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EFFECT OF SEED WEIGHT AND LIGHT ON *In vitro* GERMINATION OF *Afzelia africana* SM. EX PERS

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ABSTRACT

The micropropagation potential of *Afzelia africana*; a threatened multipurpose tree plant was investigated in this study with the aim to determine the effect of seed weight on its *in vitro* germination as well as the impact of light and dark photoperiod on the seed germination. Murashige and Skoog (MS) 1962 media was used for this study. Five categories of seed weight were used 3.3 – 3.7 g (Wt1), 2.8 – 3.2 g (Wt2), 2.3 – 2.7 g (Wt3), 1.8 – 2.2g (Wt4) and 1.3 – 1.7g (Wt5). The seeds were cultured on MS media after disinfection with H_2SO_4 for 30 minutes followed by soaking in distilled water for 120 minutes. Cultured seeds were then kept in the incubator at a temperature of 25 ± 2 ^{0}C and 16/8-hour photoperiod and under complete dark condition. Observable physiological responses of seed coat break (SB), root emergence (RE), cotyledon opening (Cop), shoot emergence (SE), and plant height (PH) of cultured seedlings were recorded after 4 weeks. Morphological data including the ratio of epicotyl to hypocotyl was also documented. Using one-way analysis of variance (ANOVA), seed weight had no significant effect on SB, RE and SE, however, it had significant effect on CoP as seeds with the highest weight Wt1 had their cotyledons opening earlier (14.50 ± 0.50^a) compared to seeds with the lowest weight Wt5 (16.50 ± 0.50^b). Also, LN of Wt1 was significantly higher than LN in other seed weights, while with PH, Wt1 (14.86 ± 0.24^a) was significantly higher than Wt4 (12.86 ± 0.43^b) and Wt5 (10.16 ± 0.19^c) at $P<0.05$. The seed weight of *Afzelia africana* has effect on its *in vitro* germination parameters, thus to produce vigorous seedlings of significant leaf numbers and plant height, seeds with weight range of 3.3 – 3.7 g should be cultured and to produce higher height seedlings, the seeds should be grown first under total darkness and transferred to light photoperiod of 16/8 hours.

Keywords: *Afzelia africana*, Seed Weight, *In vitro*, Germination.

INTRODUCTION

Afzelia africana referred to as African mahogany is one of the unconventional vegetables gotten from the wild and it augments the conventional ones obtained from farms and orchards (Lillian *et al*., 2014). *Afzelia africana* seeds contain 18–37% extractable oil which needs little purification and has a long shelf-life making it suitable for the formulation of alkyd resin and shoe polish (Gérard and Louppe, 2011). *Afzelia africana* is a hard wood tree that has provided several ecosystem services to man and the environment (Bamigboye *et al*., 2024). It is a medicinal plant because the powdery substance originating from the wood vessels consist of Kaempferol and its glycosides have antibacterial, antifungal and anti-inflammatory activities (Mohammed, 2023). Also, other important anti-nutrients and phytochemicals such as oxalate, phytic acids, micro and macro minerals have been reported to be present in it by several studies to show its anti-plasmodial, antioxidant and anti-microbial activities (Vigbedor *et al*., 2022[a]). In addition to its use in construction and furniture, the decoction from the root, stem back, leaf or seeds are used traditionally to cure several diseases including both infectious and non-infectious ones (Vigbedor *et al*., 2022[b]). Its leaves are used as forage and because its seeds contain 27% protein, 33% carbohydrate and 32% lipid, it is consumed in several dishes as soup condiment and used as a thickener, (Gérard and Louppe, 2011).

Afzelia africana is predominantly found in the rain forest and savannah in West Africa especially in the forest-savanna borders or semi-deciduous forest (Mensah *et al.,* 2020). It has been found also in North India but no information is available on its status either as exotic, domesticated or indigenous. *Afzelia africana* is a drought stress sensitive species (Atanasso *et al*., 2021) and reports showed that young individuals in open fields are more exposed to drought stresses than those in closed canopy (Biaou *et al*., 2011). *A. africana* propagates by seed but the rate of seed germination is low in the wild. Birds and rodents prey on its seeds and this has contributed to its poor regeneration in the wild (Evrard *et al*., 2019).

The rate of seed germination in the wild is low and its seedlings rarely develop into saplings hence the stock of *Afzelia africana* species has been badly depleted (Ogbimi and Sakpere, 2021). *Afzelia africana* has been heavily exploited to meet several human needs which include medicinal uses, timber production, and as food for man and animals (Bamigboye *et al*., 2022). The seeds are dormant and they become recalcitrant on storage from the physical dormancy which has been overcome by mechanical or chemical scarification (Iralu *et al.,* 2019; Ogbimi *et al*., 2020; Atanasso *et al*., 2021; Sobola, 2023). *In vitro* propagation is a widely used technique to produce large numbers of difficult-to-propagate plant tissue species (Chawla *et al.,* 2020). The technique has revolutionized various aspects of plant science, including plant conservation, commercial plant production, and genetic modification studies (Sharma *et al.,* 2021).

The role of seed size or weight in tree seedling performance has received considerable attention in recent studies. Seed size or weight has been established to have effect on germination in the field or semi-controlled field experiments (Aderounmu *et al.,* 2019; Taylor, 2020; Almuktar *et al*., 2021). The effect of different locations under different climatic conditions of humidity, light intensity and period have been investigated on the germination of *Canarium schweinfurthii*, *Xanthium strumarium* L. and many other species (Anozie and Oboho, 2019, Saeed *et al*., 2020, Yan and Chen, 2020). In the absence of light, angiosperm seedlings undergo etiolation that involves morphological, physiological, and biochemical processes that are evolutionary adaptations for efficient resource management and for increasing the chances of survival (Armarego-Marriott *et al*., 2020). This has initiated the study on the effect of photoperiod on *in vitro* germination of *A. africana* seeds for mass propagation. Moreover, the continuous increase in global warming resulting in the modification of available tree seeds in the forest is a threat to biodiversity (Iralu *et al.,* 2019; Kijowska *et al.,* 2020; Muluneh, 2021) and *Afzelia africana* is already enlisted as endangered (IUCN, 2020). Hence this study investigated the effect of different seed weight and light on *in vitro* germination of *Afzelia africana* with the aim of providing information on its *in vitro* propagation using seeds as starting material for mass propagation.

MATERIALS AND METHOD

Seed collection and Preparation

Viable, freshly harvested seeds of *Afzelia africana* were collected from Obafemi Awolowo University Campus, Ile-Ife, Osun State, Nigeria (Lat. 7^0 $31^{'}$ 8.4", Long. 4^0 $31^{'}$ 15.96"). They were authenticated at the IFE-herbarium, Department of Botany, Obafemi Awolowo University, Nigeria. A total of 100 viable seeds were selected for the study, the seed weight considered were 3.3 – 3.7 g (Wt1), 2.8 – 3.2 g (Wt2), 2.3 – 2.7 g (Wt3), 1.8 – 2.2 g (Wt4) and 1.3 – 1.7 g (Wt5). 10 seeds each were weighed for each category and kept separately in labelled containers for culturing after disinfection.

Disinfection of Viable Seeds

The cap on the seeds of *Afzelia africana* were removed mechanically with the crucible tong after which seeds were subjected to chemical treatment by soaking in concentrated H_2SO_4 for 30 minutes followed by soaking in distilled water for 120 minutes(reference?). Thereafter, the distilled water was decanted and then seeds were washed again with three rinses of distilled water. Seeds were then cultured on MS media. All manipulations were carried out under the laminar flow hood.

Media Preparation

Murashige and Skoog (MS) 1962 was prepared using the standard methods and the prepared media was dispensed into sterile bottles, covered and preserved in the incubator for 48 hours before use.

Assessment of the Effect of Seed Weight on *In vitro* Germination of *Afzelia africana* Seeds

All seed weights considered were subjected to the same growth conditions in the incubator at a temperature of 25 ± 2 ^{0}C and 16/8-hour photoperiod. Disinfected seeds were cultured on the prepared MS media. Ten (10) seeds each per weight (Wt1, Wt2, Wt3, Wt4 and Wt5) were cultured and the experiment was repeated. The cultures were maintained in an incubator at a temperature of 25 ± 2 ^{0}C and 16/8-hour photoperiod, and the date of commencement of culture was recorded.

Assessment of the Effect of Light on *in vitro* Germination of *Afzelia africana* Seeds

Another set of seed with weights; (Wt1- Wt5) were disinfected, cultured on the prepared MS media, incubated under the same temperature of 25 ± 2 ^{0}C but were subjected to total darkness by wrapping growth bottles with foil paper and placing them in a wrack covered with black cloth throughout the period of germination. Ten (10) seeds each per weight (Wt1, Wt2, Wt3, Wt4 and Wt5) were also cultured and the experiment was repeated.

Data Analysis

Physiological data of *in vitro* grown seedlings under light including seed coat break (SB), root emergence (RE), cotyledon opening (CoP), shoot emergence (SE), and leaf number (LN) were collected after four (4) weeks and subjected to one-way ANOVA. Except for LN that was counted, SB, RE, CoP and SE were recorded in number of days to their response (i.e. number of days from the first day of seed culture), the best response being the one observed within the shortest period of days.

Other parameters under both light and dark photoperiod including plant height (PH), hypocotyl length (Hypo), epicotyl length (Epi) were measured in centimeters and presented in ratio while germination period (GP) was also measured in number of days. Leaf sprout (LS) was observed and descriptively analyzed. All the quantitative data collected were subjected to one-way ANOVA using Statistical Analysis Software (SAS) version 9.2 and the means were separated using Duncan multiple range test at 0.05 confidence limit (alpha level).

RESULTS

Effect of Seed Weight on *in vitro* Germination of *Afzelia africana* Seeds Under Light Photoperiod

The weight of the seeds cultured *in vitro* had significant effect on the cotyledon opening, leaf number and plant height of the germinated seedlings as shown in Table 1. Early seed coat break recorded in Wt1 was accompanied by early root emergence. In seed Wt1, cotyledon opening was significantly faster (14.50 ± 0.50^a) compared with seed Wt5 (16.50±0.50^b). Seeds of Wt1 responded with significantly highest leaf number (7.75 ± 0.25^a) compared to other seed sizes (Wt2; 6.25 ± 0.25, Wt3; 6.25 ± 0.25, Wt4; 5.50 ± 0.29 and Wt5; 5.50 ± 0.29). Height of seedling of Wt1 (14.86 ± 0.24cm), Wt2 (14.62 ± 0.48 cm) and Wt3 (14.36 ± 0.43 cm) were significantly taller than seedlings of Wt4 (12.86 ± 0.43cm) and Wt5 (10.16 ± 0.19 cm) which were shortest in height at $p \leq 0.05$ (Figure 3). The epicotyl length was significantly shorter in cultured seeds of Wt4 (2.88±0.14) and Wt5 (2.03±0.0) compared to its corresponding hypocotyl length (9.98±0.38, 8.13±0.15) in a ratio of 3:1 and 4:1 respectively (Table 2). The hypocotyl length of Wt3 (9.08±0.86) was in ratio 2:1 with its epicotyl length (5.28±1.21) which was significantly shorter when compared to the epicotyl of Wt2 (7.72±0. 30) and Wt1 (7.91±0.15) which were both in ratio 1:1 with their epicotyl length (Table 2).

The germination period (GP) of the seed cultured under light photoperiod, Wt1 (13.20±0.37), Wt2 (13.00±0.32) and Wt3 (14.20±0.37) had significantly faster growth period followed by Wt4 (15.60±0.51) and Wt5 (16.80±0.37) which was significantly the slowest as presented in Table 2.

Under light, the root architecture in cultured seeds of Wt3, Wt4 and Wt5 (Figure 2) were scanty unlike in cultured seeds of Wt1 and Wt2 (Figure 1). A partial seed coat break in cultured seeds of Wt4 and Wt5 (Figure 2) was observed while in cultured seeds of Wt1, Wt2 and Wt3, seed coat break was total (Figure 4).

Effect of Photoperiod on *in vitro* Germination of *Afzelia africana* Seeds

The results obtained (Table 2) revealed that complete dark photoperiod had effect on the germination and seedling growth of *Afzelia africana*. Under the dark photoperiod, higher plant height was recorded in Wt1 (19.47±0.32 cm) and Wt2 (18.98±0.64 cm) which was significantly different from plant height in Wt4 (16.70±0.55 cm) and Wt5 (13.16±0.24 cm) with the shortest plant height being Wt5. Under dark photoperiod, the length of hypocotyl (HYPO D) and epicotyl (EPI D) were unequal in all seeds of different weights grown with Wt1 (15.57±0.26) cm and Wt2 (15.18±0.51) cm having significantly highest hypocotyl which are in ratio 4:1 with their epicotyl, while Wt4 (13.69±0.45) cm and Wt5 (10.79±0.20) cm which are in ratio 5:1 with their epicotyl.

Epicotyl length followed the same pattern of decreasing as seed size decreases with Wt1 (3.89±0.06) and Wt2 (3.80±0.13) significantly longer than in Wt3 (3.28±0.13), Wt4 (3.01±0.10[b]) and Wt5 (2.37±0.04) which has the shortest epicotyl. Again, under dark photoperiod, germination period (GP) was faster with larger seed sizes; while GP increased significantly as seed size reduced. GP was fast in Wt1, followed by Wt2 and Wt3 taking 30.50±0.65, 31.00±0.71, and 31.50±0.65 days respectively. Wt4 (33.50±0.29) and Wt5 (36.00±0.41) required more days to germinate.

Leaf sprout was hooked (Figure 5B and 5C) under the dark unlike under light photoperiod (Figure 5A). Seedlings grown in the dark were also cream colored unlike those under light photoperiod that showed deep green coloration (Figure 5A). In addition, the root system of seedlings grown under dark photoperiod was not well-developed (Fig. 5C) unlike seedlings grown under light photoperiod (Figures 1 to 4 and 5A).

Table 1: Effect of Seed Weight on i*n vitro* Germination of *Afzelia africana* Seeds Under Light

Size (g)	SB (days)	RE (days)	Cop (days)	SE (days)	LN (Ave)	PH (cm)
Wt1 (3.3 – 3.7 g)	3.40±0.24[a]	8.40±0.24[a]	14.50±0.50[a]	21.50±0.50[a]	7.75±0.25[a]	14.86±0.24[a]
Wt2 (2.8 – 3.2 g)	3.40±0.24[a]	8.40±0.24[a]	14.75±0.48[ab]	21.75±0.48[a]	6.25±0.25[b]	14.62±0.48[a]
Wt3 (2 .3 – 2.7 g)	3.40±0.40[a]	8.60±0.40[a]	15.75±0.75[ab]	22.50±0.65[a]	6.25±0.25[b]	14.36±0.43[a]
Wt4 (1. 8 – 2.2 g)	3.80±0.37[a]	9.00±0.32[a]	16.00±0.41[ab]	22.50±0.29[a]	5.50±0.29[b]	12.86±0.43[b]
Wt5 (1.3 – 1.7 g)	3.80±0.37[a]	9.20±0.20[a]	16.50±0.50[b]	22.25±0.25[a]	5.50±0.29[b]	10.16±0.19[c]

Means with different letters along the column are significantly different at $p \leq 0.05$.
SB – Seed coat Break, RE – Root Emergence, Cop – Cotyledon Opening,
SE – Shoot Emergence, LN – Leaf Number, PH – Plant Heig

Table 2: Effect of Photoperiod on *in Vitro* Germination of *Afzelia africana*

Size (g)	PH L (cm)	PH D (cm)	HYPO L (cm)	HYPO D (cm)	EPI L (cm)	EPI D (cm)	HL EL Ratio	HD ED Ratio	GPL(days)	GP D(days)
Wt1 (3.3 – 3.7 g)	14.86±0.24^{a}	19.47±0.32^{a}	6.95± 0.14^{c}	15.57±0.26^{a}	7.91±0.15^{a}	3.89±0.06^{a}	1:1	4:1	13.20±0.37^{c}	30.50±0.65^{c}
Wt2 (2.8 – 3.2 g)	14.62±0.48^{a}	18.98±0.64^{a}	6.90±0.20^{c}	15.18±0.51^{a}	7.72±0. 30^{a}	3.80±0.13^{a}	1:1	4:1	13.00±0.32^{c}	31.00±0.71^{c}
Wt3 (2 .3 – 2.7 g)	14.36±0.43^{a}	18.23±0.73^{ab}	9.08±0.86^{ab}	14.95±0.60^{ab}	5.28±1.21^{b}	3.28±0.13^{b}	2:1	5:1	14.20±0.37^{c}	31.50±0.65^{c}
Wt4 (1. 8 – 2.2 g)	12.86±0.43^{b}	16.70±0.55^{b}	9.98±0.38^{a}	13.69±0.45^{b}	2.88±0.14^{c}	3.01±0.10^{b}	3:1	5:1	15.60±0.51^{b}	33.50±0.29^{b}
Wt5 (1.3 – 1.7 g)	10.16±0.19^{c}	13.16±0.24^{c}	8.13±0.15^{bc}	10.79±0.20^{c}	2.03±0.04^{c}	2.37±0.04^{c}	4:1	5:1	16.80±0.37^{a}	36.00±0.41^{a}

Means with different letters along the column are significantly different at $p \leq 0.05$.

Wt- Seed weight. PH L- Plant height in Light photoperiod, HYPO L- Hypocotyl in Light photoperiod, EPI L- Epicotyl in Light photoperiod, HL EL Ratio- Hypocotyl in light: Epicotyl in light, PH D- Plant height in Dark photoperiod, HYPO D- Hypocotyl in Dark, EPI D- Epicotyl in Dark, HD ED Ratio- Hypocotyl in Dark: Epicotyl in Dark, GP D- Germination Period in Dark, GP L- Germination Period in Light

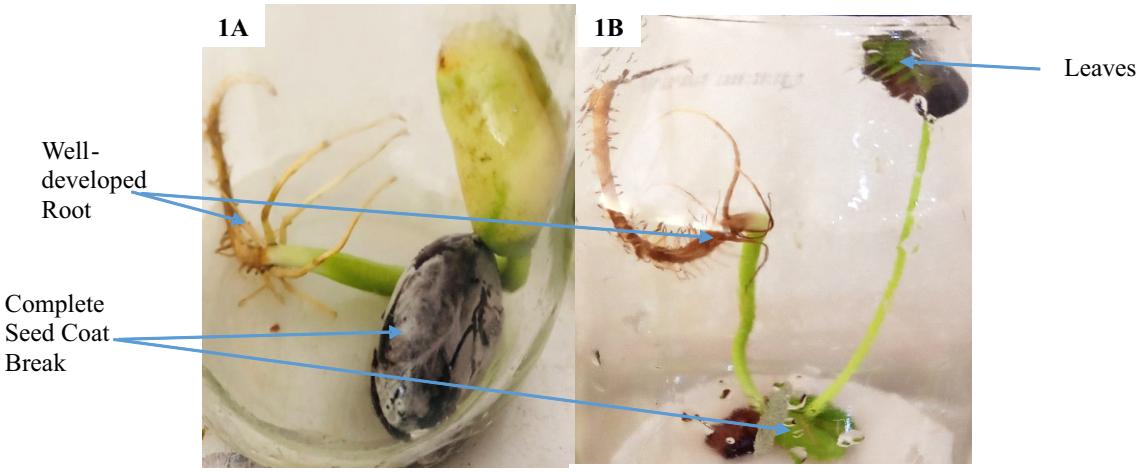

Figure 1A – Growing Seedling of Seed Weight Wt1

Figure 1B – Growing Seedling of Seed Weight Wt 2

Figure 1: Well Developed Roots of Cultured Seeds of *Afzelia africana* under Light Photoperiod

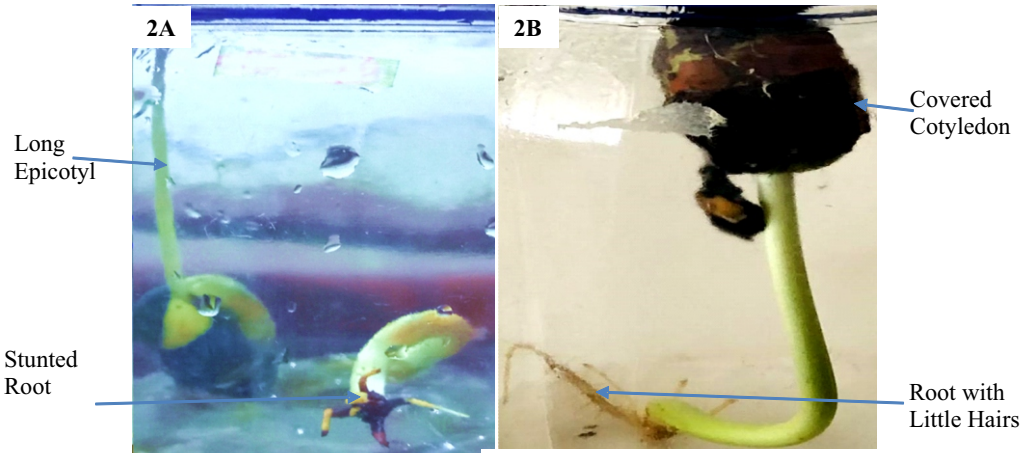

Figure 2A – Growing Seedling of Seed Weight Wt3

Figure 2B – Growing Seedling of Seed Weight Wt4

Figure 2: Seed Coat Break of Cultured Seeds of *Afzelia africana* under Light Photoperiod

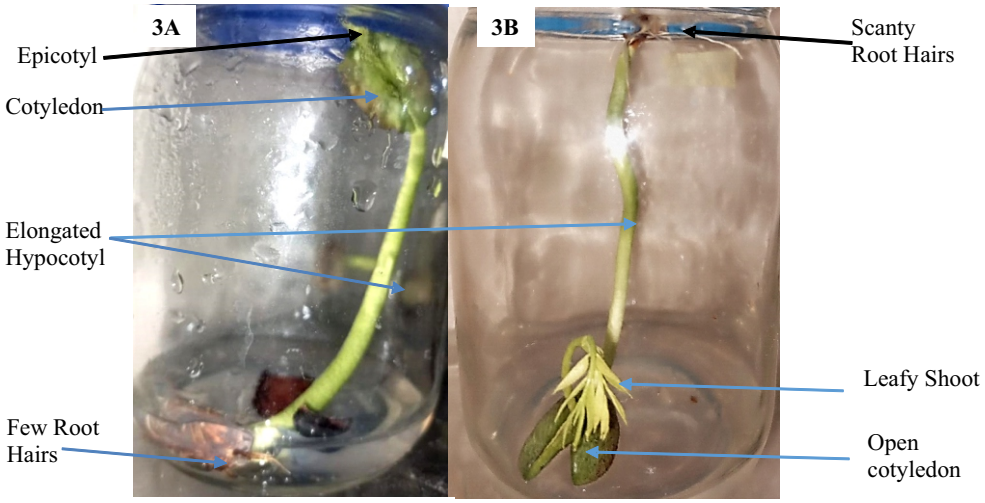

Figure 3A – Growing Seedling of Seed Weight Wt5

Figure 3B – Growing Seedling of Seed Weight Wt3

Figure 3: Cotyledon Opening and Shoot Emergence of Cultured Seeds of *Afzelia africana* under Light Photoperiod

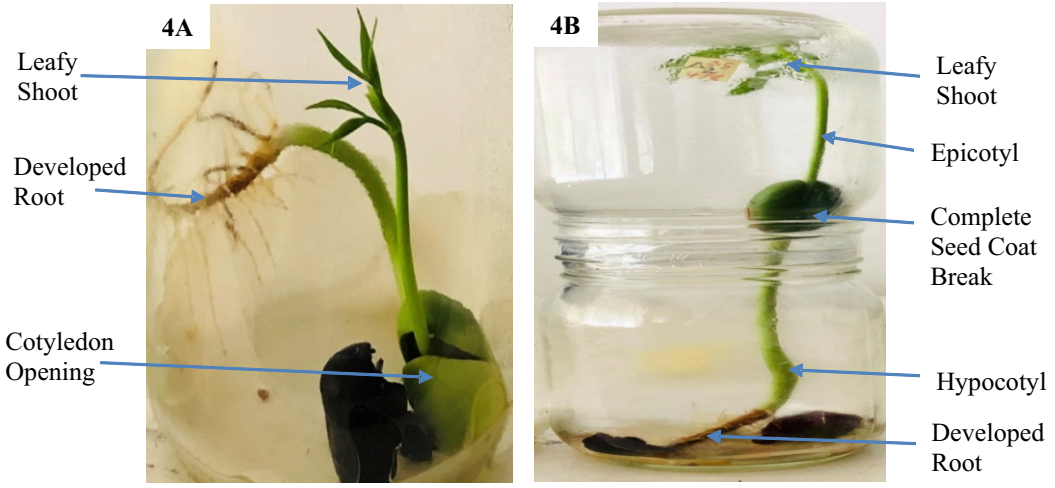

Figure 4A – Growing Seedling of Seed Weight Wt2

Figure 4B – Growing Seedling of Seed Weight Wt1

Figure 4: Leaf Number and Plant Height of Cultured Seeds of *Afzelia africana* under Light Photoperiod

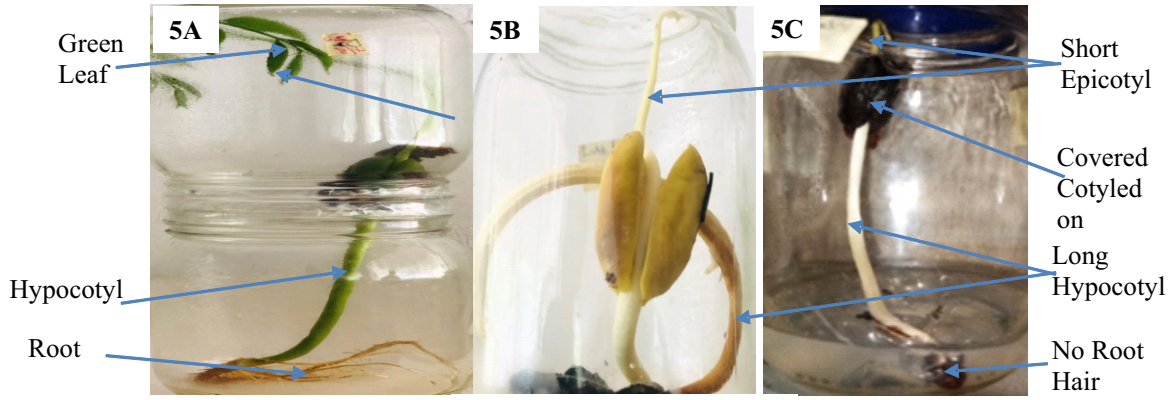

Figure 5A – Growing Seedling of Seed Weight Wt1 under Light

Figure 5B – Growing Seedling of Seed Weight Wt1 under the Dark

Figure 5C – Growing Seedling of Seed Weight Wt5 under the Dark

Figure 5: Effect of Dark Photoperiod on *in vitro* Germination of Different Seed Weights of *Afzelia africana*

DISCUSSION

A. africana is widely used for various purposes ranging from timber, food, medicine and forage. As a result of this multiple uses, there is a permanent pressure on *A. africana* in the wild. Coupled with its low rate of regeneration from seeds, *A. africana* is exposed to high risk of extinction in the wild. Hence, *in vitro* germination considering the effect of seed weight is a recommended alternative for mass multiplication, conservation and continued utilization of this threatened species. In addition, because *in vitro* plant systems are season and climate independent, there will be a continuous availability of plant material for propagation of *A. africana* with this *in vitro* germination of seeds. This study revealed that the seed coat performs functions of protecting the seed as well as play roles in the metabolic responses that converts seeds to seedlings during the process of germination. In seeds with higher weights; Wt1, Wt2 and Wt3, the first visible sign of pre-germination which is seed coat break occurred within shorter number of days, and finally resulting to significant plant height in these seed sizes. Coen and Magnani (2018) reported that seed coats act as channels for transmitting environmental cues to the interior of the seed which primes the seed to adjust its metabolism in response to changes in its external environment. Under *in vitro* environment, the seed weight and its interaction with molecular composition of the media needs to be considered

as the metabolic energy required for the cotyledon to break from the seed coat may not be enough in the stored energy of seeds of smaller weights (Tymoszuk and Wojnarowicz, 2020). Dormancy can either be embryo dormancy where the agents inhibiting germination are within the embryo or coat-imposed dormancy where the agent inhibiting germination are primarily attributed to physical or chemical barriers imposed by the seed coat. These barriers can be impermeability, hardness, or the presence of inhibitory chemicals within the seed. This explains the longer days needed for RE and SB in the seeds of *A. africana* of smaller sizes indicating smaller embryos yet having equally hard seed coats compared to large seeds with large embryos. In *Zingiberaceae lindl*, Khuat *et al.* (2022), used mechanical scarification, immersion in hot or cold water, acid scarification, and the application of plant growth regulators (gibberellic acid (GA_3) and 1-naphtaleneacetic acid (NAA)) on seed germination and subsequent seedling growth.

The shorter duration of cotyledon opening in Wt1 can be attributed to cotyledonary node induction, which accelerates cotyledon opening due to hormonal stimulation such as melatonin that is reported to stimulate cotyledon opening under dark and high light intensity (Wang *et al.*, 2022). This effect is more prominent in larger seeds due to the higher accumulation of stored nutrients. Moreover, according to Wang *et al.,* (2020), light is important for cotyledonary node opening as observed in *Arabidopsis thaliana*, where darkness promoted apical hook development and cotyledon opening. This could have been responsible for the late cotyledon opening in the seeds cultured under dark photoperiod which responded with late shoot emergence followed by shorter epicotyl. Shoot emergence that is accompanied by cotyledon opening is initiated by endogenous hormone that is abundant in the seeds of higher weight (Wt1, Wt2 and Wt3) compared to the seeds of smaller weight (Wt4 and Wt5). Similarly, Ahmad *et al.*, (2021) reported that the content of zeatin + zeatin riboside, IAA, and GA was positively correlated with seed weight of maize under semiarid conditions by the treatment of melatonin. Moreover, the cotyledon opening is also possible because of the 16/8-hour photoperiod that the seeds were subjected to which can equally trigger some kind of metabolic reactions within the seed. Ge *et al.*, (2020) observed an increased concentration of endogenous hormone including gibberellic acid (GA) during the seed germination of *Anemone rivularis* with shoot emergence occurring faster under light photoperiod than under complete dark photoperiod condition. Hence the result of shoot emergence giving rise to varying plant height points to a higher concentration of GA in the bigger-sized cultured seeds of *Afzelia africana*. This accounts for the formation of more leaves as confirmed by Cornea-Cipcigan *et al.* (2020) that gibberellic acid can improve seed germination and ornamental quality of selected *Cyclamen* species grown under short and long days.

Again, the concentration of constituent elements in the MS media such as the high nitrogen level reported by Phillips and Garda (2019) would have also enhanced the endogenous cytokinin production which in turn enhanced cell division and cell elongation that gave rise to higher number of leaves in larger-sized seeds cultured under light photoperiod. Hu *et al.* (2020) reported that in *Camellia sinensis*, the addition of high concentration of nitrogen increased cytokinin synthesis leading to vigorous growth. Also, Nunes *et al.* (2024) reported a correlation between the weight of seeds and the amount of nutrient stored in their cotyledon. Consequently, bigger cotyledons supply more nutrients to growing seedlings resulting into significantly higher plant height regardless of whether it is cultured under dark or light photoperiod. Also, Yisau *et al.* (2023) studied the effect of seed source and seed size on the early growth of *Anacardium occidentale* seedlings and attributed higher seedling height to larger seed sizes with more food reserves in the cotyledons of the seeds. Seeds grown in the dark lacked root hairs indicating that light affects both shoot and root development. In the absence of light, hormone secretion is affected hence preventing the photomorphogenic development that should have occurred in the developing cells of the roots resulting in poor root development as shown in the figure showing seedling cultured in the dark. This is confirmed by the finding of Li *et al.*, (2024) that root-specific photoreception directs early root development by the HY5 gene under light condition. Both endogenous and exogenous

hormones are required to induce proper growth and development of roots in *in vitro* seedlings of *A. africana* under dark photoperiod. Also reporting on the effects of light intensity on root development, Miotto *et al.* (2021) opined that roots respond to the light intensity applied to the shoot by changes in primary and lateral root development. Root architecture was influenced by photoperiod in this study, well developed roots emerged from large seeds while scanty roots were observed in small-sized seeds. The bigger cotyledons present in large seeds will supply more growth resources to their roots than in small seeds with small cotyledons. Singh *et al.* (2021) reported a similar observation in four tree species where seed size affected root length with larger- sized seeds having significantly longer roots than small and medium-sized seeds.

The longer germination period observed under the dark photoperiod was as a result of the absence of light which could impose stress on growing seedlings. Different species respond differently to light and dark photoperiod depending on their light requirements. The significant plant height observed in seedlings of *A. africana* cultured under dark photoperiod was as a result of etiolation. Martinsyah *et al.*, (2024) reported a similar response of increased plant height in the absence of light compared to light treatments in *Solanum tuberosum*. The relative long hypocotyl to epicotyl length ratio of seeds grown in the dark is a survival mechanism during the etiolation process. Etiolation can be manipulated to achieve desired plant response as reported by Armarego-Marriott *et al.* (2020) who reviewed recent studies of etiolation and de-etiolation. Etiolation is associated with the developmental pathway of seedlings grown in the dark and it is characterized by long hypocotyls, closing of cotyledons for longer period or not opening at all and formation of apical hooks as observed in this study. Also, the development of proplastids into etioplasts especially in the absence of light-induced auxin signaling is also a response of etiolation (Weller and Kendrick; 2015; Cheng *et al.*, 2021; Li *et al.*, 2022 and Yun *et al.*, 2023). Seedlings grown under dark conditions had a creamy appearance in contrast to the greenish appearance of seedlings exposed to light. This is caused by the low chlorophyll pigmentation due to absence of light in the dark photoperiod condition. This is confirmed by Liu *et al.*, (2020) who observed in the model plant *Arabidopsis thaliana* that in the dark grown seedlings, the phytochrome was light-deficient hence preventing the process of photosynthesis that should provide the hormonal requirement for the synthesis of plastids and other metabolic reactions. In this study, though seedlings grown under dark appeared non-green because of no photosynthesis, yet the formation of buds were observed which is attributed to the interactions of changing concentrations of endogenous hormones within the plant coupled with the nutrients supplied by the culture media. Deepika *et al.*, (2020) reporting dark-induced hormonal regulation of plant growth and development, emphasized on dark mediated changes in plant hormones, regulation of signaling complex and the transcription factors which also affects developmental events such as apical hook development, elongated hypocotyls, photoperiodic flowering, shortened roots, and plastid development.

CONCLUSION

Seed weight has significant effect on *in vitro* germination of *Afzelia africana* and seed weight range of 3.3 – 3.7g grew highest under 16/8-hour photoperiod and was established enough to be acclimatized in the outside environment. Though, seed grown under light photoperiod gave the best response for seed germination, when more explant material is needed for micropropagation, seeds can be grown under dark photoperiod to produce elongated hypocotyls.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest

AUTHORS' CONTRIBUTIONS

Ejeoghene Rita OGBIMI: Conceptualization, supervision and revision

Babajide OMISOPE: Data collection, analysis and writing

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