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Study of Germination and Seedling Growth of *Piper guineense*

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Abstract

Natural regeneration of black pepper (*Piper guineense* Schumach and Thonn) through seeds is poor and cultivation by local farmers is very limited since it is usually collected from the wild. High demand has put a pressure on its population in our declining forests and a call for domestication of non-timber forest plant species is ongoing. This study identified germination requirements, germination characteristics and seedling growth of *Piper guineense* with a view to providing information on its propagation from seeds. There were two major pre-sowing treatments - Fresh or dry fruits or seeds. Germination and seedling growth parameters were measured for all treatments. Results showed that *Piper guineense* seeds undergo epigeal germination, the radicle developing into three-legged hairy rootlets. The highest percentage germination, germination rate and germination index were recorded in the treatment with fresh seeds planted in petri dish. The dry seeds and fruits recorded no germination even after 38 days of planting. For seedling growth, the developing leaves have a higher sink strength between the fourth and fifth month of development and this stage may be crucial to the growth of the seedling. *Piper guineense* seeds must be sown fresh for germination to take place.

Keywords: Ashanti pepper, deforestation, non-timber forest plant species, propagation, recalcitrant seeds

Introduction

The increasing loss and degradation of native forests is now accepted as a global environmental crisis. Primary forests have decreased by an estimated 47 million ha globally since 2000 (FAO, 2022), with forest area decreasing, particularly in the tropics; the highest net losses in 2010–2020 were reported to be in South America and Africa. Protection of biodiversity is essential in the fight to achieve sustainable development, therefore, during afforestation or reforestation, there is a need to consider all forest species for conservation measures to be holistic since there are other inhabitants of the forests apart from trees. FAO (2022) estimated that about 3.5 billion to 5.76 billion people use non-timber forest products. One of the non-tree forest plant species that have great economic potentials due to their medicinal and nutritional values is *Piper guineense* (Schumach and Thonn) whose origins is from tropical regions of Central and West Africa. In West Africa, it is called Ashanti pepper or African black pepper (Okunade et al., 2019). It is known in Nigeria as 'Uziza' in Igbo and 'Iyere' in Yoruba. Other common names are Benin pepper, false cubeb. *Piper guineense* leaves (Figure 1A) are used as vegetable while the fruits (Figure 1B) are used as spice in preparation of local dishes in many communities in Nigeria. Traditionally, the leaves and seeds of *Piper guineense* are important ingredients in the preparation of special soups for post-partum women in Igbo communities (Okoye and Ebeledike, 2013), as they claim that it encourages or stimulates uterine contractions, therefore aiding the return of uterine muscle to the original shape. In Yoruba communities in Nigeria, the dried fruits are used in the preparation of herbal drug and ashes of burnt plants are used as salt substitute for medicinal preparations. It has been reported to have antimicrobial properties (Ojimelukwe, 2023), Aphrodisiac, food preservatives and antioxidant properties (Okunade et al., 2019), antiparasitic agent (Martins et al., 2021), insecticide (Adetunji et al., 2020; Hasan et al., 2020) among other uses.

Piper guineense is in high demand in many communities in Nigeria and the society depends on the forest for its supply. However, since it is collected in the wild, increase in population and urbanization has brought about increase in deforestation and therefore decimation of populations of the species. There is an urgent need to carry out conservation studies to ensure survival and continuity of the species. Though listed as 'Least Concern', IUCN (2023) declared that it is subject to *ex-situ* conservation. It has been reported that the seeds fail to germinate (Udosen and Sam,

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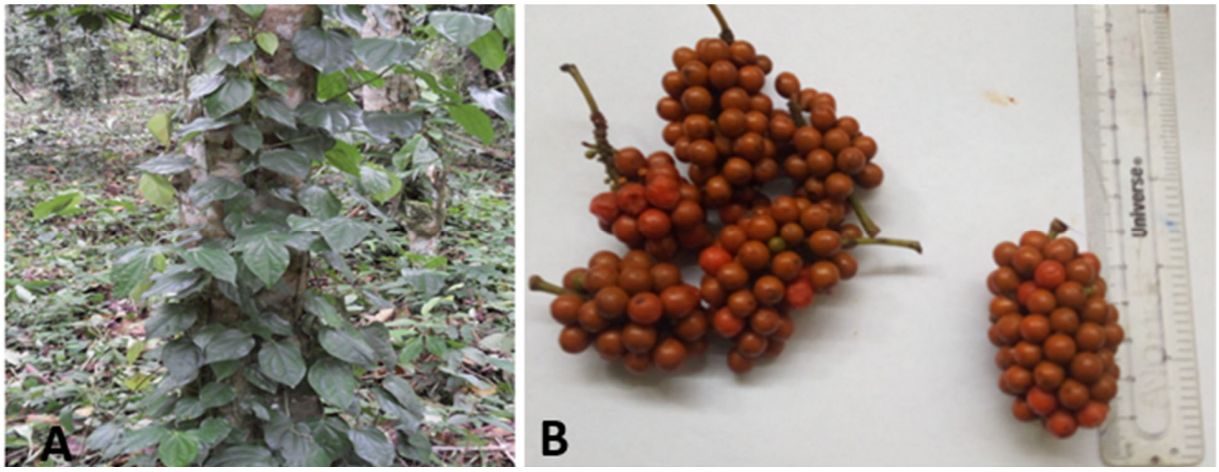

Figure 1. *Piper guineense* in its natural habitat (A) and the ripe fruits (B)

2015) and that viable seeds are not readily available (Alaje et al., 2022). Cultivation by local farmers is very limited because its growth requirements are yet to be understood as people still lack knowledge of mode of regeneration and proper management of *Piper guineense* plant (Udofia et al., 2016). There exists a lot of contradictions from the locals regarding what they think should be the growth requirements, part of plant to be used and the methods of cultivation of *Piper guineense*, while some simply believe that it cannot be propagated. Besides these, natural regeneration through seeds is very poor.

Several studies have been carried out on the phytochemical, nutritional and pharmacological properties of the plant but not much has been reported on its propagation. There is a need to determine and report more information regarding the propagation of this very important plant. This is one of the ways to ensure that the plant is conserved and its uses to mankind sustained. Therefore, the objective of this study was to identify germination requirements and characteristics of *Piper guineense* seeds and evaluate seedling growth.

Materials and Methods

The experiment was carried out in the Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. Fresh and ripe *Piper guineense* fruits were sourced from Akinola market, Ipetumodu, Osun State and authenticated at Ife Herbarium, Obafemi Awolowo University, Ile-Ife, Osun State. The seeds and fruits were subjected to six treatments categorized into two – fresh and dry.

Fresh: Forty fresh fruits were de-pulped, washed and surface sterilized with 10% commercial bleach (Sodium hypochlorite 3.5% m/v) for 5 minutes and rinsed twice with distilled water. Twenty seeds were planted in petri dish lined with filter paper and moistened with distilled water. The remaining twenty seeds were planted in a perforated plastic bowl filled with topsoil. Another forty fresh fruits from the same lot were not de-pulped. They were surface sterilized as stated above and used for the following experiments: Twenty fruits were planted in soil medium in a perforated bowl. The remaining 20 fresh fruits were planted in a petri dish lined with filter paper and moistened with distilled water. All experiments were triplicated.

Dry: Some fresh fruits were de-pulped and air-dried for 2 weeks while some were not de-pulped but air-dried for 4 weeks. These were used for dry seed and dry fruits germination tests, respectively. Dry seeds and fruits were surface sterilized with 10% commercial bleach (Sodium hypochlorite 3.5% m/v) for 5 minutes and rinsed twice with distilled water. Twenty dried seeds were planted in a petri dish lined with filter paper. Another 20 seeds were planted in perforated plastic bowl filled with topsoil. Twenty dried fruits were planted in a petri dish lined with filter paper. Another set of 20 dried fruits were planted in perforated plastic bowls filled with topsoil.

Germination measurements were conducted in the petri dishes. Seeds displaying radicle emergence greater than 1 mm were considered germinated. The emergence of plumule indicates seed germination in the plastic bowls with topsoil. Germination count was taken daily till there was no more germination. The following germination parameters were determined: number of days to first germination, number of days to 50% germination, mean germination, germination rate, germination index and percentage germination were calculated for all treatments.

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Mean germination $= \frac{\text{Total number of germinated seeds}}{\text{Number of replicates}}$

Germination rate (per day) = $\frac{Nt}{Tt}$ (Torres and Frutos, 1990). Where Nt is the total number of germinated seeds recorded for the interval time; Tt is the total time of counting (in days).

Germination index (%) = $\frac{GT}{DT}$x100

Where
GT = Number of seeds emerged after planting
DT = Day of emergence.
Germination index was determined at the 29th day after planting.

Percentage germination (%) = $\frac{\text{Total number of germinated seeds}}{\text{total number of seeds planted}}$ x 100

Information on type of germination and characteristics of germinating seeds were documented.

Seedling growth rate was conducted three weeks after germination. The seedlings were transplanted into big planting bags (50 cm in diameter and 29.5 cm in depth) filled with topsoil, 2 seedlings per bag and moved to the screen house. The seedlings were allowed three weeks to establish, and growth parameters were measured. Thereafter, the following growth parameters were taken every four weeks for a period of 24 weeks.

The shoot height of the seedlings was measured in cm using thread and meter rule from the distance of the soil level to the tip of the terminal bud of the plant. Measurements were taken in ten replicates and mean value recorded.

The number of leaves per plant was counted manually and recorded.
Leaf area: The leaf area of all the leaves on the plant were calculated using modified Hoyt and Bradfield (1962) formula as follows:

Leaf area =L x W x C.F

Where
L = represents leaf length measured as the distance between the base of the leaf and the leaf apex using meter rule.
W = represent the width of the leaf measured as the most expanded portion of the leaf using meter rule.
C.F. = Correction Factor = 0.67

The correction factor was determined as follows; twenty leaves were taken randomly from a few stands of *Piper guineense* plants, and their area measured using graph paper glued to a glass plate. The leaf area outline was marked, and the boundary was determined by counting the squares to the nearest 1 mm. The length (L) and width (W) of each of the 20 leaves were also measured. The ratio of the leaf area determined by using graph to that obtained by L x W method was calculated for the 20 leaves and the correction factor obtained.

Results

Seed Germination Characteristics

Piper guineense seeds undergo epigeal germination as they push their cotyledon above the ground with the hypocotyl elongating (Figure 3A). The radicle developed into three (3) hairy rootlets that are whitish in color (Figure 3B). The seedlings develop standing on these rootlets and carrying the cotyledon up as shown in Figure 3C.

Seed Germination as Influenced by Pre-sowing Treatments

The different treatments carried out on *Piper guineense* seeds had a significant effect on the

Figure 2. Germinating *Piper guineense* seeds in a petri dish (A), a germinated seed with hairy roots (B) and developing seedlings (C).

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germination parameters of the seeds after thirty-eight days. De-pulped fresh fruits (fresh seeds) planted in petri dishes germinated before fresh fruits planted in petri dishes while fresh seeds planted in soil also germinated before fresh fruits planted in soil. Dried seeds planted in petri dishes, dried fruits planted in petri dishes, dried seeds planted in soil and dried fruits planted in soil, recorded no germination as at thirty- eight (38) days after planting (Table 1). The maximum mean germination was recorded with fresh seeds (de-pulped) planted in petri dishes. As seen in Table 2, no significant difference ($P < 0.05$) was observed with mean germination of fresh seeds (De-pulped fresh fruits) planted in petri dishes, fresh fruits (fresh fruits with pulp intact) planted on petri dishes and fresh seeds planted in soil after 28 days but, they were all significantly different from fresh fruits on soil (Table 1).

Apart from dried seeds planted in petri dishes, dried fruits planted in petri dishes, dried seeds planted in soil and dried fruits planted in soil which recorded zero percentage germination, fresh fruits planted on soil recorded lowest percentage germination. Maximum percentage germination in fresh seeds (De-pulped fresh fruits) planted in petri dishes was significantly higher than other treatments (Table 2).

Seedling Growth Rate Measurement

Piper guineense plants are climbers, so the seedling strikes adventitious roots at the nodes as it grows (Figure 3A). After three months in the screen house, the young plants were taken out and staked under tree shades (Figure 3B).

The mean shoot height of *P. guineense* was measured for six months starting when the plants were six weeks old. At the first month (September; six weeks old), it was observed to be 3.5 cm ±0.25, at six months (February), when the plants were 26 weeks old, the mean shoot height was recorded to be 188.5 cm ± 5.53. The monthly increase in shoot height was not uniform. The highest change in shoot height recorded in the entire six (6) months was observed in the 18th week of growth (fourth month) and the 22nd week of growth (fifth month). The lowest increase in shoot height was observed in the second month (10th week of growth) as shown in Figure 4A.

A similar trend was recorded in the number of leaves produced by *P. guineense* as reported in shoot height. The highest change was recorded in the fifth month (22nd week of growth) (Figure 4B). *P. guineense* seedlings started producing branches after the third month (14th week of growth). The increase in number

Table 1. Number of days to germination of *Piper guineense* seeds as influenced by different pre sowing treatments.

Treatments	Days to germination	Days to 50% germination
Fresh seeds (depulped) in petri dish	9	13
Fresh fruits in petri dish	21	25
Fresh seeds in soil	18	25
Fresh fruits in soil	25	30
Dried seeds in petri dish	-	-
Dried fruits in petri dish	-	-
Dried seeds in soil	-	-
Dried fruits in soil	-	-

Table 2. Germination characteristics of *Piper guineense* subjected to different pre-sowing treatments.

Treatments (%)	Germination rate (day $^{-1}$)	Germination index (%)	Percentage germination
Fresh seeds (depulped) in petri dish	15.13±1.43^{a}	60.92±0.09^{a}	88.35^{a}
Fresh fruits in petri dish	8.13 ±0.25^{b}	58.62±0.03^{b}	85.00^{b}
Fresh seeds in soil	8.13±0.71^{b}	58.62±0.03^{b}	85.00^{b}
Fresh fruits in soil	4.40±0.62^{c}	44.83±0.09^{c}	60.95^{c}
Dried seeds in petri dish	0.00±0.00^{d}	0.00±0.00^{d}	0.00^{d}
Dried fruits in petri dish	0.00±0.00^{d}	0.00±0.00^{d}	0.00^{d}

Note: Means with the same letter in a column are not significantly different at $p<0.05$

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Figure 3. Young *Piper guineense* plant showing adventitious roots at the node (A) and a mature plant staked under a tree (B)

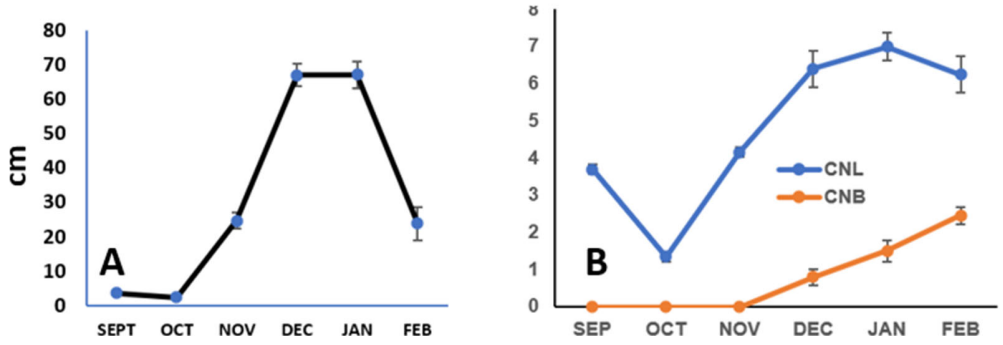

Figure 4. Shoot height (cm) (A) and number of leaves and branches of *Piper guineense* seedlings (B) for a period of 6 months after transplanting. CNL: changes in the number of leaves; CNB: number of branches

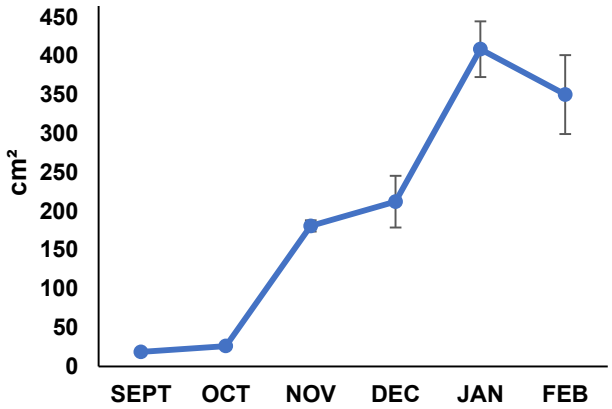

Figure 5. Changes in the the total leaf area of area of *Piper guineense* seedlings for a period of 6 months after transplanting

of branches from the fourth to the fifth month was significantly progressive (Figure 4B).

For the leaf area, a significant difference was observed in the increase in leaf area between the second month and third month, however, the highest increase in the change in leaf area was observed between the fourth and the fifth month (18th to 22nd week of growth) as shown in Figure 5.

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Discussion

Epigeal germination is exhibited by some dicotyledonous plants and a few monocots (Parolin et al., 2003). It has been reported that *Piper* plants are neither monocot nor eudicot (true dicotyledon) (Ravindran 2000, Haggerty, 2011). Germination of *Piper guineense* seed was observed to be epigeal as the cotyledon was pushed above the ground with elongation of the hypocotyl. The radicle developed into three-legged hairy rootlets which is nothing like the single strand radicle of *Piper nigrum* seedling described in the work of Ramakrishnan and Dutta, (2007). *Piper guineense* seedlings developed standing on these rootlets and carrying the cotyledon up, with the testa (seed coat) still in place. The cotyledon developed into two leaflets still retaining the testa in a cap-like manner, this observation is also seen in *Piper nigrum* (Ramakrishnan and Dutta, 2007). As the two young leaves increase in size, the testa held them together at the tip like a peg but was finally dropped when the leaves unfolded.

Zero percentage germination observed in all the dried tested seeds indicates that *Piper guineense* seeds are likely to be recalcitrant. This could have been what happened with the seeds used by Udosen and Sam (2015), who reported no germination for *Piper guineense* even after several pre-sowing treatment were employed. Recalcitrant seeds do not survive drying and freezing during *ex situ* conservation, these seeds die rapidly and need water to survive (Tchokponhoué et al., 2019). Okafor (1997) de-pulped and air-dried the seeds and reported 42.6% germination compared to 85% germination from fresh seeds observed in this study. Their results could have been due to air drying the seeds which could have brought about loss of viability since moisture is a factor to be reckoned with in maintaining the viability of seeds that are recalcitrant (Sakpere et al., 2016). Drying rate could also be a factor since most recalcitrant seeds from the tropics can tolerate only a small amount of water loss when slowly dehydrated before potentially lethal damage occurs (Bharuth et al., 2020). There is a need to investigate the minimal moisture content required for germination of *P. guineense* seeds. However, it is evident from this study that sowing *P. guineense* seeds while fresh is crucial to achieving good germination.

The significant difference observed in the percentage germination of fruits that were not de- pulped (fresh fruits) before sowing in soil (61%) when compared with the de-pulped ones (fresh seeds) sown in soil (85 %) showed that the fleshy part of *P. guineense* fruits has some inhibitory effects on the germination of the seeds. This effect can also be seen in the germination rates and the number of days it took each seed lot to germinate, as the seeds sown both in petri dish and in soil germinated earlier (9 day and 18 days, respectively) compared to the fruits that were not de-pulped (21 days and 25 days, respectively). The percentage germination of fruits that were not de-pulped (fresh fruits) before sowing in petri dishes (85%) when compared with those de-pulped (fresh seeds) before sowing in petri dishes (88%) was significantly lower (3.4%). However, there was a 24% decrease in the percentage germination of fruits that were not de-pulped (fresh fruits) before sowing in soil (61%) when compared with those de-pulped (fresh seeds) before sowing in soil (85%). This suggests that there may be additional factors influencing the germination of fresh fruits (not de-pulped) germinated in soil apart from the pulpy mesocarp. These factors, which include soil microorganisms, pathogens and soil moisture among others, likely contribute to the differences in the percentage germination observed. From this study, de-pulping is one of the efficient pre-sowing treatments for obtaining maximum seed germination from *P. guineense*.

With regards to seedling growth, a sharp increase in leaf area was observed between the 18th week of growth (December) and the 22nd week of growth (January). At the same period, a decline in the rate of increase in shoot height and leaf number was observed (December to January). These results indicate that the developing leaves have a higher sink strength at this stage of development and this stage may be crucial to the growth of the seedling. Leaf area growth has been reported to determine light interception which is an important parameter to determine plant productivity (Mahakosee et al., 2022). Information on seedling growth is important in the light of the fact that studies on growth and development makeup the basic criteria needed to evaluate the success of forest re-establishment effort (Höhl et al., 2020). According to Löf et al. (2019), a deeper understanding of limiting factors that affect plant establishment will facilitate nursery and site preparation systems to overcome inherent restoration challenges. Most reforestation efforts center on timber trees and has led to the promotion of domestication of non-tree forest plant species. Evariste and Bernard-Aloys (2016) opined that optimizing production through domestication of Non-Timber Forest Products should be considered as a priority intervention.

Conclusion

This study has confirmed that *Piper guineense* seeds are recalcitrant and must therefore be fresh for germination to take place and that de-pulped fruits

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germinate best. *Piper guineense* seeds undergo epigeal germination which takes place between nine (9) and eighteen (18) days after sowing, the radicle developing into an unusual three-legged hairy rootlet. The seedlings can grow up to 188.47 cm in length with a leaf area of 1197 cm^2, 28.85 number of leaves and 4.8 number of branches within six (6) months. The 18^{th} week to the 22^{nd} week of growth are critical in the seedling growth and development of *Piper guineense*. This information will be important for reforestation and afforestation programs which include non-tree forest plant species and for the domestication of the species which is becoming a priority. To ensure success of plant establishment, fresh de-pulped fruits should be planted, and growing conditions should be optimal between the 18^{th} week to the 22^{nd} week of growth.

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