

Full Length Research Paper

Regeneration studies on *Pentaclethra macrophylla* Bth

J. E. Ehiagbonare^{1*} and H. I. Onyibe²

¹Department of Biological Sciences, Igbinedion University, Okada, Edo State, Nigeria.

²Department of Botany, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

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To provide a basis for sustainable management of a multipurpose tropical plant – *Pentaclethra macrophylla*, a study was carried out to determine the optimum method(s) of seed germination and seedling growth. Of all the methods evaluated in the seed germination studies the optimum method was soaking seeds in tap water for 10 days with daily replacement of water. This yielded mean value of 86.30% germination at 4 weeks while the control had 10.00% germination for the same period. A comparative method was soaking seeds in coconut milk which yielded mean value of 60% seed germination at 42 days after sowing in light condition and 30% germination in dark. The control had 20% germination in light and 10% germination in dark. The stem cuttings treated with 2% IBA rooted and mean value of 50% rooting was observed while the untreated cuttings had 35% rooting. The optimum growth medium was 1 part sand to 5 parts topsoil rich in organic manure. At 12 weeks the mean value of seedlings growth in 1 part sand to 5 parts topsoil had a total height of 36.91 cm, number of leaves 25.05, root length 10.01 cm and shoot length 26.0 cm. There was no seedling abnormality. The study concluded that *P. mycrophylla* can be regenerated thus providing basis for its sustainable management.

Key words: *Pentaclethra mycrophylla*, seed germination, seedling growth, growth media, sustainable forest management.

INTRODUCTION

Pentaclethra macrophylla Bth, is a tropical tree with many uses. It is dehiscent and its habitat is usually the forest and border regions of Savanna and forest. The seeds are shown in plate 1. The tree is generally called the oil bean tree. The local names are – in Yoruba – “apara apagha”, in Urhobo – “Okpaghan, in Bini – “Okpagha”, in Ibo – “Ugba”, and in Esan – “Ugbe” (Gill, 1992). As reported by Gill, (1992) the parts used for medicinal purposes are leaves, bark and root. The main phytochemical constituents are saponius, tannis and paucine. The leaves are boiled with *Piper guineensis* and a wine-glassful given twice daily for the treatment of fever, stomach – ache, healing lotion for sores e.t.c.

The seeds are of great delicacy in Eastern Nigeria. It is prepared and eaten with stock fish. If domesticated, a large plantation of it can provide source of raw materials to support can food industry. The flowers produced by

this plant are attractive to bees. The taxon is recommended for planting to increase honey production (Paul, 2007). The plant when burnt gives off unpleasant smell, good charcoal and the ash from the seed pods is used as cooking salt. The seed has up to 30-36% oil rich in prote, suitable for soap and candle making.

The species usually grows in an elevation of 0 - 500 m in a forest, or well drained soils and rainfall of about 1500 - 2700 mm. It is distributed usually in West Africa from Angola to Senegal. The flowering period is January to May. The flower is yellow or pinkish white, sweet smelling, 7 - 20 cm long. The fruits (pods) are very persistent, 35 - 45 cm long, hard and woody, splitting open explosively and curling up, containing 8 flat glossy brown seeds up to 7 cm long. The tree grows up to 21 m high and a girth of 6 m, branching low down and forming spreading crown. It is usually crooked with wide buttresses. It is greyish to dark reddish brown, flaking off in irregular patches, slash is reddish orange.

The contributions of wild fruits, nuts, seeds, and other class of edible products to local diet in developing count-

*Corresponding author. E-Mail: drehiagbonare@yahoo.com.



Plate 1. The seeds of *Pentaclethra macrophylla*.

ries and their potentials in overcoming or ameliorating prevailing food problems are enormous (Getahun, 1974; Okafor, 1980a, 1980b, Okigbo; 1997). In many cases there is no clear cut between food and medicinal plants. Many edible plants from the forest are also used in traditional medicine, e.g. *Pterocarpus* spp, *Vitex doniana*, *Garcinia kola* which are reputed as poison antidote are also used in the treatment of coughs and hepatitis (Iwu, 1987).

This plant has the potential of being a continuous source of supply of raw material to support charcoal Industry, can food industry, phytomedicine industry, vitamin supplement etc. This great plant of value has not been domesticated it is still growing in the wild. If this trend continues the values of the plant would have been lost with the current rate of deforestation in the tropics, wild forest fires, uncontrolled forest exploitation etc. The only possible way to save this important endemic tropical forest tree species from possible extinction is to domesticate the taxon. This research was undertaken to provide information on its domestication and basis for its sustainable management. To quicken the process of seedlings production, raising seedlings from seed and cuttings was investigated.

MATERIALS AND METHODS

Plant materials

The seeds were obtained from the mother trees. These were some of the seeds released by matured fruits when they split open. Seeds without physical deformity were gathered into a basket and taken to the university laboratory at the Igbinedion University, Okada, Edo State, Nigeria. Cuttings of 15 cm were prepared from young stems and carried in black poly bags. Black poly bags were used to avoid drying and possible death of the cuttings. These were for seedling production experiment (rooting stem cuttings). Forest topsoil rich in humus and sand were collected from the ecological zone of the plant under study in wheel barrows and deposited in a temporal tree nursery in the university compound at Okada. Black polythene bags with drainage holes were purchased locally.

Two watering cans locally made with fine outlets were purchased locally. The incubators used are the ones in the university laboratory in Okada. Suitable containers into which seeds were steeped

for soaking in water and acids were locally obtained. Sulphuric acid (H_2SO_4) was obtained from the university laboratory at Okada. Coconut from which milk was obtained was purchased locally

Seed viability tests

Floatation method was used. Seeds were steeped into a suitable container containing enough water. The water was stirred with a suitable stick and allowed to settle for a few minutes. The seeds that sank were regarded as viable and the ones that floated as non viable and they were discarded. From the viable seeds samples were taken for germination experiments.

Effect of temperature on seed germination of *P. macrophylla*

Seeds were incubated for 30 ± 2 , 35 and $40^\circ C$ for a duration of 1 h each. After each incubation period, seeds were washed in tap water and allowed to cool down over night before putting out for germination. Fourteen seeds were sown in poly pots filled with only topsoil and replicated three times. Untreated seeds were also sown in poly pots filled with only topsoil and replicated three times. All the poly pots were arranged in randomized block designed under light shade of palm leaves. The pots were watered to field capacity. Subsequent watering was done morning and evening as necessary. Germination was monitored for 30 days. Mean percent germination was recorded. And other set of the same experiment was kept under dark shade. Germination was also monitored for 30 days. Mean percentage germination for the period of observation was recorded.

Effect of temperature on radicle length during seed germination of *P. macrophylla*

The method described by Truong and Hans (2007) was used.

Effect of soaking *P. macrophylla* seeds in tap water on germination

The effect of 10 days soaking regime with daily replacement of water was evaluated in this part of the study. Treated seeds (from soaking) were sown in top soil in poly pots and replicated three times. The control was not soaked. Germination was monitored on weekly basis for 4 weeks.

Effects of sulphuric acid (H_2SO_4) and coconut milk on seed germination of *P. macrophylla*

The method described by Ehiagbonare (2004) was used. The seeds were steeped into a suitable container and removed after 1, 3, 5 min respectively under four treatment regimes-absolute 90, 70, 50 concentrations of sulphuric acid (H_2SO_4). The control was not treated with acid.

Effect of coconut milk on germination was evaluated. Seeds were soaked in coconut milk for 2 h before sowing. Germination was monitored weekly for 6 weeks.

Rooting stem cuttings

Ten stem cuttings were inserted into black polythene bags filled with top soil. These first sets of cuttings were treated with growth hormone. The polythene bags were replicated three times. The stem cuttings had one end dipped into 2% IBA (indole butyric acid) for 5 min. The poly pots were arranged in complete randomized

Table 1. Effect of temperature regime on seed germination of *Pentacletra macrophylla*.

| Temperature (°C) | Incubation period | Mean germination (%) | |
|------------------|-------------------|----------------------|------------------|
| | | Under light shade | Under dark shade |
| 30 | 1 h | 43.30* | 35.0 |
| 35 | 1 h | 63.30 | 36.0 |
| 40 | 1 h | 30.10 | 22.0 |

*Observation recorded after 30 days after sowing.

Table 2. Effect of temperature on seed radicle elongation during germination of *Pentacletra macrophylla*.

| Temperature (°C) | Growth of radicle (cm) | |
|------------------|------------------------|----------|
| | Light | Dark |
| 22 | 12.2±0.10* | 2.0±0.20 |
| 30 | 18.3±0.30 | 2.1±0.10 |
| 37 | 14.2±0.10 | 2.1±0.11 |

*Observation recorded after 30 days after sowing block design.

Watering was done as necessary. Rooting was monitored every seven days for a period of 30 days. A second was set up in above but the cuttings were not treated and thus served as the control.

Growth media and seedling growth evaluation

The effect of growth media was evaluated in this part of the study. The sand and the topsoil collected were sieved to remove coarse sand, rock/stone particles and roots. After sieving they were mixed in the ratio of sand to topsoil as follows: 1:1, 1:2, 1:3, 1:4 and 1:5. These were used to fill polythene bags and replicated three times each and arranged in a complete randomized block design under light shade of palm leaves. Watering was done with the watering can as necessary but avoiding over flooding to prevent plant pathogens. Three seedlings at two leaves stage were transplanted into each polythene bag. Nursery phyto sanitation was done regularly manually by using hand to weed round and within the polythene bags. Growth was assessed after 12 weeks in terms of total plant height, number of leaves, root length, stem length and normality of the structures.

Statistical analysis

Where necessary ANOVA was used to analyse results.

RESULTS AND DISCUSSION

Viability

From the flotation method used, 90% of the seed used were found viable. Observation of the non viable 10% showed that the seeds were smaller in size than others and some have perforations. These probably could be due to immaturity and insect attack.

Effect of temperature on seed germination

The result is presented in Table 1. Temperature was observed to have significantly affected seed germination in light condition. Seeds incubated for a period of 1 h in 35°C had the highest mean germination of 63.30% in light and 36.00% in the dark. The implication of this is that the species under study is photoblastic and the seedlings will grow better and healthier in light condition than in dark condition. The values obtained from seeds incubated under 35°C for 1 h were higher than others.

Effect of temperature on seed radicle length

The result obtained from this experiment is presented in Table 2. The result showed that significant differences exist in effect of the various temperatures used. Temperature of 30°C had optimum influence on radicle length (cm). It resulted in 18.3 ± 0.30 cm. This value was also higher than others (Table 2).

Effect of soaking seeds in tap water on germination

The result of this is presented in Table 3. Evidence from the result showed that significant differences exist in the effect of the soaking regime used. Optimum value of 86.30% germination was obtained from soaking durations of 10 days with daily replacement of water. Other values were lower than this value for 10 day. The result is so because the seed coat is hard and it probably took longer days to imbibe water. The enhanced imbibitions process culminates in the seed coat rupturing and thereafter the emergence of the radicle. This probably could be due to the fact that the species is from rain forest ecological zone and may have been adopted to wet condition.

Effect of sulphuric acid (H₂SO₄)

The result of the effect of sulphuric acid on seed germination is presented in Table 4. Although the result obtained here is better than the control; however, the values are lower than that obtained from the soaking in water regime. Sulphuric acid does not seem to adequately enhance seed germination of *P. macrophylla*. Nevertheless, germination increased with increased soaking

Table 3. Effect of soaking in water on the seed germination of *Pentacletra macrophylla*.

| Soaking duration (days) | Mean germination (%) after | | | |
|-------------------------|----------------------------|---------|---------|---------|
| | 1 week | 2 weeks | 3 weeks | 4 weeks |
| 1 | - | 10.00 | 20.00 | 23.30 |
| 2 | - | 10.00 | 20.00 | 23.30 |
| 3 | - | 10.00 | 20.00 | 23.30 |
| 4 | - | 20.00 | 28.00 | 33.33 |
| 5 | - | 23.00 | 23.00 | 33.33 |
| 6 | - | 23.00 | 33.30 | 43.30 |
| 7 | - | 33.30 | 33.30 | 43.30 |
| 8 | - | 43.30 | 53.30 | 66.60 |
| 9 | - | 50.00 | 60.60 | 76.66 |
| 10 | 30.00 | 33.30 | 66.60 | 86.30 |

Table 4. Response to germination of *Pentaclethra macrophylla* seeds treated with various concentrations of sulphuric acid (H₂SO₄).

| H ₂ SO ₄ | Treatment durations (min) | Mean germination (%) |
|--------------------------------|---------------------------|----------------------|
| Absolute | 1 | 11.1±0.10 |
| | 3 | 20.0±0.23 |
| | 5 | 33.0±0.25 |
| 90% | 1 | 16.0±0.22 |
| | 3 | 21.0±0.23 |
| | 5 | 35.0±0.42 |
| 70% | 1 | 15.0±0.13 |
| | 3 | 11.2±0.26 |
| | 5 | 16.1±0.21 |
| 50% | 1 | 6.2±0.06 |
| | 3 | 15.0±0.45 |
| | 5 | 20.0±0.23 |
| Control | | 7.14±0.12 |

duration in all concentrations. Muhammad and Amusa (2003) reported a similar trend when the seeds of *Tamarindus indica* L were treated with various concentration of H₂SO₄.

Effect of coconut milk on seed germination

The result of the effect of coconut milk on seed germination is presented in Figure 2. The result of this experiment showed that the seeds of the species under study responded well to growth hormone (coconut contains IAA) under light condition. From Figure 2, treated seeds yielded 60% mean germination in light condition and 30% in dark, while 30% was obtained in light and 20% in dark for the control. This result of this experiment further confirms that the species under study is photoblastic. The result is therefore indicative that quality seedlings can be grown in light condition.

Effect of 2% IAA (indole acetic acid) on root stem cuttings

This result is presented in Figure 1. 50% stem rooting was obtained from the treatment while 35% was observed in the control. The ability of growth hormones to root cuttings has been established in this study and the result of the study is thus in consonance with the findings of Al-Rawi (1976), Davis et al. (1982) and Rathore et al. (1975).

Effect of growth media on seedling growth

The result of this experiment is presented in Table 5. The result in Table 5 showed that the less the proportion of sand in the mixture of growth medium the better the plants should be produced to meet domestication requirements. The seedlings did not manifest any abnormality.

The result of this study is in agreement with our pre-

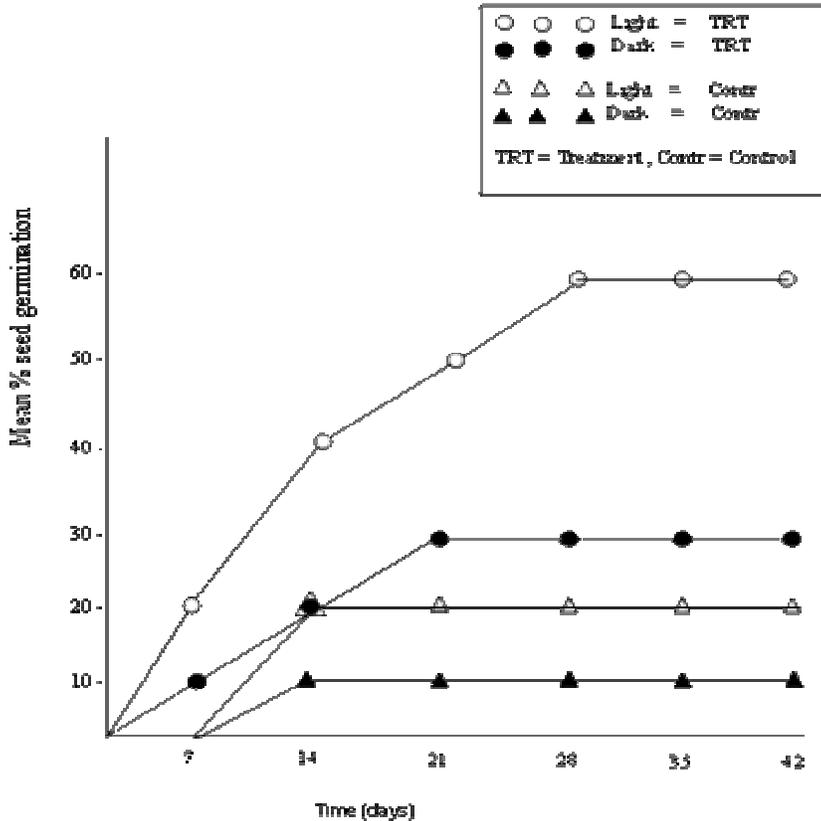


Figure 1. Effect of coconut milk on the seed germination of *Pentaclethra macrophylla*

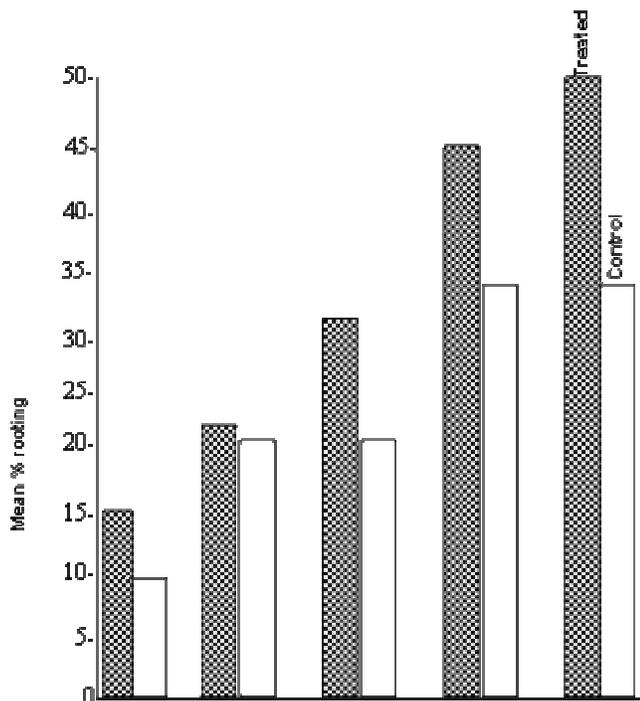


Figure 2. Effect of growth hormone (2% IBA) on seedling of *Pentaclethra macrophylla*.

growth. A mixture of 1:5 yielded the best result in the study while a mixture of 1:1 had the poorest values (Table 5). Tropical seedlings are slow growing and hence enhanced growth medium is indispensable if quality seed-study (Ehiagbonare, 2005) optimum seedling growth of *Tamarindus indica* in forest top soil rich in humus was observed. The seedlings from seed germination and rooted stem cuttings did not manifest any form of abnormalities in this study temperature observed in the study was 35°C for 1 h which resulted in 63 and 36% germination in light and dark (Table 1). This result is at variance with that of Truong and Hans (2007) where he reported that the seeds of *sesbania* did not germinate in temperatures of 45 and 130°C, respectively. The possible conclusion here is that species are temperature specific. While seeds of *P. macrophylla* germinated in light, Lopez and Sanchez (1989) noted that *Sesbania* seeds germinated without light. Cisneros and Zedler (2001) and Raven et al. (1999) reported that light may inhibit seed germination in certain cases; they reported the germination of *Phalaris aurundinacea* seeds to do without light. The length of the radicle was observed to respond positively to light and temperature (Table 2). This is against the observation of Taiz and Zeiger (1998). To obtain good and healthy seedlings of *P. macrophylla*, they should be produced under light condition. *P. macrophylla* can be regenerated,

Table 5. Effect of growth media (mixture of sand and top soil) on seedling growth, 12 weeks after transplanting.

| Ratio of sand/topsoil | Total plant height (cm) | Number of leaves | Root length (cm) | Stem length (cm) | Abnormality of structures |
|-----------------------|-------------------------|------------------|------------------|------------------|---------------------------|
| 1:1 | 4.80 | 7.00 | 1.50 | 2.50 | - |
| 1:2 | 4.01 | 7.00 | 2.05 | 2.05 | - |
| 1:3 | 6.00 | 15.00 | 1.50 | 4.50 | - |
| 1:4 | 25.02 | 18.00 | 8.02 | 17.00 | - |
| 1:5 | 36.01 | 25.05 | 10.01 | 26.00 | - |

thus providing basis for its sustainable management and conservation.

REFERENCES

- Al-Rawi Ak (1976). Influence of IBA on rooting of soft wood cuttings of *Olea europea* Cr. Ashrosy, Mesopotamia J. Agric. 11: 37-43.
- Cisneros RT, Zedler J (2001). Effect of light on seed germination in *Phalaris arundinaceae* L. plant Ecol. 155: 75-78.
- Davis FT, Lazarire Jr, Joiner JN (1982). Initiation and development of roots in juvenile and mature leaf cuttings of *Ficus pumila* L. Am. J. Bot. 69: 804-811.
- Ehiagbonare JE (2004). Study of the germination of *Delonix regia* (BOJER EX. HOOK) RAF seeds in Nigeria. J. Nat. Adv. Sci. 3: 123-126.
- Ehiagbonare JE (2005). A study of vegetative propagation of *Tamarindus indica* L. Nig. J. Appl. Sci. 23: 84-87.
- Getahun A (1974). The role of wild plants in the native diet in Ethiopia. Agro-ecosystem, 1: 45-46.
- Gill LS (1992). Ethnomedical uses of plants in Nigeria. University of Benin, Benin City, Edo State, Nigeria. pp. 184-185.
- Iwu MM (1987). Evaluation of the antihepatotoxic activity of biflavonoides of *Gacinia kola* seed. J. Ethno-pharmacol., p. 21.
- Lopez G, Sanchez P (1989). Germinacion de dos variedades de pitaya *Stenocereus griseus* (Haworth) Buxbaum. Cactaeas Suculentas. Mexican. 34: 35-40.
- Muhammad S, Amusa NA (2003). Effect of sulphuric acid and hot water treatments on seed germination of tamarind (*Tamarindus indica* L) Afr. J. Biotechnol. 2(9): 276-279.
- Okafor JC (1980a). Trees for food and fodder in the Savanna areas of Nigeria. Tree crops J. 1: 10-12.
- Okafor JC (1980b). Edible indigenous woody plants in the rural economy of the Nigeria forest Zone. Forest Ecol. Manage., 3: 15-24.
- Okigbo BN (1997). Neglected plants of importance in traditional farming systems of tropical Africa. Acta Horticulturae, 53: 131-150.
- Paul L (2007). The oil bean tree: Agricultural value. Bees for Development Journal No. 84 September. pp. 1-4.
- Rathore HS, Singh SM, Chabra AD (1975). Effect of plant regulators and their concentrations on the performance of soft wood cutting of guava. Haryana Agric. University J. Res. 5(1): 46-147.
- Raven PH, Ever F, Eichhorin ES (1999). Biology of plants 6th Edition Freedman WH, Company Worth Publishers, USA. pp. 26-336.
- Taiz L, Zeiger E (1998). Plant physiology, 2nd Edition. Sinauer Association Ins. Publisher, Sunderland, Massachusetts. U.S.A.
- Truong HD, Hans B (2007). The influence of temperature, light, salinity and seed pre-treatment on the germination of *Sesbania sesban* seed. Afr. J. Biotechnol. 6 (19): 2231-2235.