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Determination of Chlorophyll Content of Six Provenances of *Faidherbia albida (Delile) A. Chev* Using SPAD-502 Chlorophyll Meter and Spectrophotometer

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Abstract: The objective of this study was to assess the chlorophyll content of the seedlings of six provenances (Awassa, Chinzombo, Lake Koka, Maseno, Taveta, and Wagingombe) of *F. albida* using the soil plant analysis development (SPAD) meter and Spectrophotometer and to determine the relationship between the chlorophyll contents of the six provenances as determined by the two instruments. SPAD measurements were made on the leaves of nine (9) randomly selected seedlings per provenance and averaged. Readings were made at three locations on the leaves ranging from the youngest to senescent (when available). SPAD readings were taken from the second to fifth month after transplanting. Chlorophyll concentration was determined by subjecting the leaves to a spectrophotometric analysis. There was no significant difference among provenances in SPAD readings at months 2 and 3, but significant differences were observed at months 4 and 5. Highest SPAD readings were found in Chinzombo at month 2 and 3 and in Wagingombe at month 4 and 5 while lowest SPAD reading were found in Lake Koka at months 2, 4 and 5 and in Awassa at month 3. There was significant variation among provenances in chlorophyll contents using Spectrophotometer. Total chlorophyll was highest for Awassa and lowest for Wagingombe provenances. There was a nonlinear relationship between the chlorophyll contents of the six provenances as determined by the two instruments.Whereas the Southern African provenances (Chinzombo and Wagingombe) had the highest chlorophyll content using the SPAD meter, an Eastern African provenance (Awassa) had the highest total chlorophyll content followed by Chinzombo when the Spectrophotometer was used. The nonlinear relationship observed between the chlorophyll contents of the provenances as determined by the two instruments (SPAD and Spectrophotometer) could be attributed to provenance effect. Therefore, the SPAD meter cannot be relied upon as an alternative to spectrophotometric analysis for chlorophyll content determination in *F. albida.*

[Charity Fredrick, Catherine Muthuri, Kamau Ngamau, Fergus Sinclair, Uzoma Darlington Chima.**Determination of Chlorophyll Content of Six Provenances of *Faidherbia albida (Delile) A. Chev* Using SPAD-502 Chlorophyll Meter and Spectrophotometer.** Nat Sci 2021; 19(10);39-46].ISSN1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 6.**doi:**10.7537/marsnsj191021.06.

Keywords: *Faidherbia albida,* SPAD meter, Chlorophyll content, spectrophotometric analysis

Introduction

Faidherbia albida (Del.) A. Chev (Syn. *Acacia albida* Del.) commonly called Apple-ring acacia is a multipurpose leguminous tree species belonging to the mimosoideae subfamily (Barnes and Fagg, 2003; Fredrick *et al.,* 2015). It is widespread and mostly found in all tropical Africa from Senegal to Sudan and as far as Natal or Angola (Fredrick et al. 2016). Being so widespread and utilized by man, *F. albida* has a very large number of common names (Barnes and Fagg, 2003). It grows at low to medium altitudes (270 - 2700 m) in areas of low to medium rainfall (250 – 1200 mm/yr) and average temperatures from 18°C to 30°C (Barnes and Fagg, 2003). The leaves remain green during the dry season due to its long tap root (30-35 m) which is able to utilize underground water (Dupuyl and Dreyfus, 1992). The species is also known to have high medicinal value. It provides food, furniture, fuel, and can be used for making fences. It also provides shade and a highly nutritious fodder for livestock (Hadgu *et al.,* 2009). In eastern, southern, and central Africa, the species is often found growing near sources of water, such as along rivers, on the shores of lakes, and in gullies and ravines while in western Africa, it prefers deep, lighter sandy or silt soils, although it can also be found on lateritic soils with a shallow pan (Wood, 1992). Generally, it occurs on Coarse-textured well-drained alluvial soils and tolerates seasonal water logging and salinity but cannot withstand heavy clayey soils (Orwa *et al.,* 2009). The tree has an extensive root system and

can be planted close together in rows in a contour to stabilize eroded soils (Adamu, 2012)

Determination of chlorophyll concentration is one of the common measurements made by plant scientists (Markwell *et al.,* 1995). Ling et al. (2011) noted that leaf chlorophyll concentration is an important parameter which is measured as an indicator of chloroplast development, photosynthetic capacity, leaf nitrogen content, or general plant health. Such measurements in the laboratory have been determined spectrophotometrically following extraction of the pigments using an organic solvent, such as acetone or dimethyl formamide (Ling *et al.,* 2011). The spectrophotometric method is however lengthy and expensive (*Coste et al.,* 2010), time consuming, destructive and involves the use of toxic or inflammable chemicals (Ling *et al.,* 2011). Non-destructive methods have been developed which provides an alternative method for the measurement of relative leaf chlorophyll levels. The SPAD-502 meter (Konica-Minolta, Japan) is a hand-held, self-calibrating, convenient, inexpensive, and non-destructive lightweight device used to calculate the amount of chlorophyll present in plant leaves (Minolta, 1989; Ian and Grady, 2000; Ling *et al.,* 2011). It uses two light-emitting diodes (650 and 940 nm) and a silicon photodiode receptor which measures transmittance of leaf in the red and infrared regions of the electromagnetic spectrum (Markwell *et al.,* 1995; Ling *et al.,* 2011).

Ling et al. (2011) reported that the relationship between SPAD values and chlorophyll concentration has been monitored in different species, and has been found to display considerable interspecific variation and this variation may be due to structural differences between the leaves of different species. Monje and Bugbee, (1992) noted that the relationship between the output of the SPAD-502 meter and leaf chlorophyll concentration is nonlinear. Similar relationships have been obtained with leaves of wheat, rice and soybean (Markwell *et al.,* 1995).

Information on the relationship between SPAD readings and Spectrophotometeric analysis will create an informed decision on the use of the SPAD meter to determine the chlorophyll content of a plant. Therefore the objective of this study was to assess the chlorophyll content of the seedlings of the different provenances of *F. albida* using the SPAD meter and Spectrophotometer and to determine the relationship between them. Knowledge of chlorophyll concentration of a species will provide an accurate estimation of its vitality (Percival *et al.,* 2008).

Materials and Methods

Study sites

This study was carried out at the World Agroforestry Centre in Nairobi, latitude 1°33'S, longitude 37°14'E and altitude of 1580m above sea level. ICRAF is located about 20km north-east of Nairobi, Kenya with a mean annual rainfall of between 500 mm and 1370 mm and mean temperature of 21°C.

Seeds Collection

The seeds of *F. albida* provenances, namely, Awassa (Ethiopia), Chinzombo (Zambia), Lake Koka (Ethiopia), Maseno (Kenya), Taveta (Kenya), and Wagingombe (Tanzania) used in this study were obtained from the ICRAF germplasm laboratory. The samples were representative of the natural distribution range of the species in East and Southern Africa. The six provenances were tested because of the demand of the scaling out provenances from these countries to Rwanda, Burundi and Uganda from an on-going Australian funded tree for food security project operating in Rwanda, Ethiopia, Uganda and Burundi. In this study, the term provenance denoted the original geographic zone from which seeds were collected (Fredrick et al. 2015, 2016).

Experimental Design

This experiment had six (6) treatments consisting of 6 provenances and three (3) replicates, and was laid out in a completely randomized design (CRD). A total of 360 seeds were randomly selected (i.e. 20 seeds x 3 replicates x 6 provenances = 360 experimental units). Seeds were nicked and directly sown in a germination tray measuring 21cm x 15cm x 8cm and filled with sterilized sharp sand. Germination process was monitored every day from the date of sowing for 30 days. Following germination, 180 seedlings were selected (i.e. 10 seeds x 3 replicates x 6 provenances = 180 experimental units). Each excavated seedling was transplanted into a polybag measuring 15.5 cm x4 0cm and filled with forest soil. The experiment was carried out in a greenhouse under a 50% light shade net. No fertilizers or bacterial and/or mycorrhizal inoculation was used. Watering was done daily while weeding was carried out regularly and when required.

Chlorophyll Content Determination.

SPAD measurements were made on the leaves of nine (9) randomly selected seedlings per provenance (i.e. 3 seedlings per replicate) and averaged. Readings were made at three locations on the leaves ranging from the youngest to senescent (if any). Initial SPAD readings were taken one month after transplanting and monthly thereafter on the same leaves for three other months (i.e. month 2 to 5). The leaves were then

collected and sealed in labelled polythene bags, placed in an icebox and stored in the freezer prior to spectrophotometric analysis (Rodriguez and Miller, 2000) to prevent destruction of chlorophyll pigments following exposure to light, heat or oxygen (Lawson *et al.,* 1998).

Chlorophyll determination by spectrophotometric analysis was done following extraction in acetone (Leegood 1993). One gram (1g) fresh weight sub-samples from the sampled leaves were ground with a pestle using a mortar and pure quartz sand and 40 ml of 80 % acetone as the extraction solvent. The resulting suspension was diluted to 100 ml using 80 % acetone and centrifuged at 6000 rpm for three minutes (tubes covered with aluminium foil). Optical density (absorbance) was read and recorded using a spectrophotometer (UVmini-1240, Shimadzu Corporation, Japan) set at 645 and 663 nm. The samples were read against an 80 % acetone blank; three readings were taken for each sample and averaged. Chlorophyll a, b and total chlorophyll concentrations (mg g^{-1} tissue) were determined as follows:

Chlorophyll a = (12.7(D663) - 2.63(D645)) x V/1000xW (Eq. 1)
Chlorophyll b = (22.9(D645) - 4.68(D663)) x V/1000xW (Eq. 2)
Total Chlorophyll = Chlorophyll a + Chlorophyll b (Eq. 3)

Where D represents absorbance, V denotes volume and W is the measured tissue fresh weight.

Data Analysis

The relationship between Chlorophyll concentrations and SPAD measurements was determined by plotting a scatter diagram and fitting a regression line using the Microsoft excel 2007. Pearson's correlations were also performed among SPAD readings at different ages of growth.

Results

Chlorophyll Content Determination using the SPAD Meter (SPAD-502 Readings)

There was no significant difference among provenances in SPAD readings at months 2 and 3, but significant differences were observed at month 4 and 5 (table 1). In the second month, chlorophyll Spad readings ranged from 15.87 in Lake Koka to 21.69 in Chinzombo closely followed by 21.08 in Taveta. In the third month, highest SPAD reading (32.54) was found in Chinzombo while the lowest (22.73) was found in Awassa which was not significantly different from that of Maseno (24.14). Highest SPAD readings in the second and third months were observed in Chinzombo. Highest reading (36.51) was observed in Wagingombe and lowest (27.21) in Lake Koka in the fourth month while in the fifth month, Wagingombe exhibited highest readings (41.03) closely followed by Chinzombo (36.34) while Lake Koka exhibited lowest (28.57). Highest SPAD readings in the fourth and fifth month were observed in Wagingombe (Table 1).

Table 1: Mean chlorophyll content of six provenances of *F. albida* by SPAD meter reading

Provenances	SPAD2	SPAD3	SPAD4	SPAD5
Chinzombo	21.69b	32.54b	32.39ab	36.34ab
Wagingombe	18.94ab	29.61ab	36.51b	41.03b
Taveta	21.08b	26.02ab	29.72a	30.21a
Maseno	16.69ab	24.14a	30.52ab	33.10a
Lake Koka	15.87a	25.24ab	28.11a	28.57a
Awassa	17.71ab	22.73a	27.21a	32.68a
P value	0.086	0.065	0.034	0.019

Means followed by the same letter in a row are not significantly different at $p \leq 0.05$
SPAD 2 - 5 = SPAD meter readings for months 2 to 5

Correlation Analysis for SPAD Readings

There was a positive correlation in SPAD readings among provenances in the second to fifth month. No significant correlation was recorded between SPAD readings at month 2 and other months. SPAD readings at month 3 had a strong correlation with SPAD readings at month 4 while SPAD readings at month 4 were highly correlated with SPAD readings at month 5 (Table 2).

Table 2: Pearson correlation coefficient (r) of SPAD readings of leaves of six *F. albida* provenances.

Parameters	SPAD 2	SPAD 3	SPAD 4	SPAD 5
SPAD 2	1	0.332	0. 255	0.254
SPAD 3		1	0.531*	0.461
SPAD 4			1	0.854**
SPAD 5				1

* = significant and ** = highly significances at the 0.05 and 0.01 levels respectively. Where SPAD 2 to SPAD 5 = SPAD readings at month 2 to month 5.

Spectrophotometeric Analysis

There was significant variation among provenances in chlorophyll determination using Spectrophotometer. The mean total chlorophyll varied from 2.55 to 2.87 (figure 1). Total chlorophyll was highest for Awassa (2.87) and lowest for Wagingombe (2.55) provenances.

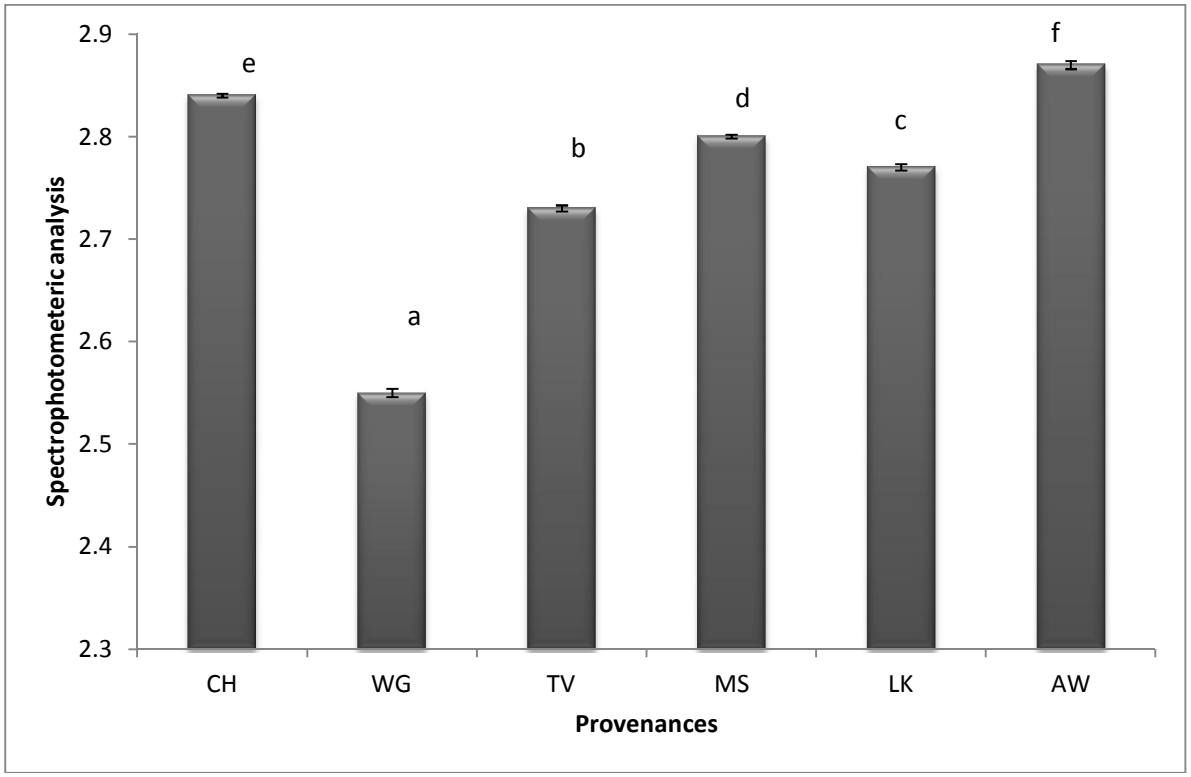

Figure 1: Total chlorophyll content of six provenances of *F. albida*.
CH – Chinzombo; WG = Wagingombe; TV - Taveta; MS – Maseno; LK - Lake Koka; AW - Awassa.

Relationship between Total Chlorophyll Content and SPAD Readings

There was a nonlinear relationship between total chlorophyll content and SPAD readings at month 5 for the six provenances using the linear, polynomial and exponential functions. Summary of this result is presented in figures 2A, B and C.

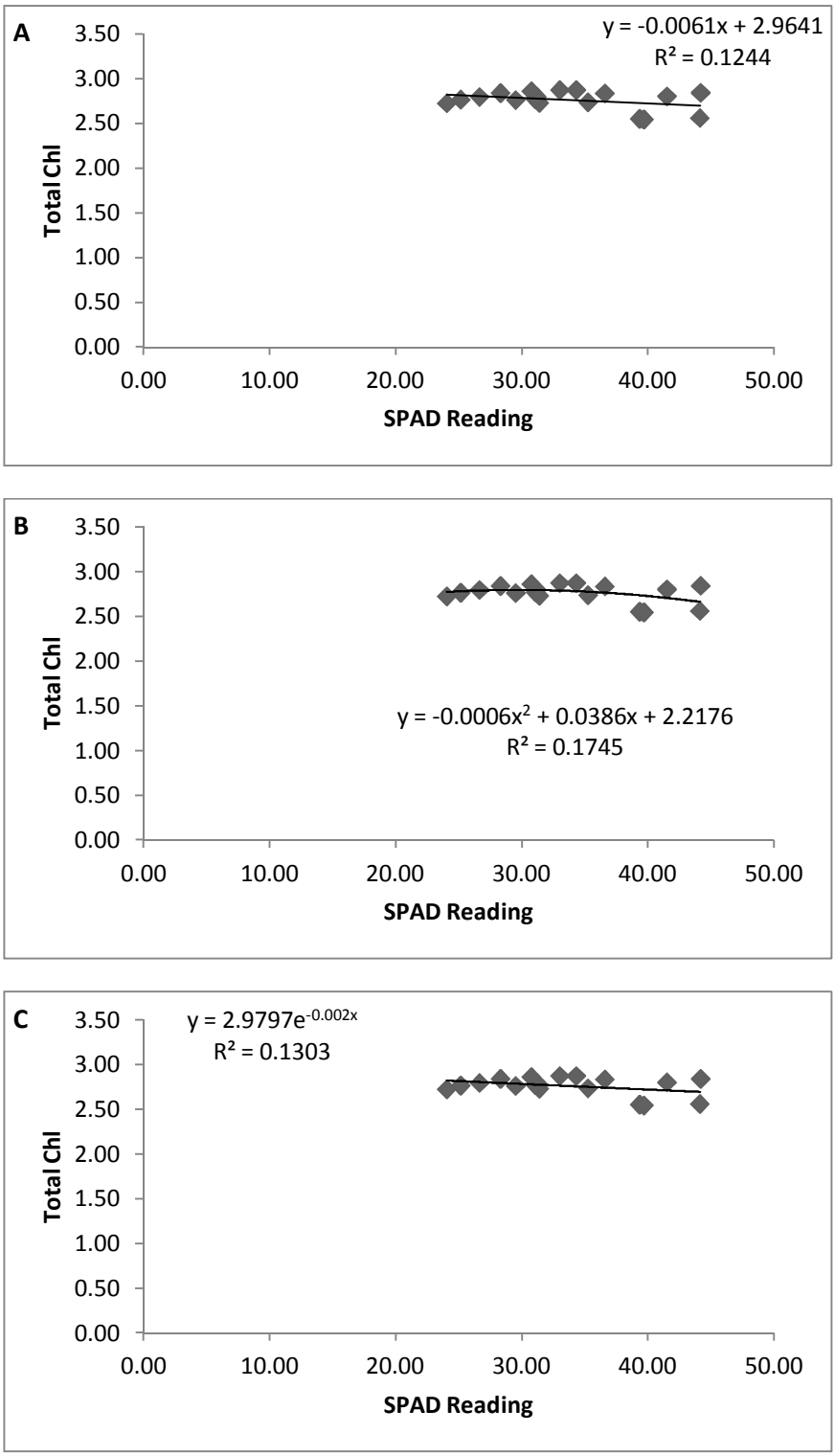

Figure 2: Relationship between total chlorophyll and SPAD readings of leaves of *F. albida* provenances at month 5. The data are shown with lines representing fits provided by linear (A), polynomial (B) and exponential (C) functions.

Discussion

Provenances displayed non-significant differences ($p \leq 0.05$) in SPAD readings at month 2 and 3and a significant differences at month 4 and 5. Naus et al. (2010) noted that SPAD meter readings can be influenced by changing growth conditions that may lead to a redistribution of chloroplasts within mesophyll cells. Provenances also displayed significant differences ($p \leq 0.05$) in total chlorophyll content at month 5. The differences observed among provenances may also be related to differences in the distribution of chlorophyll in leaves as a result of the structural organization of chlorophyll molecules in chloroplasts, chloroplasts in cells, and cells in leaves (Coste *et al.,* 2010). Differences observed could also be due to veins and veinules network in leaves (McClendon and Fukshansky, 1990). SPAD readings did not appear to follow regional differences in the second month but appeared to do so in the third to fifth months with the southern Africa provenances (Chinzombo and Wagingombe) exhibiting higher SPAD readings than the Eastern Africa provenances (Taveta, Maseno, Lake Koka and Awassa). A non-significant correlation between SPAD readings at Month 2 and other months could be due to establishment of the young seedling and changing growth conditions (Ling *et al.,* 2011).

There was a negative nonlinear relationship between SPAD readings and leaf chlorophyll concentration. Uddling *et al.,* (2007) noted that with increasing heterogeneity of chlorophyll distribution inside a leaf, the absorption of an amount of chlorophyll decreases. This tends to affect the relationship between SPAD values and Chlorophyll content (Coste *et al.,* 2010).The observed nonlinear relationship between SPAD-502 meter and leaf Chlorophyll concentration could also be attributed to provenance effect. On the contrary, Rodriguez and Miller, (2000) reported positive correlations between SPAD readings and chlorophyll concentrations for all three cultivars of St. Augustinegrass used in their study but concluded that the usefulness of SPAD readings for chlorophyll management of St. Augustinegrass seems limited.

According to Coste et al. (2010), 11 out of 13 tropical trees studied had a pronounced non-linear SPAD-chlorophyll relationship. Monje and Bugbee, (1992) reported a nonlinear relationship between the output of the SPAD-502 meter and leaf Chlorophyll concentration in three species (*Oryza sativa, Glycine max* and *Tritium aestivum*) studied but were able to fit the curve using a second-order polynomial functions. Similar relationships have also been obtained with leaves of maize and soybean but where fitted using both polynomial and exponential equations (Markwell *et al.,* 1995). Ling *et al.,* (2011) observed a much stronger fit using second-order polynomial functions. The use of an exponential function has been reported to provide more accurate relationship between the SPAD values and chlorophyll concentration (Markwell *et al.,* 1995; Ling *et al.,* 2011). Hawkins et al. (2009) also reported that polynomial functions are known to best describe the relationship between SPAD values and chlorophyll concentration. Although the polynomial functions best described the relationship between SPAD values and chlorophyll concentration when compared to the exponential and linear functions. The relationship for the three functions was nonlinear.

The relationship between SPAD values and chlorophyll concentration has been monitored in different species, and has been found to display considerable interspecific variation (Uddling *et al.,* 2007) and this variation may be due to structural differences between the leaves of these species (Ling *et al.,* 2011). According to Uddling et al. (2007), the effect of non-uniformly distributed chlorophyll is likely to be more important in explaining the non-linearity in the relationships between the two instruments.

Conclusions

Whereas the southern African provenances (Chinzombo and Wagingombe) exhibited highest chlorophyll content determined using the SPAD meter, an Eastern African provenance (Awassa) exhibited highest total chlorophyll content followed by the southern African provenance (Chinzombo) via spectrophotometric analysis. The nonlinear relationship observed between them could be attributed to provenance effect. Although the SPAD-502 meter provides a simple, non-destructive method for estimating foliar chlorophyll that quickly reports a large number of readings, it cannot be relied upon as an alternative to spectrophotometric analysis for the determination of chlorophyll content of *F. albida*.

Acknowledgments

Authors are thankful to the World Agroforestry Centre (ICRAF, Nairobi) for providing the necessary materials for this work. This work was carried out under the 'trees for food security' project led by ICRAF and funded by the Australian Centre for International Agriculture Research (ACIAR). The project is operating in Ethiopia, Rwanda, Uganda and Burundi and *F. albida* is one of the important tree species being studied under the project. We also want to thank Lucy Mwaura of the ICRAF seed laboratory for availing the seeds and Julius Njoroge, Agnes Gachuiri, Julius Kimani and Valentine Gitonga for their support in setting up the experiment.

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8/25/2021