 *Leguminosae*[10]. The tree species is well distributed in all regions of Malawi and it has been prioritized as
55 one of the species for conservation in Malawi to enhance its contribution to livelihood of communities. The
56 morphological description, its uses and application in Malawi has been well explained by [11]. Most of the
57 studies on *Albizialebbeck* and other species in Malawi have concentrated on germination [8, 11]. In both
58 studies, it was reported that seed size had no influence on seed germination of *Albizialebbeck*

Comment [M9]: Conclusion should not be specific to *A. lebbeck* but rather an aspect of how seed science relates to seedlings quality.

Comment [M10]: From my ealier comment, your keywords were the study subject and should have appeared earlier in the abstract.

Comment [M11]: I assume you mean correlates

Comment [M12]: Between compares 2 variables

Comment [M13]: Yous gap is already clear from these studies. Was *A. lebbeck* a subject in both studies and was seed size effect on germination also investigated??

59 and *Bauhinia thonningii*. There is no information on effect of seed size on seedling growth attributes of
60 *Albizia lebeck*. *Albizia lebeck* seeds collected from natural population are of different sizes, ranging from
61 0.3cm to 1.1cm long and 0.2cm to 0.9cm broad and the weight ranges from 0.06g to 0.2g [12]. To fill the
62 knowledge gap, this study was conducted to investigate the influence of seed size variation on seedling
63 growth attributes of *Albizia lebeck* in the nursery.

Comment [M14]: The best justification for your study should be either seed sizes of *A. lebeck* vary widely or seedling quality vary widely. The two can also apply and should be tied up and if possible references should be used

Comment [M15]: A method statement

65 2. MATERIAL AND METHODS

66 2.1 Seed Acquisition and Study site

67 *Albizia lebeck* seed from Salima (13°53'S, 34°26'E, and about 540m above sea level), was supplied by
68 Forestry Research Institution or Institute? of Malawi (FRIM) in March 2014. This study was conducted at
69 Malawi College of Forestry and Wildlife (MCFW) nursery (14°19'S, 34°17'E, and 1591 m above sea level)
70 which is located in Malawi near the tropical savannah region in Southern Africa. MCFW receives 1200 mm
71 to 1800 mm rainfall per annum, with annual temperature ranging from 7 °C to 25 °C. It is situated about 85
72 km south east of Lilongwe ~~the capital~~.

74 2.2 Experimental design and treatments

75 Seeds were categorised into four treatments; large, medium and, small ~~and control (mixture of large,~~
76 ~~medium and small) in terms of according to both seed~~ length and weight (Table 1). The treatments were
77 completely randomized in four replicates. Each treatment had 50 seeds making a total of 800 seeds that
78 were used for the whole experiment. ~~In order to~~ To improve germination, All experimental seeds were of
79 seed, mechanically nicking nicked of seeds using with secateurs was applied to improve germination as the
80 technique has been effective in other studies. This technique was applied because in most studies in
81 Malawi it has proved to be effective [8, 11, 13].

Comment [M16]: Scientifically, there is no need to emphasise that a method has worked in Malawi or any other place as Malawi or other places were not the subject of study, instead it was seed pre-treatment for the case at hand.

83 Seed were sown on 31st March 2014 in 10 cm black polythene tubes filled with soil collected from a natural
84 woodland (Brachystegia woodland stand) to as which contain adequate nutrients for seedlings growth
85 (ref) ensure availability of nutrients for growth. One seed was sown per tube at a depth of 2cm, as
86 previously recommended by [14]. All pots were watered Regular regular watering was done as per
87 requirement to maintain adequate moisture necessary for germination and seedling growth.

Comment [M17]: If watering frequencies are known, specify them

88 | In data collection, you have two experiments but they are not adequately described in this section.

89 | Consider elaborating the two experiments as follows:

90 | 1. Germination experiment: what was it about and what was assessed see below comment on a
91 | suitable statement under this section.

92 | 2. Growth, biomass production and survival experiment

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93 |
94 | **Table 1** Seed size categories in terms of length and weight
95 |

Seed size category	Range of length (cm)	Average length (cm)	Range of seed weight (g)	Average weight (g) of 100 seeds
Small	≤ 0.50	0.32	≤ 0.10	0.09
Medium	0.51 – 0.79	0.64	0.11 – 0.19	0.13
Large	≥ 0.80	0.98	≥ 0.20	0.22
Control	0.20 – 1.10	0.67	0.06 – 0.25	0.17

Comment [M18]: Instead of having seed dimensions as a range, it's probably better to have them as $\pm$, to demonstrate that experimental seed dimensions were indeed significantly different. I hope that data at hand can show this

96 |
97 |

98 | 2.3 Data collection and analysis

99 | Seed Germination germination was recorded daily for 30 days until germinated seeds occurred no
100 | more and the experiment concluded after 30 days when no more germination occurred. The A seed was
101 | considered germinated by a visible protrusion of split seed coat with the cotyledons, hypocotyls, and
102 | epicotyl on the soil surface of the soil. Daily germination percentages were summed up to obtain cumulative
103 | germination for each treatment. After the completion of seed germination experiment, seedling growth
104 | attributes were monitored for 150 days to assess the seed size variation effect on growth. All the seedlings
105 | were measured for total shoot height and root collar diameter once for every 30 days. Survival of the
106 | seedlings was also assessed at the same interval. Total shoot height was measured by using a 30 cm
107 | ruler and collar diameter by using a microcalliper to the nearest 0.01 mm. The measurements were taken
108 | just below the cotyledons. The number of seedlings that survived was also counted in each and every
109 | treatment unit. At the termination of the experiment root length, number of leaves, leaf area and dry weight
110 | of the seedlings were determined.

Comment [M19]: The statement is better under experimental design and treatments

Comment [M20]: See earlier comments and review throughout the manuscript

Comment [M21]: Too abrupt and not described under experiments. Should be a new paragraph

Comment [M22]: Specify after how long was this part of the experiment concluded

111 |

112 | Germination percentage was calculated by dividing the total number of seeds that germinated in each
113 | treatment by the number of seeds sown (excluding dead seeds) and multiplied by 100. Survival
114 | percentage of seedlings that survived was also calculated based on the seed germinated. Data on

Comment [M23]: How were dead seeds determined. Look at your very good example of germination and have a statement to describe dead seeds.

germination percentage, seedling height, root collar diameter, survival percentage, root length, number of
 leaflets, leaf area and dry weight. ~~data normality was tested with~~ ~~were subjected to~~ Kolmogorov-Smirnov D and
 normal probability plot tests using Statistical Analysis of Systems software version 9.1.3[15]. ~~This was~~
~~done in order to check the normality of the data.~~ After that the data was subjected to analysis of variance
 (ANOVA) using the same Statistical Analysis of Systems software ~~version 9.1.3 and means.~~ ~~Differences~~
~~between treatment means were~~ separated using Fischer's least significant difference (LSD) at the 0.05
 level. Pearson's correlation ~~coefficients (r) were~~ ~~was used to~~ ~~calculated to~~ determine the relationship ~~that~~
~~existed~~ between seed size and the parameters studied.

3. RESULTS AND DISCUSSION

3.1 Germination

~~The results for cumulative germination percentage are given in Table 2. The results shows that there~~
~~There were~~ ~~was~~ no significant ($P > 0.05$) ~~differences in~~ ~~differences in~~ cumulative germination percentage
~~between among the three seed sizes (Table 2).~~ the seed size variation, although large seeds had a slightly
 higher (3.7% – 4.9%) cumulative germination percentage than the other seed size variation. ~~The present~~
~~results are consistent with the previous study [8, 911].~~ This could mean that all the ~~four~~ seed size
 categories can germinate provided that high physiological quality seeds are used [9]. According to [16]
 some of the desirable seed physiological qualities include plumpness, high purity, freedom from pests and
 diseases, and being dried to a right moisture content. ~~The present results are consistent with the previous~~
~~study [11] and also in agreement with those reported by [8, 9].~~

3.2 Seedling growth attributes

There were significant ($P < 0.001$) differences ~~in seedling height Among the three~~ between the seed
 sizes ~~variation in all the seedling growth attributes~~ studied (Tables 2 and 3). ~~The results indicate that large~~
~~Large~~ seeds produced ~~seedlings with~~ higher ~~height, RCD than~~ values while small seeds had lowest
 values, ~~while medium seeds produced seedlings with medium height RCD (Table..).~~ The findings of
 the present study are in agreement with ~~those in literature~~ ~~previous studies~~ [1, 2, 17, 18]. ~~Production of~~
~~superior seedlings by Large-large seed~~ ~~produced robust seedlings due to greater amount of~~ ~~has been~~
~~attributed to growth enhancement by higher~~ carbohydrates ~~reserves~~ in their endosperm ~~when compared to~~

Comment [M24]: Three if you opt to do away with the control – see my earlier comments.

or cotyledons than medium and; smaller and mixture seeds, ensuring early development to produce faster growing plants [2, 19]. On the other hand, Mwase and Mvula [8], Agboola [20], Malavasi and Malavasi [21] reported no significant influence of seed size on seedling growth in a few tropical tree species, while [2] reported that medium sized seeds produced better seedling growth than those of smaller and larger ones. These differences may be due to the fact that seedling growth is highly controlled by many intrinsic and extrinsic factors and is species specific [22]. Although seedling growth is not always correlated to seed size (all relevant refs here), Therefore the present study has shown that for large seeds should be used for production of better superior seedling in terms of seedling vigour, large seeds of in *Albizia lebbbeck* should be encouraged in the nursery.

Comment [M25]: Throughout you have avoided this style, why have it now??. You should stick to ne that is recommended to authors by the journal.

Comment [M26]: You do not have to spell out the species after the first instance except in tables??.

Comment [M27]: Recall earlier comment where this attribute should be statistically demonstrated? Without such data, its erroneous to state this as fact both in text and tables.

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Table 2 Effect of seed size variation on germination percentage, root length, shoot length, seedling length and shoot root ratio with standard errors in parenthesis of *Albizia lebbbeck* at nursery

Seed size	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Shoot root ratio
Large	100.0(0.0) ^a	29.7(1.5) ^a	58.4(1.4) ^a	88.1(1.5) ^a	0.51(0.01) ^a
Medium	96.3(1.5) ^a	20.2(1.3) ^b	43.7(2.7) ^b	63.8(1.9) ^b	0.46(0.03) ^b
Small	95.1(1.6) ^a	12.6(1.3) ^c	29.3(1.3) ^c	41.9(1.3) ^c	0.43(0.03) ^c
Control	96.2(1.3) ^a	18.8(1.2) ^b	40.5(1.5) ^b	59.3(1.3) ^b	0.46(0.03) ^b
CV%	5.2	4.6	7.3	8.9	6.3

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Note: ^{a,b,c} means with different subscript within a column significantly differ ($P < .001$)

Table 3 Effect of seed size variation on root collar diameter, number of leafs, leaf area, dry weight and survival percentage with standard errors in parenthesis of *Albizia lebbbeck* at nursery

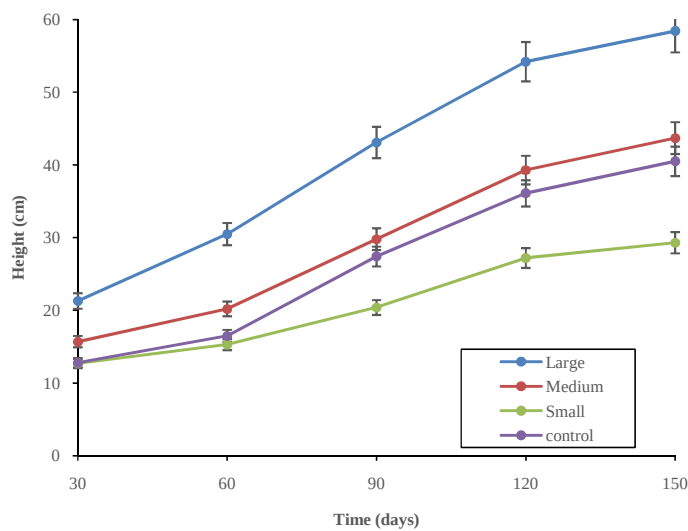
Seed size	Root collar diameter (mm)	Number of leafs	Leaf area (cm ² plant)	Dry weight (g plant)	Survival (%)
Large	5.97(0.01) ^a	8.21(0.04) ^a	405.0(2.4) ^a	11.3(0.6) ^a	89.6(2.5) ^a
Medium	4.03(0.02) ^b	5.93(0.03) ^b	207.3(3.2) ^b	7.8(0.3) ^b	65.2(2.3) ^b
Small	2.01(0.02) ^c	3.66(0.03) ^c	136.8(2.2) ^d	5.1(0.3) ^c	46.1(2.4) ^c
Control	3.97(0.02) ^b	5.82(0.02) ^b	195.5(3.2) ^c	5.9(1.1) ^c	52.9(2.4) ^c
CV%	5.3	4.6	6.8	8.9	7.2

Note: ^{a,b,c} means with different subscript within a column significantly differ ($P < .001$)

Shoot length and root collar growth trend of *Albizia lebbbeck* for the period of 150 days are presented in Figures 1 and 2 respectively. The shoot length and root collar growth of *Albizia lebbbeck* was significantly ($P < .001$) higher in large seeds than in other seed size variations. Large seeds gave the highest seedling height growth which increased rapidly between 30 and 120 days, then increased at the decreasing rate between 120 and 150 days. A similar trend was also observed for the other seed size variations and for the root collar diameter growth (Figure 2). According to [6], the higher growth rate of large seeds could be

Comment [M28]: Seen this for the first time. Should appear in methods – see earlier comment.

166 due to the reason that large-seeded species are predicted to be better adapted to the catastrophic events
167 encountered by seedlings because they can compensate for damage using seed reserves. The growth
168 increased at an increasing rate between 60 and 120 days because the seedlings were using the
169 photosynthesized food for growth, as the food reserves were being invested in the tap root of the plants.
170 This was indicated by the plants changing of cotyledons colour from brown yellowish to right green in the
171 nursery.
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173 **Figure 1** seedling height growth of *Albizia lebbek* from different seed size categories
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Comment [M29]: See how bad your control has interfered with your good results in this and other graphs

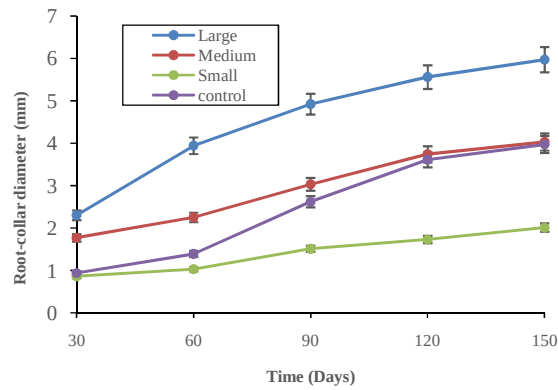


Figure 2 Seedling root collar growth of *Albizia lebbbeck* from different seed size categories

3.3 Seedling survival

A log survival percentage against time was constructed (Figure 3). Survivorship curves of seedlings from all the four seed size variation were close to Deevy Type II curve with more or less constant mortality rate. However, survivorship curves for medium, small and mixture seeds were steeper than that for large seeds. There were no significant ($P > .05$) differences between curves of small seeds and mixture seeds. The results are in conformity with other researchers [23, 24]. Survival of seedlings is dependent on the amount of the reserves at the time of re-growth, such that those with greater amount of food reserves are able to regenerate new shoots [24].

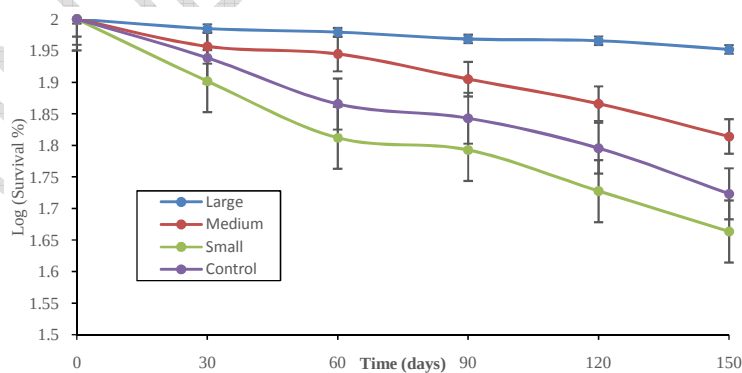


Figure 3 Survival trend of *Albizia lebbbeck* seedlings from different seed size categories

3.4 Correlation of seed size with seedling growth attributes

Correlation and regressions equations results between seed size and seedling growth attributes are presented in Table 4. The results shows that there was a strong positive significant ($P=.05$) relationship ($r>.80$) between seed size and seedling growth attributes. This imply that seed size could be used as parameter for predicting seedling growth rates in the nursery. The present results are in agreement with the results of [9] who reported a strong relationship between seed size and seedling growth attributes (height and root collar diameter growth) of *Azelaquanzensis*.

Comment [M30]: Specify as commented eralier

Table 4 Regression equations between seed size and seedling growth attributes of *Albizia lebbeck*

Comment [M31]: You can also attempt multiple regression and see which variables are influenced more as some growth attributes also correlate e.g. height and diameter

Shoot length (cm)	Root length (cm)	Root collar diameter (mm)	Number of leafs	Leaf area (cm ² plant)	Dry weight (g plant)	Survival (%)
$Y=197.98X+12.78$	$Y=117.71X+2.37$	$Y=27.62X-0.22$	$Y=31.68X+1.07$	$Y=1891.6X-52.32$	$Y=40.08X+1.41$	$Y=283.07X+20.28$
$r=.918$	$r=.926$	$r=.950$	$r=.948$	$r=.901$	$r=.808$	$r=.822$
$P=.022$	$P=.021$	$P=.013$	$P=.016$	$P=.031$	$P=.050$	$P=.046$

4. CONCLUSION

The current study has revealed that seed size has a clear effect on seedling growth attributes and survival of *Albizia-A. lebbeck* in the nursery. Larger seeds produced high quality seedlings and high survival rate. This confirms merits of grading seed to enhance transplant quality. There was a strong positive significant relationship between seed size and seedling growth attributes. This indicate This indicates that seed size could be used as parameter for predicting seedling growth rates in the nursery. Therefore, it is recommended to use large sized seeds for production of high quality transplants of *Albizia-A. lebbeck* in the nursery.

Comment [M32]: Which ones

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