Observations on seed embryo and germination, seedling morphology and development of Vitellaria paradoxa (C. F. Gaertn.)

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Observations on seed embryo and germination, seedling morphology and development of *Vitellaria paradoxa* (C. F. Gaertn.)

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**ABSTRACT**

Cotyledon morphology and subsequent establishment of *V. paradoxa* seedlings that account for its cryptohypogeal germination were studied. To identify the embryonic axis, fresh and partially dry seeds were deshelled and their distal ends transversely cut off. Cut seeds were immersed in 1.0 \% tetrazolium chloride (TTC) solution for 24 hours and kept in the dark for staining. To study cotyledon morphology, 50 seeds were deshelled, air-dried for 3–5 days and split open from the distal end. Another batch of 50 seeds were sown directly. The sprouted seeds or seedlings were sampled at 5-day intervals and their cotyledonary tubes sectioned to observe morphological and anatomical features. Morphologically the seed bears a cotyledonary raphe, which lies vertically with distally schizocotylous and proximally syncotylous cotyledons. Large seeds sprouted earlier (7 days) followed by seedling emergence within 61 days, while small seeds sprouted almost a week later (12 days) with seedling emergence within 75 days. The location of the embryos at the proximal ends cause germinating seeds to produce long cotytedonary tubes which bury the plumules deep into the soil, a protection against bushfires. This germination study will enhance nursery establishment for plantation development of *V. paradoxa*.

**KEYWORDS**

Germination; seedling emergence; shea; cryptohypogeal; establishment; cotyledon; *V. paradoxa*

**Introduction**

*Vitellaria paradoxa* is an economically important oleiferous crop indigenous to interior savannah regions of sub-Saharan Africa (Hall et al. 1996). It plays a significant economic role in the livelihood of the rural women (Elias and Carney 2007). The shea butter, extracted from the kernels or nuts has characteristics similar to cocoa butter used to manufacture confectionery (Masters et al. 2004) and as base for medicines and lotions in the pharmaceutical and cosmetic industries respectively. Additionally, the fat is used locally as cooking oil, for soap making as well as fuel for lighting lamps. The importance of the shea fruit as a food security crop is observed in rural areas where it is most frequently consumed as a staple when food supply is low (Yidana 2004).

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V. paradoxa grows in arid marginal Sahelian soils and can therefore combat desertification and mitigate against climate change due to its pyrophytic features and high longevity. The tree ameliorates the microclimate and recycle nutrient through annual leaf shedding (Dianda et al. 2009). Despite these ecological benefits, V. paradoxa is not planted yet but still grows in the wild (Nyarko et al. 2012; Okao et al. 2012) and very little effort has been made to domesticate it on a commercial scale. The lack of domestication of the shea tree has been attributed to the recalcitrant nature of its seed, which loses viability rapidly after harvest, and to its slow seedling establishment (Jøker 2000; Ugese et al. 2010).

The germination of V. paradoxa is described as cryptogeal (Jackson 1968) which is a characteristic of pyrophytes growing in arid regions. It involves the emergence of a cotyledonary tube through the seed coat which buries the plumule deep into the soil during the development of the radicle which grows further into the soil (skotomorphogenic germination). The buried plumule develops into a rudimentary shoot which emerges through a slit on the cotyledonary tube (photomorphogenic germination).

The embryonic axis of the seed cannot be located with accuracy because it is buried within fatty tissues. Secondly, the morphology, anatomy and the functional role of the cotyledonary tube also remains undescribed; thus, it has been commonly referred to as a pseudoradicle. A precise description of the germination process and the seedling morphology in V. paradoxa is therefore needed to enhance nursery establishment for domestication of the shea tree. Also, the identification of the exact location of the embryonic axis is necessary to allow for its excision for plant regeneration through embryo culture. In this paper, we aimed at determining the exact location of the embryo using tetrazolium (TTZ) test and we studied its subsequent germination and seedling establishment. Morphological features of the cotyledonary tube are also described.

Materials and methods

Collection and preparation of shea fruits

Mature shea fruits were collected from agroforestry parklands growing at an altitude of 305 m at Ga and Tanina in the Wa West District of the Upper West Region of Ghana (Figure 1). At each location, 20 fruits per tree were picked from 24 farmer-identified plus trees. All the fruits were bulked together and transported by road to the Biotechnology Centre of the Biotechnology and Nuclear Agriculture Research Institute (BNARI). Six hundred (600) fresh fruits were depulped manually and seeds were washed under tap water. The seeds were divided into three groups based on size (defined in seed size and germination section below) and used for the morphological and germination studies.

Identification of the embryonic axis using tetrazolium (TTZ) test

Triphenyl tetrazolium chloride (TTC) solution is an International Seed Testing Association (ISTA) recommended method for evaluating viability of seeds especially when the seeds are dormant, slow growing, recalcitrant or when quick estimate of viability is needed (ISTA 2004). Tetrazolium (TTZ) test was performed as described by Yu and Wang (1996)
to determine the location of the embryonic axis in the seed. Six (6) fresh and 6 partially dry seeds (air-dried for 72 hours) were deshelled and a quarter of their distal ends were transversely cut off. The remaining portions were washed and soaked in distilled water for 6 hours before immersion in 1.0 % tetrazolium chloride (TTC) solution in a Petri dish. The dish with its contents was wrapped tightly using parafilm and placed in a closed cabinet for 24 hours. Thereafter, the seeds were observed for staining of the embryonic axis by TTC solutions and photographs were taken using a 16-megapixel Sony digital still camera.

*Morphological studies on the cotyledon*

Cotyledon morphology was studied by using 100 fresh seeds. Fifty (50) seeds were deshelled and air-dried for 3–5 days and this constituted treatment I (TI) whilst the remaining 50 seeds were sown on a seedbed made up of weathered sawdust and soil mixed in the ratio 5:1 respectively (treatment II, TII). The sprouted seeds or seedlings were sampled at 5-day intervals beginning 7 days after sowing (DAS). Five (5) seedlings were sampled at each time point by carefully uprooting and washing them using tap water. The partially dried or sprouted seeds were then split open manually by pulling apart the cotyledons beginning from the distal end. The cotyledonary tubes of the seedlings were transversely or longitudinally sectioned to observe the morphological
and anatomical features. Transverse sectioning of the cotyledonary tube was done by cutting the tube sharply using a scalpel at 3–5 cm away from the seed. Longitudinal sectioning was done by carefully dividing the cotyledonary tube along the raphe of the cotyledons. The morphological features were observed using magnifying lenses and a stereomicroscope (Leica ZOOM 2000, Cole-Parmer, Wetzlar, Germany).

Seed size and germination

The linear dimensions (length, breadth and width) of 180 seeds were measured with vernier callipers and the results were used to categorize seeds into small (13–15 cm³), medium (19–21 cm³) or large (25–27 cm³). The breadth is obtained as the linear dimension of the two adjacent sides of the hilum. The seeds were then sown on seedbeds made up of topsoil and well-decomposed sawdust in a ratio 5:1, in randomized complete block design with 60 seeds in each of the 3 replicates. All seeds were sown 2 cm deep with the hilar side down. Watering and hoe-weeding were done as and when necessary to ensure adequate supply of moisture and control of weeds respectively. Germinating seeds and seedlings were sprayed with Cydem Super and Akape 20 SC (IPROCHEM Co. Ltd, Shenzhen, China) to protect against insects. Seeds were observed at a 5-day interval for signs of germination for 30 days and subsequent shoot emergence 30 days after sowing (DAS). Seeds were considered germinated when their cotyledonary tube had become visible. Germination percentage (GP), mean germination time (MGT) and two emergence parameters were calculated from the data obtained. Germination percentage was calculated as follows:

\[
GP = \frac{GS \times 100}{TS}
\]

where GP = germination percentage, GS = number of germinated seeds and TS = total number of sown seeds,

and mean germination time (MGT) was computed as:

\[
MGT(\text{days}) = \frac{\sum (t_i \times n_i)}{\sum n_i}
\]

where \(t_i\) is the number of days beginning from the date of sowing and \(n_i\) is the number of germinated seeds at each day (Bewley and Black 1994; Yeboah et al. 2011).

Emergence percentage (EP), emergence index (EI) and emergence rate index (ERI) were computed using the formulae described by Adetimirin et al. (2006) as follows:

\[
EP = \frac{\text{Number of emerged seedlings}}{\text{Total number of seeds sown}} \times 100\%
\]

\[
EP = \frac{\sum (\text{Emerged seedlings on a day})(\text{DAS})}{\text{Total number of emerged seedlings}}
\]

\[
ERI = \frac{\text{EI}}{\text{EP (in decimal)}}
\]

Emergence index measures the rate of seedling emergence and emergence rate index estimates the duration to the emergence of all seedlings in the absence of other limiting conditions. Observations on stages of growth of the seedlings were made using
a method described by Ugese et al. (2010) with modifications and the duration of each stage was measured (in days).

**Statistical analysis**

Data collected were subjected to analysis of variance (ANOVA) using the Genstat statistical package (9th Edition). Percentage data on germination and on emergence were transformed using square-root transformation before analysis. Means were separated where appropriate at 5% significance level using least significant difference (LSD) test.

**Results**

**Identification and location of the embryo in V. paradoxa seed**

All the fresh seeds immersed in the TTC solution showed a red coloration in the slit along the two cotyledons with the staining being deeper towards the proximal end and visible at the exerted spot suggesting the presence of embryos (Figure 2(a)). A longitudinal section through a stained seed showed two differentially stained regions: a lighter portion suggesting the presence of the radicle and a deeper section indicating the presence of the plumule (Figure 2(b)). Partially dry seeds did not show any red coloration indicating that they were dead. Microscopic examination of freshly split open seeds without staining with TTC solution revealed the embryonic axis as a small linear structure surrounded by two cotyledons with no differentiation yet into plumules and radicles (Figure 2(c)).

**Cotyledon morphology of vitellaria paradoxa seed**

Fresh cotyledons of the seeds used for this study were usually unequal with the embryo appearing as a yellow thrusted spot at the proximal end of the smaller one and its notch on the bigger one (Figure 2(c)). Deshelled seeds showed cotyledonary raphes at the distal end which are oriented either parallel or perpendicular to the embryo. Seeds whose raphes are parallel to the embryo were classified as Type 1 seeds while Type 2 refers to seeds with raphes perpendicular to the embryo (Figure 3(a–c)). The size of the cotyledonary raphe increases as the moisture content of the seed decreases and this desiccation often creates a depression in between the cotyledons.

A fully split-open seed of either air dried or sprouted seed shows two distinct sections on the cotyledons (Figure 3(d)). One of the sections broadens at the distal part but narrows sharply and ends bluntly just close to the proximal end. At the distal section, the cotyledons are separable and the seed is therefore referred to as schizocotylous. At the proximal side, the cotyledons are fused, thus they are referred to as syncotylous.

Longitudinal section through a fresh seed showed latex being exuded only from the spot where the embryo is located (Figure 4(a)). Conversely, transversely sectioned seeds exuded latex from the edges as well as from the slit in between the cotyledonary raphe (Figure 4(b)). Partially dry seeds did not exude latex on their cut surfaces when sectioned in either direction.
Germination of seeds

Germination of *V. paradoxa* seed was initiated by a swelling followed by the protrusion of a root-like structure, pseudoradicle, through the testa with a visible embryo at the tip (Figure 5(a)). As germination continued, the pseudoradicle grew deeper into soil, pushing the radicle into the soil, and forming a bulge or swelling at 2–8 cm. After 2–3 weeks, the true roots developed from the radicle below the bulge and continued its positive geotropic growth (Figure 5(a)) while the shoot appeared from a slit on the bulge of the pseudoradicle and then grew upwards to emerge above the soil (Figure 5(b)). The seed remains under the soil surface after germination.

Features of the cotyledonary tube

A transverse section through the pseudoradicle showed a hollow tube surrounded by a sheath and laticiferous vessels (Figure 6(b,c)). The sheath enveloped the vessels into
a tubular geotropic structure. The laticiferous vessels ranged from 6 to 8 extending from the seed to the base of the bulge of the pseudoradicle. The plumule of the embryo moves through the central hollow tube during germination until it reaches the bulge (Figure 6(b)).

The plumule is single or branched with rhizoids or hair-like structures. It is white in colour but turns pink as it matures into a rudimentary shoot (Figure 6(c,e)). The bulge, a swelling of 0.5–0.7 cm long at the base of the cotyledonary tube, is formed when the descended plumule develops into the rudimentary shoot. Microscopic examination revealed that the rudimentary shoot has nodes and internodes with scale leaves numbering 5–7. As germination continues the rudimentary shoot protrudes from the cotyledonary tube via a slit and grows upwards through the soil until it emerges above the soil surface suggesting a cryptogeal type of germination.

Figure 3. Seed of *V. paradoxa* showing morphology of the cotyledon. (a) Seed showing embryo spot (arrowed), (b) Type 1 seed with a raphe (arrow) parallel to the embryo; (c) Type 2 seed with a raphe perpendicular to the embryo; (d) split seed showing where cotyledons are appressed (x) and adpressed (y) to each other. PE means proximal end and DE means distal end.
Figure 4. Latex exudation (a) from embryo spot (b) transversely sectioned surface and cotyledonary raphe (arrowed).

Figure 5. *Vitellaria paradoxa* seedling; (a) sprouted seed showing pseudoradicle (ps), cotyledonary slit (cs) and true radicle (r) and (b) seedling showing cotyledonary node (cn) and emerged shoot.
Effect of seed size on germination and emergence of V. paradoxa seedlings

Seed size had a significant influence on the duration to germination (protrusion of the pseudoradicle), pseudoradicle elongation, bulging, shoot appearance, shoot elongation, emergence and establishment of the seedlings (Tables 1 and 2). All the small and medium-size seeds sprouted or germinated compared to 95% of the large seeds but statistical analysis did not show any significant (P > 0.05) effect of seed size on germination. Similarly, emergence percentage was high and varied from 93.30% for large-size seeds to 95.79% for small-size seeds (Table 1).

Duration to sprouting varied significantly (P < 0.05) from 7 days in large seeds to 12 days in small seeds (Table 2). Statistically, the rate of elongation of the cotyledonary tube (CT) also differed significantly (P < 0.05) with large seeds elongating faster (12 days), followed by medium (16 days) and small seeds (22 days). Similarly, seed size also had significant influence on bulging of the pseudoradicle. Bulging was observed on the pseudoradicle of large seeds 21 days after sowing and this was significantly (P < 0.05) different from medium (26 days) and large seeds (28 days). Shoot appearance also differed depending on the size of seeds sown. For large seeds, shoot appeared 25 days after sowing which was 7 days earlier than medium seeds (33 days) and 14 days earlier than small seeds (38 days).
However, subsequent days to shoot elongation (SE), defined as the difference between days to emergence of seedlings above the soil and days to shoot appearance (SA) on the cotyledonary tube was not significantly influenced by seed size (Table 2).

Seed size, had a significant effect (P < 0.05) on days to emergence (SE). Seedlings produced by large seeds significantly reduced seedling emergence to about two months (61 days) (Table 2) but significantly delayed seedling establishment to almost 4 months (114 days). In contrast, seedlings produced by medium and small seeds delayed emergence to only about 2 (65 days) and 2.5 months (75 days) respectively but they significantly enhanced early seedling establishment (Table 2). A seedling was considered established if its cotyledonary tube withered completely. The cotyledonary tube is functionally haustorial, implying that an established seedling would depend on food reserves that have already been translocated from the seed to the base of the bulge or those mobilised during photosynthesis. The corresponding emergence rate indices (EI) calculated for seedlings produced by large and medium seeds were 65 and 70 days respectively (non-significant difference). The EI of seedlings from both seed sizes were significantly shorter than seedlings from small seeds (78 days) (Table 2).

### Discussion

#### Identification of the embryonic axis

The embryonic axis of *V. paradoxa* is deeply buried in fatty tissues which makes it very difficult to identify. Thus, excision of the embryonic axis, which presents a good source of totipotent cells for regeneration via *in vitro* culture is very difficult. To overcome this challenge, we used tetrazolium test to accurately identify location of the embryonic axis in the seed.

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**Table 1.** Effect of seed size on germination, emergence percentage and emergence rate index of *Vitellaria paradoxa* seedlings.

<table>
<thead>
<tr>
<th>Size class</th>
<th>Germination percentage</th>
<th>Emergence percentage</th>
<th>Emergence rate index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>100a</td>
<td>95.79a</td>
<td>78.15b</td>
</tr>
<tr>
<td>Medium</td>
<td>100a</td>
<td>93.15a</td>
<td>70.25a</td>
</tr>
<tr>
<td>Large</td>
<td>95a</td>
<td>93.30a</td>
<td>65.07a</td>
</tr>
<tr>
<td>LSD(0.05)</td>
<td>31.8</td>
<td>35.0</td>
<td>7.37</td>
</tr>
</tbody>
</table>

Means in the same column followed by the same letters are not significantly different (P < 0.05).

**Table 2.** Effect of seed size on development of *Vitellaria paradoxa* seedlings.

<table>
<thead>
<tr>
<th>Seed size</th>
<th>Days to Sprouting</th>
<th>CTE</th>
<th>Bulging</th>
<th>SA</th>
<th>*SE</th>
<th>Emergence</th>
<th>EST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>11.52c</td>
<td>21.52c</td>
<td>27.86c</td>
<td>37.99c</td>
<td>37.03c</td>
<td>75.02b</td>
<td>97.42a</td>
</tr>
<tr>
<td>Medium</td>
<td>9.15b</td>
<td>15.89b</td>
<td>25.69b</td>
<td>33.31b</td>
<td>32.16b</td>
<td>65.47a</td>
<td>99.29a</td>
</tr>
<tr>
<td>Large</td>
<td>6.98a</td>
<td>11.55a</td>
<td>20.76a</td>
<td>25.36a</td>
<td>35.34a</td>
<td>60.70a</td>
<td>114.26b</td>
</tr>
<tr>
<td>LSD(0.05)</td>
<td>1.95</td>
<td>5.78</td>
<td>1.85</td>
<td>3.49</td>
<td>--</td>
<td>5.57</td>
<td>6.55</td>
</tr>
</tbody>
</table>

Means in the same column followed by different letters are significantly different (P < 0.05); CTE: Cotyledonary tube elongation; SA, shoot appearance; SE, Shoot elongation; EST, Establishment and *SE = Emergence – SA. Days to sprouting are mean germination times (MGT).
The central parts of fresh seeds immersed in the TTC solution stained deep red after 24-hour incubation suggesting the location of the embryo. According to Yu and Wang (1996), embryo tissues stain dark red on contact with TTC solution due to presence of dehydrogenase enzymes which reduces 2,3,5-triphenyl tetrazolium chloride to a stable, bright red triphenyl formazan. Thus, we conclude that the darker stained portion of the seed of *V. paradoxa* is the embryo which could be excised as explant for *in vitro* culture.

**Morphology of vitellaria paradoxa cotyledons**

The seed of *V. paradoxa* consists of two large cotyledons which are distally free (schizocotylous) but proximally fused (syncotylous). This observation is contrary to Nikiema and Umali (2007) who reported that the seeds of *V. paradoxa* are fully syncotylous. The proximally fused cotyledons develop into long hollow cotyledonary petioles deep into the soil which has often been incorrectly described as pseudoradicles. Thus, the proximally syncotylous morphology of *V. paradoxa* seeds explains their cryptohypogeal germination. While partial or full syncotyly may be a pyrophytic adaptation, it impedes emergence of the cotyledon from the seed coat thereby delaying germination (Flores 2002). In terms of cotyledonary traits, the *V. paradoxa* seedlings are remote cryptocotylar hypogeal types (skotomorphomatic). Tillich (2007) used the term remote to describe hypogeal germination of palms (Arecaceae) which produce cotyledonary nodes that push the embryo below the seed into the soil to avoid bush fires associated with the arid ecological habitats.

The protrusion of the cotyledonary petiole through the testa marks the initiation of skotomorphogenic growth and it is followed by elongation of the petiole, bulging, shoot appearance and elongation, emergence above the soil and establishment (Figure 7). Although Jackson (1968) has described germination of *V. paradoxa* seed, he did not classify it into these distinct stages. Ugese et al. (2010), however, made a description similar to ours, although they considered shoot appearance and shoot elongation as a single stage event while seedling establishment was excluded. These multi-stages of germination account for the long period of seedling establishment in *V. paradoxa*, which prolongs nursery development.

A longitudinal section through the embryo of a fresh seed revealed latex exudate from the embryo whilst a transverse section showed latex oozing from the edges of the seed and the embryo surfaces suggesting the presence of laticiferous vessels. Also, a transverse section through the cotyledonary petiole showed an inner hollow tube surrounded by cylindrical bundles of laticiferous vessels running parallel to each other. Functionally, the sheath and the laticiferous bundles protect the plumule located in the cotyledonary petiole against drought and may also play an additional function of translocating food reserves from the seed to the developing rudimentary shoot as it develops in the petiole. The increased number of laticiferous vessels may facilitate rapid translocation of food reserves to developing shoot.

Seedling traits are evolutionary conserved reflecting phylogenetic relationships among species (Ibarra-Manríquez et al. 2001). Cryptogeal germination in *Vitellaria paradoxa* might have evolved to offer an ecological advantage. The elongating cotyledonary tube of *V. paradoxa* buries the plumules and radicles deep into the soil. Jackson (1968, 1974) has thus described this germination as plumule burying which is a characteristic
feature of pyrophytes to protect their developing shoots against both bush fires and prolonged drought. Additionally, pyrophytes bury their seed below the soil surface even after the emergence of the shoot and hence their germination is referred to as crypto-cotylar hypogeal.

**Influence of seed size on seedling development**

Seed germination was independent of the size, however, subsequent seedling development was significantly influenced by the size of seeds sown. Jøker (2000) has reported that fresh *V. paradoxa* seeds usually have a very high germination percentage but it decreases with loss of moisture, owing to recalcitrant nature of the seed. The high germination percentage could be attributed to the freshness of the seed sown. Sprouting of large seeds was significantly (P < 0.05) earlier (7 days) than medium (9 days) and small seeds (12 days). Subsequently, the cotyledonary petioles of large seeds elongated faster producing bulges quicker than those of medium and small seeds. According to Ugese et al. (2010), *V. paradoxa* seeds often sprout within 7 days when sown. The faster rate of germination of large seeds may be attributed to the presence of more food reserves in the seeds while delayed germination of small seeds may be attributed to little food reserves present.
Similarly, seedling emergence was also significantly earlier (61 days) in large seeds than in small seeds (75 days). Variation in seedling emergence in *V. paradoxa* seeds have been reported by different authors (Yidana 2004; Ugese et al. 2007; Asante et al. 2012). Whereas we observed an emergence range of 61–75 days, Ugese et al. (2010) reported 51–79 days emergence. Contrastingly, Yidana (2004) reported a very early emergence of 28 days after sowing of seeds. These varied stages of seedling development could be attributed to the time of sowing after harvesting the seeds, season of harvest or seed pre-treatment before sowing. Shoots developing from large seeds took almost four months to be established while small and medium sized seeds took three months. Seed size is an important trait that affects germination time, percentage and seedling establishment (Murali 1997; Mäkken et al. 2005; Yanlong et al. 2007). Large seeds have more nutritional reserves than smaller seeds (Primack 1987), which increases the probability of successful seedling establishment in habitats with limited resources (Geritz 1995).

**Conclusion**

The location of the embryonic axis in *V. paradoxa* seed was accurately identified at proximal end using TTZ test, but the axes is not well differentiated into plumule and radicle. The cotyledons of the seed are fused at the proximal end but are free at the distal end. The proximal syncoytly makes the seeds exhibit cryptogal germination by producing a long cotyledonal petiole which buries the embryo deep into the soil, where it is protected from bush fires which is a characteristic of its habitat. Thus, the plumule develops into shoots from below the ground. Seed size has a significant effect on emergence, emergence rate index and subsequent seedling development. The identification of the exact location of the embryo and its germination will be useful in the propagation of the shea tree under both in vivo and in vitro conditions for nursery establishment and domestication of the tree.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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