REPRODUCTIVE PHENOLOGY AND PROPAGATION OF *Pycnanthus angolensis* (WELW.) WARB. IN SOUTHWESTERN NIGERIA

BY

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A Ph.D Thesis in the Department of Forest Production and Products Submitted to the Faculty of Renewable Natural Resources in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF IBADAN

MARCH, 2021.

CERTIFICATION

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DEDICATION

This work is dedicated to Almighty God (Giver of life) who led me to this academic height and my daughter Favour Atinuke Bello.

ACKNOWLEDGEMENTS

I wish to acknowlege my renowned supervisor Dr. Adejoke O. Akinyele for her constructive/criticisms and encouragement, during the course of study.
I am also grateful to my Head of Department, Prof. Y.K. Ogunsanwo, the Dean of the Faculty of Renewable Natural Resources Prof. E.K. Ajani and Dean of the Postgraduate School Prof. J.O. Babalola for their joint responsibilities as supporters of tranquility, honesty and sustainable human growth.

My gratitude goes to Prof. A. O. Akinrinde Head of Agronomy Department, Dr. Esther Uchendu, and Laboratory technologist, Mrs. Bola Oyelami for their assistance during the work. I also express my gratitude to members of my supervisory committee for their support.

I appreciate Dr. P.O. Ige, Mr. Adedoyin Bamidele and Ajisefinni Olajumoke for analyzing and editing this work for me, entire staff members of the Department of Forest Production and Products for their supports and assistance during this study.

I appreciate Prof. I.O. Oyewole, efforts of my siblings, Oyedokun Timothy and Alhaji Nafiu Usman.

The family of Dr. Niyi and Dr. Taiwo Ogunjobi, Prof. Ayo Aiyeloja and Mr. Durodola Kayode are recognized.
Special thank to Favour Olaitan Atinuke Bello and my parents Mr. & Mrs. J. B. Adetunji for for their strong support during the course of study.

ABSTRACT

Pycnanthus angolensis is one of the overexploited indigenous tree species used in phytomedicine. Various parts of the tree are harvested from wild populations for ethnobotanical purposes, thus threatening the sustainable utilisation of the species. However, information on phenology and propagation techniques pre-requisite for *ex-situ* conservation of this species is limited. Therefore, flowering and fruiting patterns, germplasm characteristics and *in vitro* propagation protocols of *Pycnanthus angolensis* were investigated.

Matured trees of *Pycnanthus angolensis* (20.0±5.0years) were purposively selected from Osun (Gbongan, Ajaba), Ekiti (Otun, Ayetoro) and Oyo (Idito, Adewumi) States, based on availability. Population frequency, onset and duration of flowering and fruiting (months) and period of fruit colour change (days) were monitored for 24 months. Fruit and seed morphology were determined by measuring weight (g), width (cm) and length (cm) of 100 samples per location. One hundred seeds per location were sown in the nursery to determine germination speed (day^{-1}), total germination and seedling survival. In a completely randomised design experiment, 10 uniformly growing seedlings were selected from each location and used to assess seedling height (cm), nodulation and Number of Leaves (NL) for four months. Four growth hormones: Naphthalene Acetic Acid (NAA), 6-Benzyl Amino Purine (BAP), Kinetin and Indole Butyric Acid (IBA), were used for *in vitro* propagation of embryo and nodes of seedlings following standard procedures. Shoot height (cm), Number of Nodes (NN), NL, plantlet survival, Shoot Formation (SF, %) and callus induction were assessed for four months. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05.}$

Population frequency was highest in Gbongan (10.5%) and least in Idito (1.8%). Flowering onset and duration were September-November and 5-8 months, respectively. Fruiting onset and duration were January-June and 8-10 months, respectively. Four stages of colour change: brown (30-150), green (30-105), greenish yellow (30-89) and yellow (90-202) were observed. Highest fruit weight (19.9±0.2), width (5.3±0.0) and length (4.2±0.0) were obtained from Ajaba, Otun and Gbongan, respectively. Ayetoro had the

least weight (12.5±0.2), while Idito had the least width (3.9±0.0) and length (2.7±0.1). Seed weight varied from 2.1±0.1 (Ayetoro) to 4.4±0.1 (Ajaba), while width increased from 1.4±0.1 (Ajaba) to 2.4±0.1 (Gbongan). Otun had highest seed length (3.4±0.0), while Idito had the least (2.9±0.0). Germination speed, total germination and seedling survival ranged from 27-49, 13-81% and 11-56%, respectively. Highest seedling height (22.9±1.4), nodulation (15.0±0.8) and NL (12.9±0.7) were from Gbongan; while the least (8.0±1.4, 5.0±0.8 and 2.6±0.7) were from Ayetoro. For *in vitro* propagation of embryos, highest Shoot height (2.1±1.0), NN (3.2±1.0), NL (2.4±0.8) were from Ayetoro, Ajaba and Gbongan, respectively. Idito had the least shoot height (1.6±1.0) while Adewumi had the least NN (2.3±1.0) and NL (1.4±0.8). Plantlet survival varied from 4-25%. Shoot formation (20%) were obtained for nodes exposed to 1.0-3.0 mg/L NAA+10mg/L IBA and 30% callus induction was observed for nodes treated with MS+1.0 mg/L NAA+10 mg/L IBA.

Pycnanthus angolensis exhibited extensive flowering and fruiting period. Germplasm characteristics influenced seedling growth. Embryo and nodal culture for *in vitro* propagation were feasible using MS+1.0mg/L NAA and MS+1.0mg/L NAA+10mg/L IBA, respectively.

Keywords: Floral and fruiting, Tree morphology, *In vitro* regeneration, *Pycnanthus angolensis*

Word count: 498

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INTRODUCTION

1.1 Background of the study

According to Osemeobo (2005), medicinal tree species contribute to Nigeria economy. The average annual value of harvested wild plants products from the forest (including items consumed, sold, given out to neighbours, and damaged after harvest) per household was estimated at One Million Six Hundred and Fourteen Thousand, One Hundred and Thirty Three Naira (₦1,614, 133.00), or US$ 11,956.54. Hoareau and DaSilva (1999) reported that medicinal plants also contribute to the world economy at large with an estimated market value of plant- based drugs put at US$ 2.5 billion. In fact, almost all the people on earth use plants for primary health care (Eloff, 1998). Therefore, ensuring the availability of these useful plant species through research will be addressing the economic and health needs of more than half of the world community.

Pycnanthus angolensis, an evergreen tree, chiefly growing in tropical African countries. *Pycnanthus angolensis* is normally identified as false nutmeg, a huge tree with a little trunk of twigs at correct angles to the stem. *Pycnanthus angolensis* can be called; English: false nutmeg, pycnanthus, cardboard, boxboard, African nutmeg; Igbo: oje, akwa-mili; Luganda: munaba, lunaba; Spanish: calabo; Trade name: *pycnanthus*, lunaba, ilomba; Yoruba: akomu.

The tree is 40 meters tall and a meter wide, occasionally it could be 1.5 meters or more. It has a straight and cylindrical trunk with fissures and flaking bark, branches are in whorls. The leaves are leathery up to 31cm lengthy and 9cm width, leaf blades have pointed tips, heart-shaped bases, and thick midribs. The leaves have signs of insect damage, a common attribute to the species (Foahom, 2002). Fruit has a black kernel with a red aril (Stannard, 1997) which looks like that of nutmeg (Mapongmetsem, 2007) and ripens for a long period enduring into the subsequently flowering season, which starts around October

(Orwa *et al.,* 2009). *Pycnanthus* consist of 3–4 varieties in Africa. *Pycnanthus angolensis* is variable, particularly in the fluffiness of the leaves, the size and shape of the fruits (Orwa *et al.,* 2009). It is a non-timber forest product that local people make use of it for treatment of wounds, stimulation of lactation, colds, skin diseases and measles and lastly for canoe making and paper making. The aril of the fruit can be used as bait for fish and to attract wild animals towards the trap (Onana, 2000).This plant is highly useful and thus calls for scientific study.

1.1.1 Forest Conservation

Forests are generational heritage bequeathed to man. However, this versatile relocation has consistently witnessed abused. Henne and Theis (2001) reported that of the original forest cover of the world, half is gone as only one-fifth remains as large tracts of ancient forest- that is, forest ecosystems shaped mainly by nature where human impacts have been comparatively great.

Tropical forests are so important because they harbor at least 50 percent, and perhaps more, of the world's biodiversity. Direct observations reinforced by satellite data, documents that these forests are declining. The original extent of tropical rainforests was 15 million square km but it now remains about 7.5-8 million square km. The most recent Food and Agriculture Organisation of the United Nations (FAO) State of the world's Forests report in 2012 estimated world-wide deforestation between 2000 and 2010 at 130 million hectare (about 3.2% of the total forests area in 2000). Forests in the tropics have suffered most of this devastative exploitation. Despite the pressure of economic development, which elicited the unrestrained exploitation of natural relocations for foreign exchange earnings, these relocations must be conserved for sustainability and posterity purposes.

Elliot (1996) described conservation as the prudent use of nature's bounty, in opposition to the unrestrained forest exploitation. Wohlfahrt (1996) attempted to reconcile forest conservation and forest utilization and submitted that the relationship between the two terms is not one of constrasting interests but rather one of reciprocal dependence and support. Forest relocations can also be conserved *in situ* or *ex-situ* (Ola-Adams, 2000). *In situ* conservation involves keeping plants and animals in their original habitat and is

highly desirable in cases of tree species where there is danger of over-exploitation and where the appropriate propagation techniques for their perpetuation are unknown (Ola-Adams, 2000). *In situ* conservation areas include Strict Nature Reserve, Game Reserve, National Park, Managed Nature Reserve, Arboreta and Anthropogenic Reserves (Sacred Groove or Juju Shrine). *Ex-situ* conservation refers to maintaining organisms outside their original habitat in facilities such as botanical gardens, kernel gene banks and field gene banks.

In vitro conservation can either be short or long timing (Engelmann, 1991). For short period of conservation, it involves storage of plants part in flasks or tubes in artificial media, under controlled environments, normally in sterile conditions. In this case, the aim is to reduce growth, thus increasing intervals between subcultures and it is normally under slow growth storage (SGS) i.e. in conditions of controlled light, temperature and growth retardant chemicals. Cryopreservation is the *in vitro* conservation of plant materials for long-term (LTS). Micropropagation is the word makes use of for the series of procedures employed to preserve and raise plant callus, cells, protoplasts and organ (stems, roots, embryos) in germ-free environment. The method is used for breeding, genotype amendment, and biomass making of biochemical derived products, lessons in plant pathology, germplasm protection, and logical investigations.

It is employed to reproduce new plants, such like those that have been hereditarily adapted through usual plant propagation. Adequate number of plantlets for plant structure does not produce seed, or does not react well to vegetative imitation or when seed cannot be stored (recalcitrant seeds). This method involves establishment (involves selection, explants preparation and test-tube culture, assortment of plant substance to be used), multiplication, pre-transplant (hardening-preparation of plantlets for natural environment which involves weaning) and transfer from culture. It can be used to make germ-free plants and saves time for the breeders. It has potentially higher propagation ratio. A huge deal of attention has been articulated in current years about the use of test-tube culture to plant inbred maintenance. Some of the reasons for this upsurge of interest are the inherent 'recalcitrance (loss of viability) of kernels of many important crops, including many

timber, food and commercial crops such as *Elaeis guineensis* (oil palm), *Hevea brasilensis* (rubber), Citrus species and coffee species.

1.2 Statement of problem

Pycnanthus angolensis is an indigenous tree species threathened by extinction due to over exploitation, relevance in traditional medicine and other human activities. There are no known large scale plantations of the species anywhere in Nigeria.

In addition, there is inadequate information concerning the fruiting pattern of this species in the Southwestern geographical zone of Nigeria. Adequate information on aspect of the species reproductive biology will contribute to its domestication for large scale plantation development through the selection of stands with desirable traits. To this end, a study on fruiting timing of *Pycnanthus angolensis* will give enough information for tree improvement, biodiversity conservation as well as tree management of the species.

Successful micropropagation of tree species is a phenomenon (Bajaj, 1997). In nature owing to the heavy leaching of phenolics *and* the *in-vitro* technique for this species conservation has not been developed. Recent climatic change has affected the flowering and fruiting patterns, therefore, availability of seed for meaningful research and development work has been impaired.

Herb collectors hinder the growth of the trees in the wild with extensive removal of the bark for sale. Regeneration, propagation as well as the improvement strategy is currently a problem. Even now, plantations of medicinal tree species such as *Enantia chlorantha Oliv., Rauwolfia vomitoria Afz., Pycnanthus angolensis Welw.,* and others with already market structure have not been established whereas, exotic tree species used for timber, pulp and paper such as *Tectona grandis*, *Gmelina* and *Pines* continue to have gained recognition more than the indigenous medicinal tree species in the afforestation and reforestation programmes of both Federal and State government in Nigeria.

1.3 Justification of the study

Hoareau and DaSilva (1999) reported that medicinal plants contribute to the world economy at large with an estimated market value of plant- based drugs put at US$ 2.5

billion. This world has over increased the request for medicines as the community is increasing Okafor and Ham (1999). The indiscriminate exploitation of *Pycnanthus angolensis* for timber and herbal condiments together with the problem of unavailability of germplasm materials constituted serious challenge in conservation. Lack of kernels resulting from irregular fruiting pattern associated with most rain forest tree species demands effective and alternative methods for growing the species.

Distinction in the form of *Pycnanthus angolensis* organs (fruits and seed) might not be due to climatic factors, especially the edaphic qualities, but also the trait variations in the community growing from various states (Bouda *et al*., 2006). Adequate information on the form qualities among these populations determined the stages of distinction. Study on the effect of seed location on germination and seedlings growth of *Pycnanthus angolensis* found out the form distinction shown from the fruits and seeds. Therefore, the *in vitro* seedlings performance showed levels of distinction among the community. *In vitro* propagation techniques have proven to be an alternative means; hence, different explants were used to produce rooted plantlets. In addition, tissue culture has been ascertained to produce clones of true-to-type plantlets for rapid multiplication, therefore *Pycnanthus angolensis* kernel explants were subjected to *in vitro* regeneration with modified MS media for rapid multiplication of planting materials for plantation establishment.

1.4 Objectives of the Study

1.4.1 Main Objectives

The main objective of this study is to generate scientific information on the natural distribution, flowering and fruiting patterns as well as assessing variability and develop *in vitro* propagation techniques for *Pycnanthus angolensis* with a view to ensuring mass production and conservation of the species.

1.4.3 Specific Objectives

The specific objectives are to:

(i) investigate the natural allocation of the species

(ii) find out the flower and fruiting duration of *Pycnanthus angolensis*

(iii) determine seeds and fruits form of *Pycnanthus angolensis*

(iv) investigate the influence of seed location on seed raising of *Pycnanthus angolensis*

(v) determine the effects of seed location, Explants types and growth regulators on *in-vitro* propagation of *P. angolensis*

1.5 Scope of the study

The studies estimated the natural distribution from three selected states. There were assessment of the flower and fruiting timing and determination of the seeds and fruits form of *Pycnanthus angolensis* coming from six dissimilar locations. The effect of seed sources on tissue culture of *Pycnanthus angolensis* was investigated. Effect of the six locations of seeds on seed germination, seedlings and *in vitro* propagation was examined. The studies involved flower biology and development of protocols for test-tube regeneration using special explants and growth hormones.

CHAPTER TWO

LITERATURE REVIEW

2.1 Botanical Features and Uses of *Pycnanthus angolensis*

Pycnanthus angolensis (Welw.) Warb., a tree species in the nutmeg family (Myristicaceae). It is known as Akomu, Oje, Aakwa-mili, Egwunond, calabo and can also be called Akujandi' in Yoruba, Igbo, Spanish and Hausa, respectively (Rahama, 2011), Walele in Cote D' Ivore and Eteng in Cameroon (Wikipedia, 2017), and lolako in Zaire (Mapongmentsem, 2007). It is native to Tropical Africa (Germplasm Relocations Information Network, GRIN, 2015). A monoecious or dioecious tree found in highlands and damp woodland and partly-deciduous forest, plentiful in woodland. The tree is usually in a direct bole with fissures, flaking bark, usually without buttresses and with red sap.

Leaves have symbols of insect damage, familiar to the species. Young fruit is brown and yellowish orange when ripe (Plate 2.1) with black seed and red aril (Orwa *et al.*, 2009) similar to that of nutmeg (Orwa *et al.*, 2009). The fruit maturity takes a long period into the subsequently flowering season, which begins just about October (Orwa *et al.*, 2009). *Pycnanthus* consists of 3 or 4 species, in Africa. The leaves are hairy *with* difference in the sizes of the fruits and shape, as well as in the value of the timber. The fruits are globose with thicker fruit wall.

Seeds of *Pycnanthus angolensis* germinate after sowing. The seeds are also aromatic. *Pycnanthus angolensis* seed has short viability. Katende *et al.* (1995) opined that cultivation by kernels and stem cuttings did not thrive. *Pycnanthus angolensis* is reputed for its analgesic, stomachic, aperative*;* carminative, haemostatic and antimicrobial events (Ancolio *et al*., 2002). Spent from seeds can be used as compost (Mapongmentsem, 2007). It is an important timber at the market in hot West Africa. Wood can be used as fuel, planks, roofing and covering houses (Orwa *et al.*, 2009). Nearly all of the parts of the trees have been utilized in herbal medicine for treatment of skin infections, cough, chest complaints and anemia, as purgative (Mapongmentsem, 2007), sap used to manage bleeding, and eye wash to treat cataract (Onocha and Otunla, 2010). Research has been

carried out into the medicinal properties of the plant with promising results. The seed oil has promising anti-oxidant properties for pharmacology as having hypoglycemic action in diabetic patients. The bark contains dihydroguaiaretic acid (Njoku *et al*., 1997) with effect on human tumour cell. Aqueous extracts of *Pycnanthus angolensis* can be used for wound curative, excellent for tummy, sore treatment, and cataract, for guinea worm (Onocha and Otunla, 2010). It is a very good plant for milk secretion.

2.2 Tissue culture

Plant tissue culture can be described as the culture of plant cells, tissues and organs under restricted setting on artificial medium, which has an important role in the production of agricultural, horticultural and medicinal plants. It consists of **Establishment** which is the collection of plant material to be used, **Multiplication** is the absorbing of tissue samples formed from the initial stage and escalating the quantity, **Pre transplant** entails taking care of the shoots formed to hold up root development and 'hardening'. This is done in germ-free condition. **Taking away from culture** is the last stage of test-tube breeding. Test-tube breeding has immensely contributed to the growth and development of many crops and it helps breeders save time during the breeding process and was used in the production and growth of quality and improved cultivars (Latitha *et al.,* 2014). It is useful for germplasm conservation, production of Genetically Modified Organisms (GMO), cryopreservation and also helps industries with production of secondary metabolites. It has proven over the years to be a dependable procedure for rapid plant production.

Theoretically, the chief aim of micro propagation attempt is to develop the *in vitro* ability of a living plant cell or tissue to give a great amount of genetically alike, physiologically homogeneous and developmentally standard population of the best plant selection that will have high photoautotrophic ability to stay alive in field. Now the most accepted purpose of micropropagation is the mass clonal increase of preferred genotypes of plants. Many micropropagation methods, phenomenal rates of clonal multiplication have been achieved.

The practical applications of micropropagation in horticulture using apical meristem culture in orchid and inhibition of apical dominance to terminate the axillary meristems from dormancy were achieved while others included the role of cytokinins in shoot morphogenesis. The production of plants by tissue culture has been an important factor in

the development of this skill. At present, the micropropagation method is specially used not only for plants that are not easy to propagate by usual practices but also for mass increase of accessible stocks of germplasm for biomass energy production and management of reasonably significant, best and uncommon plant species that are in danger or on the verge of destruction. The usual breeding practices for clonal propagation in plants are time consuming and labour demanding but in an ideal situation micropropagation phase must operate over a short time and at a low price. Above all, micropropagation is important where preparatory plant material is inadequate, where clonal progenies are essential; anywhere valued propagules are desired immensly, or where special plants (sterile somatic hybrids, transgenics) are vital for breeding and for any other research activities and development. In principle, Tissue culture is called *in vitro* breeding can be described as a set of procedures used to grow massive members of plant cells, *in vitro*, in an aseptic and closely restricted situation. I*n vitro* breeding is a true-to-type propagation of a chosen genotype in *in vitro* culture methods. It is the development of clonal lines from small tissue materials like buds, roots, and embryo removed from seeds.

2.3 Factors affecting *in vitro* propagation.

2.3.1 Media

The level of achievement of test tube procedures depends on the type of nutritional composition and growth regulator used. Therefore, it is important to appraise the nutritional and metabolic requirements of cells and tissues to be cultured prior to culture initiation. Different compositions of basic medium have been employed for test-tube propagation. In general, a test tube medium made up of inorganic salts, reduced nitrogen compounds, a carbon location, vitamins, growth regulators, amino acids (for specific purpose) and gelling agent (Lima *et al.,* 2012).

Plate 2.1: Full-grown tree and fruits of *Pycnanthus angolensis*

Several kinds of culture media have been developed such as Lloyd and Mc Cown (1980), Gamborg *et al.* (1968), Driver and Kuniyuki medium (1984) and MS medium, MS (1962). However, the most widely used is MS medium (1962) for plant propagation, and mostly for micro propagation of dicots (Lima *et al.,* 2012).

An important factor that affects the physical properties of culture medium is the kind and application of gelling material used (Ramesh and Ramassamy, 2015). Gelling agents are powdery substances used to solidify tissue culture media. They provide firmness to the medium and support plants for easy uptake or diffusion of nutrients. A good gelling agent is colourless, odorless, and holds water efficiently. Gelatin was the first gelling agent used for tissue culture (Poppe, 1997). A variety of basal media for micropropagation (Diallo *et al*., 2008), have been employed.

2.3.2 Type of explants

Type of explants is one of the essential conditions in optimizing the test tube procedure.The developmental stage of explants determines the success in the initiation. Therefore, young or juvenile explants should be used because the type of explants and their age has greater influence in tissue culture. Explants types like hypocotyls, epicotyls, embryo, internodes and root severely affect test tube procedure of plants (Kumar *et al*., 2011b) which may be as a consequence of diverse level of inbuilt plant regulators present in the plants parts. Leaf is the most commonly used explant for regeneration (Tyagi *et al*., 2001). Sujatha and Mukta (1996) used different explants like leaf, petiole, hypocotyls and greatest regeneration frequency was observed from leaf explants of *Jatropha curcas.* Apical meristem was used as explants for direct shoot regeneration. Alagumanian *et al*. (2004) used leaf and stem explants and utmost rejuvenation efficiency observed from stem explants in *Solanum trilobotam*. Gubis *et al*. (2003) used hypocotyls, epicotyls, cotyledons; leaf, petiole, internodes and maximum response were attained from hypocotyls in Tomato. Some explants like root tip could be used but they are difficult to isolate due to soil contamination that might be difficult to remove without damaging the root. Ali and Mirza (2006) used root, stem, leaf and petiole but maximum responses were observed from stem explants in *Citrus jambhiri* Lush.

2.3.3 Location of explants

Location of explants i.e. *in-vitro* and *in- vivo* is also important for regeneration (Kumar *et al.,* 2010a). *In vitro* explants are considered to be the most suitable for organogenesis (Reddy *et al*., 2008). Location of explants has different effects on regeneration of plant. However, *in-vitro* explants in general have better potential to organogenesis as matched up to conventional explants (Reddy *et al.*, 2008). Seedling explant is more meristematic than mature plants (Feyissa *et al*., 2005). An example was found in *Jatropha curcas*.

2.3.3.1 Orientation of explants

The way explants are placed in the medium affects the rejuvenation efficiency (Kumar and Reddy, 2010). In general regeneration efficiency is higher in horizontal placement as compared to erect position of explants. However, the initiation site, polarity, and efficiency of bud regeneration were altered by explants orientation in *Dionaea muscipula* (Teng, 1999).

2.3.3.2 Mineral nutrition.

Mineral deposits are vital apparatus of the medium. In attendance is a great selection of combinations of major and minor-salt. The MS medium is popularly used because most plants react to it positively.

2.3.3.3 Carbon Source

The most common source of carbon is sucrose. It helps in supply energy for the metabolism (Caldas *et al.,* 1998) and also vital for test-tube increase and progress of numerous species.

2.4 Types of Tissue Culture Techniques.

Jona (1987) listed four explants used in tissue culture: (1) meristems culture (2) callus culture (3) production of haploids and (4) protoplasts.

2.4.1 Meristerm culture

Meristerm culture is shoot tip culture (Beelen, 1990). The apical meristem is that part of a shoot distal to the youngest leaf primodium. It enables the production of virus-free plants; this is possible because the virus titre increase with increasing distance from meristem tips, which are in general free of virus.

2.4.2 Somatic embryogenesis

This is the means by which an embryo is formed from tissue or cell, competent of growing into an original plant (Razdan, 2003). Embryos formed callus which consists of a growth of newly developed undifferentiated cells, mostly proliferating from cut tissues at the cut surface. Callus is affected by the size of the explants, the type of culture, and the polarity of the explants on the standard.

2.4.3 Production of haploids

Haploid plants are produced by distant hybridization, delayed pollination, application of irradiated pollen, hormone treatments, shocks and culturing of excited anthers for induction of mutations and production of homozygous plants.

2.4.4 Protoplasts cultures

Protoplasts are plants cells without cell walls (Pierik, 1987). These can be isolated by treating plant tissues, often leaf mesophyll tissue, with a combination of cell- wall-degrading enzymes in a solution, which contain osmotic stabilizers to preserve the structure and viability of the protoplast (Beelen, 1990). FAO (1994) reported that the application of micro propagation methods in forest trees improvement permits rapid multiplication of tree species to meet up with market request.

2.5 Variation

Distinctions in nature are responsible for creating provenances, clones, races and ecotypes (Zobel and Talbert, 1984). These distinctions are important location for a tree breeder to improve species. Distinctions can be successfully utilized for adaptability of tree species e.g. drought resistance or selection of a suitable genotype for growth or fruit quality and so on (Sundaram *et al*., 2003). The genetic gain can be realized by making kernel collections from phenotypically and genotypically superior trees or stands.

UNESCO (1994) noted that within each species there exists a breadth of distinction that has been created and partitioned by evolutionary forces. Basically, three types of distinctions may be distinguished (Styles, 1976). The distinction between the individuals of any community is based on three factors (Vavilov, 1951); Environmental modification, Genetic Recombination and Mutation. Variation is the basic genetic rearrangement of

information available for domestication which may be improved by selection and breeding.

2.5.1 Seed location variability for seedling parameters

Reddy *et al.* (2007) evaluated seven seed locations of *Pongamia pinnata* (L.). Pierre from Karnataka for germination and seedling traits. They found that, seed locations had significantly higher distinctions among the seed locations. Hence, they noticed that Kolar seed location was superior with respect to all traits. Lekha and Lalji (2011) studied the distinction in seed and seedling traits of *Jatropha curcas* L. with varying zones and provenances, in which seedling height ranged from 14.45 cm to 15.36cm in different provenances within the sub-humid Sutlej Ganga alluvial plains.

2.6 Flowering and Fruiting Pattern

Floral biology has practical implications that are very important, in addition to its scientific application, in that flower characters and buds affect fruit uniqueness and yield. Yield derives from fruit quality (e.g. weight) and quantity (i.e. number), depend on amount of flower and value: (a) suitable flowers to become fruits, properly pollinated and fertilized, and must set fruits, that can grow. Not all flowers can do all of this: some flowers have aborted ovaries that are incompletely developed or having absence of flowers, depending on when the abortion occurred. These aborted ovaries cannot become fruits. Normal pistils, may not be pollinated or fertilized, but also fertilized ovaries may drop after some growth, resulting in fruit drop. Fruiting phenology plays important role in controlling the abundance of fruits.

Phenology is the study of the timing of seasonal life cycles depending on the causes of their timing with living and non living factors. It is the nature's calendar. It is a key component of life on earth. This timing affects interrelation among phases of the same or different species? In plants, flowering, fruiting, leaf flushing, and germination are the phenological events. It is certainly feasible that the plan of these events has significant effects on survival success. Temperature and humidity are not only abiotic environmental conditions that affect survival success, but biotic factors like intra specific and inter

specific competition for various resources can be selective agents for plant phenology. Low survivorships of seedlings are caused by germination, flowering, and leaf production with high predation rate. It affects plant survival in their environment; food production depends on the timing of phonological events. Phenology is used as indicator for climate change, signals to evolutionary shifts of plants, agronomic time indicator. It facilitates conservation efforts, provides functional rhythms of plants and plant communities. It is also important in tree production management strategies and helps in improvement programmes.

2.6.1 Classification of Phenology Patterns

Flowering phenology is classified in tropical forests based on duration and synchrony, flowering behaviors of plant into (a) continuous (b) extended and (c) brief and further divided the latter two into (i) synchronous and (ii) asynchronous. Flowering phenology in Bignoniaceae is classified into four flowering types based on duration, frequency, and amplitude: (i) Steady state (ii) Cornucopia (iii) Big bang and (iv) Multiple bangs.

2.7 Reproductive Phenology

Reproductive Phenology is the timing in the reproductive events. It involves plant reproduction and population persistence.It involves three stages: time of flowering, time of seed dispersal and duration of seed maturation. Reproductive Phenology focused on flowering which is stages:

(i) Onset of flowering: This is the beginning of reproductive period. (ii) Pattern of seed/ fruit maturation length. It is characterized by population level of plant. Population is the group of individuals of the same species growing in similar conditions at the same site during a given year. Reproductive Phenology is influenced by environmental factors, influenced by life history and photosynthetic pathway

2.8 Challenges in Tissue Culture

Contamination by microorganisms is considered a bottle neck in tissue culture. Therefore, the works with tissue culture need to be carried out under effectual aseptic situation. The choice of the sterilization method depends on the occurrence of contamination and death.

High concentrations and durations of the sterilizing agent prevent contamination and cause higher death percentage and vice versa. Contamination in tissue culture can originate from two sources, either through carry over of microorganisms on the surface or in the tissue of the explant as endophytes.

2.8.1 Sterilisation Protocols

The advent of *in vitro* plant culture include cell, tissue, organ and embryo culture for plant enhancement can allow the growth of important somaclonal variants, in the removal of plant diseases through regions that are capable of dividing in tissue culture technique or serve as a originator for plant enhancement during gene transfer (Sarasan *et al*., 2011). There are so many factors that can contaminate tissue in cultures which can be caused by different microorganisms viz: bacteria and fungi, which reduce the productivity and can totally disallow them from growing. Consequently, removal of contaminants by disinfection from the surface of the explant is of main concern.

There are different disinfection protocols on *in vitro* cultures that have been developed for exotic tomato cultivars but there is lack of information on sterilization protocols and *in vitro* regeneration responses of local Nigerian tomato cultivars (Durosomo *et al*., 2015). In other to establish a capable *in vitro* regeneration protocol, there must be an efficient sterilization procedure. Contamination in tissue cultures from diverse sources can completely avoid plant from thriving in culture. There are different chemicals for surface sterilization. They are mercuric chloride, Hydrogen peroxide and sodium hypochlorite for the sterilization of tomato seeds and found out that 5% sodium hypochlorite for 20 min was more effective resulting in high germination rate of 77.07% with no contamination.

The vital step for aseptic culture establishment is sterilization of explants. Successful tissue culture of all plant species depends on the removal of exogenous and endogenous contaminating microorganisms. To remove contamination during *in vitro* propagation various procedures have been developed. Bacteria and Yeast by fungi, is one of the most serious problems of commercial and research plant tissue laboratories *in vitro* contamination. Contaminated plants can reduce multiplication and rooting rates or may

die. It is necessary to remove foreign contaminants including bacteria and fungi from explants and it is very difficult to obtain sterile plant material completely free from contamination. This is a serious problem while dealing with woody plant material.

The surfaces of living plant materials are naturally contaminated with microorganisms from the environment, so surface cleaning of the materials with chemical solutions is a vital research step. Sodium hypochlorite, calcium hypochlorite, ethanol, mercuric chloride, hydrogen peroxide and silver nitrate are the major disinfecting agents. Sodium or calcium hypochlorite or various commercial bleaches are the most commonly used for surface sterilization of explants.

In view of the fact that sterilizing agents are toxic to the plant tissue, contamination removal must be done in a way not to injure the plant cells. The rate of microbes in plant cultures usually results in increased culture mortalitity. Diverse infections can manipulate variable growth, tissue necrosis, reduced shoot proliferation and rooting. Although the tissue culture techniques usually involve growing stock plants in ways that will minimize infection by treating the plant.

In the management of contaminats in plant tissue cultures there are three main issues to be put into consideration: (a) entrance of microorganisms must be prevented with the initial plant material (b) from the environment during subculturing and (c) reducing microbial contaminat in the cultures during multiplication and rooting. The best way of preventing bacterial contamination *in vitro* is by eradication of bacteria from the first plant explants that are introduced into the culture. These methods are used in reducing contaminations. They are: (1) The use of explant of donor plants under a strict clean environment, (2) Proficient sterilization of the initial explants, and (3) cutting of the size of the explants to apical meristem. The procedure of sterilizations are many, depending on plant species, part of the plant to be used as explant for sterilization, the growth environment, age and part of the plant used for micropropagation. It is difficult to determine standard sterilization procedures that apply to all plants.

2.9 Trends on Tissue Cultures of Plants

In vitro propagation has been used to culture medicinal plants. The mass of *Stevia rebaudiana* callus was formed in 3.0mg/l, 2, 4D + MS. The same way, maximum shoots of *Rehmannia glutinosa L.* was formed on medium with 1mg/L TDZ. Singi hawthorn c*rataegus sinaica* Boiss an endangered species was investigated. Fast shoot production of *Nyctanthes arbotristis* L. was achieved through 1.0-1.5 mg/LBA, 50mg/L adenine sulfate and 3% (m/v) sucrose. Micro propagation of *Ruta graveolens* was greatly influenced by the effect of plant growth regulators.

Micropropagation protocols have been used for some other plant species like *in vitro* shoot multiplication in Cinnamon *camphora* and Cinnamon *kanehirae*. Induction of multiple shoot from shoot tip/ meristem explants in sorghum, shoot growth of *in vitro* plants of sweet potato (*Ipomea batatas* L.), multiplication and regeneration of Solanum tuberosum (Potato) from nodal explants and *in vitro* propagation of elite *Theobroma cacao* cultivar (F3 Amazon). A prolific shoot multiplication system for *Plukenetia conophora* (walnut) has been reported. *In vitro* culture of *Telfaria occidentalis* under different cytokinins and auxins combination was achieved in vegetable as well as in ornamentals like cultivars of hybrid tea roses (*Rosa hybrid L.*). *In vitro* propagation has been used to regenerate, establish and conserve trees such as *Khaya grandifolia* through organogenesis and somantic embryogenesis, proliferation of nutmeg aril (mace) in a nutrient medium supplemented with NAA and BA, *in vitro* shoot production was achieved using axillary bud of *Talinum portulacifolium* that were powerfully influenced by the cytokinin (BAP) on MS Medium supplemented with 6µm, BAP and 2µM (IAA). Equally, medium with BAP had significant influence on leafy spurge (*Europhorbia esula).* Indole Acetic Acid is not only meant to induce root but also removed tufted shoots and undifferentiated callus in *Vitis vinifera*. The optimization of growth medium and priming led to survival and callus formation of *Zanthoxylum zanthoxyloides* and *Pycnanthus angolensis* from different locations (Bello and Akinyele, 2016).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Reconnaissance survey

An investigative study of the area distribution of *Pycnanthus angolensis* was carried out in the Southwestern part of Nigeria. Three states: Oyo, Ekiti and Osun states were purposively selected due to the availability of matured trees.

3.2 Study location collection procedures

According to the results from investigation study, purposive sample based on flowering and fruiting pattern was used to select two locations from each of the three selected states along rain woodland region across the southwestern part of Nigeria.

3.3 Climate and vegetation of the study region

Full-grown fruits of *Pycnanthus angolensis* were sampled from six locations down the rain woodland in South West Nigeria. The weather of Southwestern Nigeria is hot, typified by damp and arid seasons (Faleyimu *et al*., 2013) while the high temperature and annual rainfall ranged between 21^0C and 34^0C; 1500mm and 3000mm, respectively (Faleyimu *et al*., 2013).

The plant life in south west Nigeria consists of unsullied marsh and mangrove woodland at the low land woodland extend inland border.

3.4 Procedures for Data Collection

3.4.1 Study1: Natural Distribution of *Pycnanthus angolensis*

Three states, Oyo, Ekiti and Osun states were purposively selected due to the availability and abundance of the plant.

3.4.1.1 Ekiti state

It is situated on latitudes 7^0 25′ and 80^0 5′ East of Greenwich Meridian and longitude 4 ^{0} 45′ and 5′ 46^0North of the Equator (Adegboyega and Akintan, 2018).The state is surrounded to the North by Kwara and Kogi states, bordered by Osun state to the west, Edo to the East, and Ondo to the South. The state enjoys a hot weather with two distinct seasons: rainy season (April to October) and dry season (November to March) and the temperature ranges from 21°C to 28°C (Oluwasusi and Tijani 2013) with high moisture. The total land area of the state is about 6,353km^2 and it has a community of 3,930,212 (NPC, 2006) with more than 60 percent residing in rural areas.

3.4.1.2 Osun State

Sofoluwe *et al.* (2011) opined that Ekiti state is situated on latitude 7.0° and 9.0° N and longitude 2.8° and 6.8° E, with a total land area of 8,602 km^2

3.4.1.3 Oyo State

Adejumo *et al*. (2016) reported that it is geographically situated on latitude 7° 02′ N and 9° 10′ N and longitude 2°04′ E and 4° 30′ E, enclosed by Ogun state, Kwara state, partly surrounded by Ogun state and partially by the Republic of Benin, whilst Osun State on the eastern part. The state enjoys a hot weather with prominent wet and dry seasons (Adejumo *et al*., .2016).

3.4.1.4 Data collection and analysis

Information on total number of stands per location per state, percentage frequency (%), habitat distinction (lowland, upland, fallow land, farm, and river side), number of stand with flowering and fruiting within each selected state were investigated. Data collected were analysed using descriptive statistics.

3.5.1 Study 2: Flower and fruiting timing of *Pycnanthus angolensis*

3.5.1.1 Tree selection.

In each of the states, two matured trees were purposively selected. The geograghical coordinate of each tree stand in selected location was determined using GPS (Table 3.1). All the matured fruits from each one of six purposively chosen trees were collected singly and labeled. In each chosen state, twigs from two trees with flowering and fruiting phase

were randomly selected. Period of flower and fruiting maturity were monitored for 24 months.

3.5.1.2 Geocoordinates/ Geographical description of Selected Locations of *P. angolensis*

The details of the geographic origin of the fruit and kernel location with respect to special seed locations are presented in Table 3.1.

3.5.1.3 Data collection and analysis

Phenological qualities such as flowering initiation, (FI), flowering period and fruit maturation colour (FMC), Flowering timing was predictable as the number of days the plant remained in bloom. Timing of flowering was calculated as the subtraction of first and last days of flowering for each of the tree. Fruit maturation colour was observed from formation to maturity: Green fruit (GF), Yellow fruit (YF), and Orange/ Red fruit (ORF) were pragmatic. The time gap between every developmental stage was observed within every cycle period and recorded.The observations on the trees were carried out weekly in all the locations. Data were analysed with descriptive statistics.

Table 3.1: Locations of *Pycnanthus angolensis* within three Selected States in S/W Nigeria

Locations	Latitude	Longitude	Altitude(m)	Accuracy(m)	State
Gbongan	7°29'17.616"N	4°20'37.092"E	244.40	3.50	Osun
Ajaba	7°59'33.894"N	4°53'52.596"E	485.40	2.90	Osun
Otun	7°58'47.661"N	5°7'59.874"E	594.70	3.40	Ekiti
Ayetoro	7°55'59.090"N	5°81'43.182"E	569.90	3.30	Ekiti
Adewumi	7°26'57.792"N	4°2'3.381"E	207.10	2.80	Oyo
Idito	7°27'14.676"N	4°3'9.504"E	215.20	4.00	Oyo

Location: Field survey, 2017

3.5.2 Study 3: Seed and fruit morphology of *Pycnanthus angolensis*

3.5.2.1 Tree selection and seed collection.

In each of the states, two matured trees were purposively selected as described in 3.5.1.1.

3.5.2.2 Fruit selection and form

Mature fruits of *Pycnanthus angolensis* were harvested from each mother tree. One hundred (100) fruits were at random selected from each tree giving a total of 200 seeds per state

3.5.2.3 Data collection and analysis

The length (cm), width (cm) and weight (g) were measured. Fruit colour was determined by matching the fruits and seeds collected from six different locations with Munsell Colour Chart, (2009), number of the fruit per bunch was counted visually and recorded from mother tree within each location in each state. The lenght of the fruit was measured using electronic caliper. Fruit breadth was taken at the central of the fruit using electronic caliper although electronic weighing scale was used to evaluate the heaviness of chosen fruits in the laboratory.

3.5.2.4 Seed selection and form

One hundred seeds were at random selected from each location. The following variables were measured: length (cm), width (cm) and weight (g). Seed length was measured with electronic caliper. Kernel breadth (cm) was taken at the centre of the seed using electronic caliper while electronic weighing scale was used to assess the heaviness of chosen kernels in the laboratory. The experiment was setup in two levels Nested design. Level A is States while Level B is Locations.

3.5.2.4.1 Data collection and analysis

The records collated per fruit location and seed were analysed with analysis of variance (ANOVA) R version 3.3.4 to investigate the significance of variability in form traits of fruits and seeds from different locations while the Tukey Multiple Comparisons was used to separate the means at 95% Confidence Interval (CI). Simple linear regression was also used to find out the effect of fruit variables on seed variables.

Statistical models:

$Yijk = \mu + Ai + Bj_{(i)} + eijk$

Where:

Yijk = individual observation

μ = overall mean (general effect common to all effects)

Ai = Level A (Each State)

Bj = Level B (Locations within each State)

$Bj_{(i)}$ = interaction effects of locations within each State

Eijk = experimental error

3.5.2.5 Seed anatomy

The removed seeds from each location were soaked in water over night and were cut transversely with sterile surgical blade in the lamina flowhood to avoid contamination.

3.5.2.5.1 Data collection and analysis

Amount of striations was counted and categorized into long (LS) and short (SS) with their number

3.5.3 Study 4: Effect of seed locations on seed and seedling growth of *Pycnanthus angolensis*

3.5.3.1 Variation in seed germination of *Pycnanthus angolensis*

From the seeds collected in the previous study, an overall of 100 mature kernels were at random selected from every one location and sowed in well labelled polythene bags (14 x 12 cm) filled with top soil and arranged out in an open space of Agronomy screen house. A total of six hundred seeds were set for the study, arranged in 6x3 factorial experiments in a Complete Randomised Design (CRD). Factor A is locations while factor B is states. Watering to saturation point of the sown seeds was carried out every day in the morning using a watering can. Daily monitoring was carried out on sprouting. Germination was taken to have occurred when the radicle emerged and can be seen above soil level in the bags. Germination was taken to have accomplished when no additional germination takes place. Data collected were analysed using graphic statistics.

$$\text{Germination percentage} = \frac{\text{Amount of seed Germinated}}{\text{Amount of seed sown}} \times \frac{100}{1}$$

3.5.3.2 Variation in seedlings of *Pycnanthus angolensis*

At two-leaf stage (6 Weeks After Sowing (6WAS), ten uniformly growing seedlings from each seed location were picked out of germination bags and transferred into polythene bags (14 x 12 cm) packed with top soil and arranged under the shade to wean for two weeks (Plate 3.1.). Thereafter, the seedlings were laid out in an open nursery in two replicates of five seedlings each in a Complete Randomised Design (CRD).

3.5.3.2.1 Data collection and analysis

The following growth traits were measured to evaluate the Shoot Height (SH), Number of Leaves (NL), and Number of Nodes (NN). The NL and NN were counted visually, while SH was measured with metre ruler fortnightly for 4months.

The data taken were analysed. The observed means were subjected to Tukey Post Hoc to show mean separation.

Statistical models:

$Yij = \mu + Ai + Bj + AB_{ij} + eij$

Where:

Yij = individual observation

μ = overall mean (general effect common to all effects)

Ai = Factor A (Locations within each state)

Bj = Factor B (Each state)

ABij = interaction effects of AB

eij = experimental error

Plate 3.1: Transplanted seedlings of *Pycnanthus angolensis* at two-leaf stage (6 Weeks After Sowing (6WAS)

3.6 Study 5: Effects of locations, types of explant and plant regulators on *in vitro* propagation of *Pycnanthus angolensis*

3.6.1 Embryo Explants preparation procedures

The seeds were severally washed under tap water to remove dirts. Seeds were surface sterilized in 70% ethanol for 2 minutes, decanted and transferred to 50% Sodium hypochlorite solution (Clorox) for additional 5 minutes, and then rinsed thoroughly with sterile distilled water under the laminar flowhood (Bello and Akinyele, 2016).

3.6.1.2 Seed inoculation

The embryos were carefully excised together with a little endosperm using uncontaminated surgical knife blade in the lamina flowhood. Each was horizontally placed on the MS cultured media (Murashiege and Skoog, 1962) (Tables 3.2).

3.6.1.3 Culture medium and incubation conditions

Each explant was inoculated on MS medium (1962) combined with growth regulators namely: Benzylaminopurine (BAP), Kinetin and Naphthalene acetic acid (NAA) were used (Table 3.3), adjusted to a pH of 5.7 with 0.1 or 1.0M NaoH or HCl before the addition of 7 g/l Agar. The media was dispensed into sterile bottles at a rate of 28 ml / bottle, labeled and sterilised for 20 min at 121°C in an autoclave and allowed to cool before inoculation. Incubation of cultures was done at 26 $\pm$ 2^0C for 16h photoperiod in white fluorescent bulb and prearranged on the shelves.

3.6.1.4 Experimental design and Data analysis

The experimental treatments were arranged in 6x4x3 factorial in Completely randomised design (CRD) where 6 represents seed location/ location, 4 represents levels of cytokinins, and Auxins and 3 represents States, each replicated five times. All were represented in alphabets A-H (A_0-A_3, B_0-B_3…..H_0-H_3). Percentage survival was documented after 4weeks of culture by dividing the number of germinated embryo by the sum of inoculated embryo in every medium. The NR, RL, SH and NL were weekly evaluated for three months.

Table 3.2 MS media Formulation in *in vitro* culture of *Pycnanthus angolensis*

Chemical	Quantity (g/liter stock)
Nitrate stock	
Ammonium nitrate (NH_4NO_3)	165.00
Potassium nitrate (KNO_3)	190.00
Sulfate stock	
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	37.00
Manganese sulfate ($MnSO_4 \cdot H_2O$)	1.690
Zinc sulfate ($ZnSO_4 \cdot 7H_2O$)	0.860
Cupric sulfate ($CuSO_4 \cdot 5H_2O$)	0.0025
Halide stock	
Calcium chloride ($CaCl_2 \cdot 2H_2O$)	44.00
Potassium iodide (Kl)	0.083
Potassium phosphate (KH_2PO_4)	17.00
Boric acid (H_3BO_3)	0.620
Sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$)	0.025
NaFeEDTA stock	
Ferrous sulfate ($FeSO_2 \cdot 7H_2O$)	2.784
Ethylenediaminetera acetic acid, disodium salt (Na_2EDTA)	3.70
Sucrose (g)	30.00
Agar (g)	7.00
Growth Regulators	as Required

Percentage of explants regenerated were determined as [(number of plantlet regenerated from explants per treatment/ total number of each explants cultured per treatment) x100, analysed using analysis of variance (ANOVA) while mean values were carried out using the post hoc. Test using the SAS 9.1 (SAS, 199.)

Statistical models:

$Yijkl = \mu + Ai + Bj + C_k + D_L + AB_{ij} + BC_{jk} + CD_{kl} + BCD_{jkl} + ABCD_{ijkl} + eijkl$

Where:

Yijkl = individual observation

μ = overall mean (general effect common to all effects)

Ai = Factor A (Locations within each State)

Bj = Factor B (Each state) effect of provenances (location within the selected states

ABij = interaction effects of AB

C_k = effect of Explant types

D_l = effect of hormonal levels

BCjk = interaction effects of provenance

CDjkl = interaction effects of hormonal levels

BCDjk = interaction effects of plant samples, provenances, and hormonal levels.

There were 160 treatment combinations in 5 replicates giving a total number of 800 test blocks for the factorial experiment.

3.6.1.5 Hardening and acclimatization

All plantlets at 3-5 cm in heights were considered for hardening. Rooted plantlets were gently removed from the media and washing the roots under running water before transfering to14x12 cm polythene bags containing top soil. The soil was kept moist and the plantlets were covered with transparent nylon bags during the first week. After this period of acclimatization, nylon was removed to expose the plantlets to standard screen house conditions.

3.6.1.6 Data collection and analysis

The following variables, Number of Leaves (NL), Number of Nodes (NN), and Shoot Height (SH) were measured to evaluate growth. The observed means were subjected to Tukey Post Hoc. Test.

3.6.2 Sterilisation process of Nodal explants of *Pycnanthus angolensis*

3.6.2.1 Explants preparation and sterilization

Nodal stem segments were prepared from 4months old nursery-growing seedlings with leaves by discarding internodes and were then used as the experimental materials. The nodal stems and leaves were washed severally by immersing the explants into mixture containing 100ml Chlorox with 2000litres of distilled water, rinsed under running water and were surface cleaned in 70% ethanol for 2 minutes, decanted and transferred to 50% Clorox (Sodium hypochlorite solution) for additional 5 minutes, and then rinsed thoroughly with sterile distilled water (Bello and Akinyele, 2016) in the laminar flowhood. Nodal stem segments were then excised into desired size aseptically and Leaf explants were then cut with mid rib and were individually inoculated in medium enhanced with levels of auxin NAA alone and combined with cytokinin modified with 10mg/l Indole Butyric Acid (IBA) (Table 3.3).

3.6.2.2 Data collection and analysis

The NL, NR and NN were counted visually, callus formed was also counted and days of sprouting were recorded to determine the influence of explants and hormones.

Table 3.3: Effects of locations and Plant growth regulators on *in vitro* regeneration of *Pycnanthus angolensis*

CYTOKININ (Mg/l)				
		AUXINS		**NAA (mg/l)**
K	**0**	**1**	**2**	**3**
A 0	$K_0 N_0$	K_0N_1	K_0N_2	K_0N_3
B 1	K_1N_0	K_1N_1	K_1N_2	K_1N_3
C 2	K_2N_0	K_2N_1	K_2N_2	K_2N_3
D 3	K_3N_0	K_3N_1	K_3N_2	K_3N_3
BAP (B)				
E 0	$B_0 N_0$	B_0N_1	B_0N_2	B_0N_3
F 1	B_1N_0	B_1N_1	B_1N_2	B_1N_3
G 2	B_2N_0	B_2N_1	B_2N_2	B_2N_3
H 3	B_3N_0	B_3N_1	B_3N_2	B_3N_3

Where:
K=Kinetin, BAP (B) = Benzyl Amino Purine, NAA= Naphthalene Acetic Acid
A, B, C, and D = 4 levels of Kinetin
E, F, G, and H = 4 levels of BAP
KN= Kinetin and NAA
BN= BAP and NAA

Plate 3.2 Media browning of *in vitro* culture of *Pycnanthus angolensis* Occuring

CHAPTER FOUR

RESULTS

4.1 Population frequency of *Pycnanthus angolensis*

The population frequency of *Pycnanthus angolensis* across three selected states was presented in Table 4.1 Distribution varied within each state in Osun state, the highest distribution of 10.5% in Gbongan, while the least tree distribution was found in Ajaba (3.4%). In Ekiti state, Otun had the highest distribution of 8.6%. The least distribution 6.9% was found in Ayetoro Ekiti. In Oyo state, the highest distribution of 3.5% was in Adewumi, while the least (1.8 %) was found in Idito. The table revealed that highest population was found Gbongan. This indicated that Osun state had the highest population of *Pycnanthus angolensis.* The tree status varied across the states from juvenile to maturity.

Pycnanthus angolensis was found growing in fallow or abandoned land, marshy areas, farmland, river side, new site areas and Quarters. In Osun state, it was found growing on abandoned or fallow land and farmland. It was found growing in river side and new site areas in Ekiti state while it was found growing on farm land, new site, river side and quarters. This indicated that *Pycnanthus angolensis* could be found in different habitats among and within the states.

Table 4.1: Population frequency of *Pycnanthus angolensis* in three States

State/ Location	Number of Tree Stands/ Percenentage (%)	Treematurity/ Status J	M	Flowering & Fruiting	Habitat Distinction
OSUN STATE					
Gbongan	10.5	4	2	++	Fallow land, Farm land, fallow land.
Ajaba	1.8	0	1	-	Marshy area/ farmland
EKITI STATE					
Otun	8.6	3	2	++	River side/ abandoned la fallow land
Ayetoro	6.9	3	1	+	New site area/ fallow/ river si
OYO STATE					
Idito	1.8	0	1	+	Farm land /New site area
Adewumi	3.5	0	1	+	New site area

Where:

J= Juvenile

M= Mature

4.2 Study 2: Flower and fruiting timing of *Pycnanthus angolensis*

4.2.1 Floral timing

The reproductive units (flowers) are hairy, thick and scented, with inflorescence (panicles) rust in colour while the individual flowers are tightly packed pending the enlargement of stamens (Plate 4.1). The reproductive units are unisexual flowers (androecium and gynoecium are separate) formed with small, stalkless 3-lobed enclosed dim chocolate hairs (Plate 4.1). The inflorescences are basipetal (from the base to the apex) (Plate 4.2) while the male reproductive organs are fused together with no unique filaments (Plate 4.3) but they are in clusters of inflorescence.

Plate 4.1: *Pycnanthus angolensis* Flower showing 3 lobed perianth.

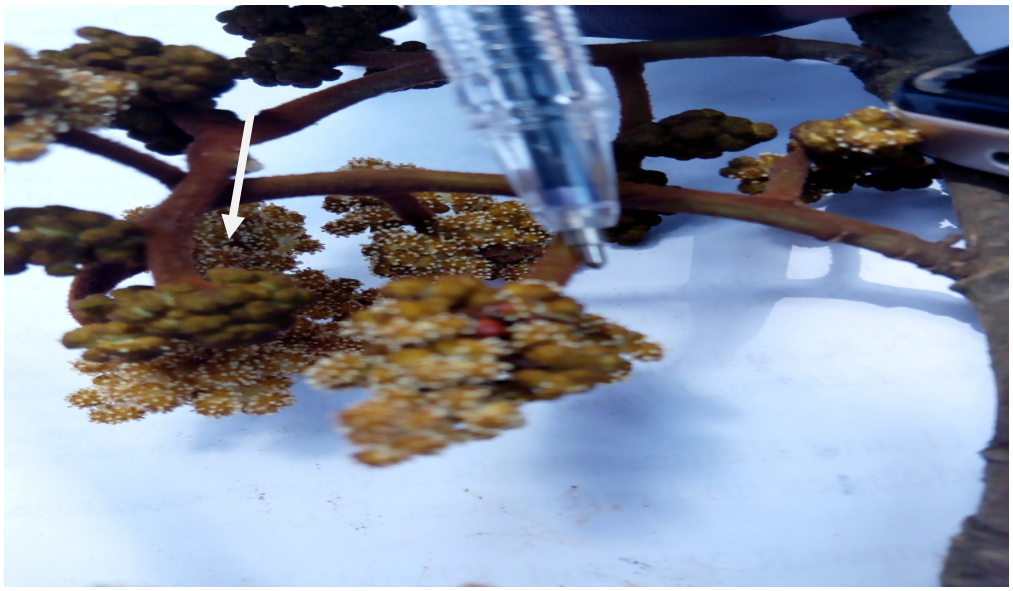


Plate 4.2: Basipetal Flowers (base to apex) of *Pycnanthus angolensis*

Plate 4.3: Male Reproductive Organs of *Pycnanthus angolensis*

In *Pycnanthus angolensis* blossoming of flowers occurred at September, 2017 and ended in June, 2018. In September, 2017, Osun state started flowering and ended in January, 2018 against Ekiti which blossomed in November, 2017 and ended in April, 2018 while in November, 2017 Oyo started flowering and ended in June, 2018. Commencement of flower began from September - November, 2017 where Gbongan: October, 2017, AJ: September while in November 2017, flower starts blossoming in Otun, Ayetoro (AY), Idito (ID), and Adewumi (AD) at similar time (Table 4.2).

Flowers were noticed at dissimilar period, it commenced in October, 2017 and ended in January, 2018. In November, 2017, GB formed flowers and spanned through December, 2017, In October 3rd Ajaba (AJ) formed flowers and ended in November 30th, 2017. In the same vein, Otun (OT) formed flowers in 8thNovember and ended in 8th December 2017; In AY, flowers were formed in 8th November and ended in 8th December, 2017, while Idito (ID) and Adewumi (AD) started forming flowersin 8th December 2017 and ended in 8th January 2018. Flowering period in *Pycnanthus angolensis* took 5-8 months while it took seven months in GB; five months in AJ and OT, respectively; eight months in AY, AD and ID, respectively.

Opening of flower buds occurred early in December to near the beginning of January during the dried up time. Falling of flowers varied from one location to another (January to June) 2018, Falling of flowers in GB occurred in April, AJ: January, OT: March, AY: April while falling of flowers in ID and AD occurred in June. Disease and ecological conditions could be responsible for the dropping of flowers of *Pycnanthus angolensis* in Otun Ekiti. Flowering initiation (FI) is 30 - 58 days while FF is 51- 282 days (Table 4.3)

Table 4.2: Floral duration of *Pycnanthus angolensis* in selected locations

LOCATION/ STATE	Flower initiation (MONTH)	Flower formation (DAYS)	Flowering period (M/D)	Inflorescence dropping
Gbongan **Osun state**	October, 2017	15th Nov – 15th Dec, 2017 (30days)	1st Oct, 2017 – 30th April, 2018 (7mths/ 211days)	April, 2018
Ajaba **Osun state**	September, 2017	3rd Oct – 30th Nov. 2017 (58days)	1st Sept, 2017 – 31st Jan. ,2018 (5mths/ 153 days)	January, 2018
Otun **Ekiti state**	November, 2017	8th Nov – 8th Dec. 2017(30days)	1st Nov,2017– 31stMarch, 2018 (5mths/ 151days)	March, 2018
Ayetoro **Ekiti state**	November, 2017	8th Nov – 8th Dec 2017(30days)	1st Nov, 2017 – 30th April, 2018 (6mths/ 181 days)	April, 2018
Idito **Oyo state**	November, 2017	8th Dec, 2017 – 8th Jan , 2018 (30days)	1st Nov, 2017 – 30th June, 2018 (8mths/ 242days)	June, 2018
Adewumi **Oyo state**	November, 2017	8th Dec, 2017 – 8th Jan, 2018(30days)	1st Nov, 2017 – 30th June, 2018 (8mths/ 242days)	June, 2018

Table 4.3: Estimated Flowering Duration of *Pycnanthus angolensis*

Location	Flowering Initiation (Days)	Flowering Formation (FF) (Days)
Gbongan	30	181
Ajaba	58	153
Otun	45	151
Ayetoro	62	181
Idito	30	242
Adewumi	30	242

4.3 Fruit phenology

4.3.1 Fruiting Duration/timing

Fruiting in *Pycnanthus angolensis* is from July of 2017–June of 2018. Nevertheless, Osun fruit timing spanned from July, 2017 through April, 2018; while (EK) Ekiti spanned from July, 2017 ended in May, 2018. Oyo fruiting time spanned from November, 2017 through June, 2018. The fruit developmental cycles phases are similar in all selected locations except time with slight relationship in various regions. Fruiting initiation (FrI) in *Pycnanthus angolensis* started from July, 2017 and spanned through November, 2017. Initiation of fruit in GB occurred in September of 2017, AJ and OT onset fruiting in July, 2017; AY: August, 2017, ID and AD: November, 2017. On the other hand, Otun (OT), Ajaba (AJ), Idito (ID) and Adewumi (AD) started fruiting at similar stage. Fruit formation (FF) started in September and finished formation at November, 2017.

4.3.2 Fruit maturation

Ripening of *Pycnanthus angolensis* occurs in the wet season with brown colour at an indicating fruit maturation designated as FrM_1 (Plate 4.9) followed by Green (FrM_2), greenish-Yellow (FrM_3) and Yellow as FrM_4. Fruit of *Pycnanthus angolensis* matures between 30 to 150 days. The FrM_1 in GB is 30days, AJ (150days), OT: (62 days), AY: (90days), ID: (61days) and AD starts fruit initiation in November 1st and ends in November 30th 2017 (30days) (Table 4.4).

In FrM_2, the time for fruit of *Pycnanthus angolensis* to change from Brown colour to Green colour differs greatly. In GB, FrM_2 took 105 days, AJ: November1st - 30th 2017 (30days). OT: In September 1st FrM_2 in Otun started changing and ended in November 30th, 2017, conversely, it started in Ayetoro from October 1st and ended in November 30th 2017, ID and AD changed their fruits from brown to green between November - December, 2017(61days), respectively. At FrM_3, fruit of *Pycnanthus angolensis* colour changed from Green to Yellow from 15th December ^{2017} - 15th January, 2018 (30 days) in GB. The FrM_3 in AJ, FrM_3 is from 15th November - 15th December, 2017 (30days), in OT, FrM3 is 1st - 30th November (30days), in AY FrM_3 is 1st November -31st December, 2017 (61days), ID FrM_3 is December1st, 2017 - January 31st, 2018 (61 days), while Adewumi (AD) FrM_3 is December1st - Feburary 29th, 2018 (89 days). At the FrM_4, fruit colour of *Pycnanthus angolensis* changed to Yellow. In GB, FrM_4 began changes from

1st January up till April 30th 2018. Ajaba FrM_4 started in December 1st, 2017 through Feburary 29th, 2018 (90days/3months), Otun (OT) FrM_4 commenced in December 1st, 2017 and turned yellow in March 31st, 2018 (121days/4months), while that of Ayetoro (AY), started changing from December 13th, 2017 and ended in May 5th 2018 (142 days), In Idito (ID), FrM_4 started appearing yellow colour in January 5th up till June 16th 2018 (160 days) while FrM_4 of AD started changing from Feburary1st up till 16th of June, 2018 (135 days) (Table 4.4).

4.3.3 Fruiting period

The fruiting period of *Pycnanthus angolensis* spanned for 8-10 months Gbongan fruiting is for 8 months (241days**)**, beginning from 1st September, 2017 and ended in 30th April, 2018. In 1st July, 2017, Ajaba fruiting period started July, 2017 and ended in 29th February, 2018, while that of Otun (OT) spanned for 9 months (274 days) from 1st July, 2017 up till 30th March, 2018. Contrarily, Fruiting period in AY extended for 10 months (304 days), starting from 1st of August, 2017 and ended in 30th May, 2018, Idito (ID) and Adewumi (AD) took 8 months (242days), from 1st November, 2017 and spanned till 30th June, 2018 but, Ayetoro experienced the longest fruiting period (304 days), Otun had 274days whereas Ajaba, Gbongan, Idito and Adewumi fruit for 8months. Fruiting period of Idito and Adewumi had 242 days; Ajaba had 243 days while Gbongan had the least 241days as seen in Table 4.4.

4.3.4 Fruit Dehiscence

In Table 4.4 and Plate 4.4, time of fruit splitting varied greatly from one location to another. Fruit splitting in GB lasted for 30 days starting from April 1st and ended in April 30th, 2018. Fruit splitting lasted for 2months in AJ, starting from March, 2018 through 30th of April, 2018. It took 121days for Fruit splitting to be completed in OT starting from 15th December, 2017 through 31st of March, 2018. Fruit splitting in Ayetoro (AY) was completed within 2months, starting from March, 2018 up till 30th of April, 2018; Fruit splitting in ID lasted for 6months in 181 days, starting from 25th of January, 2018 and ended in 30th June, 2018. Fruit dehisced for 91days in AD starting from April, 2018 till June 1st, 2018.

Plate 4.4: Fruit dehiscence of *Pycnanthus angolensis*

Table 4.4: Fruiting and maturation timing of *Pycnanthus angolensis* in selected locations

Locations	Fruit initiation /Onset (FrI)	Fruit formation (FrF)	Fruit Maturation/ colour			Fruiting Period	Fruit Dehiscent
			Fr_1 Brown-Green (days)(BG)	Fr_2 Green-Yellow(GY)	Fr_3 Yellow(Y)		
Gbongan	Sept.	Sept1 – Sept30, 2017 (30 days)	Sept 1 – Dec 15, 2017 (105 days)	Dec 15, 2017 – Jan 15, 2018 (30days)	Jan – April, 2018 (120 days)	Sept – April (8 months) 241 days	April (1 – 30, 2018) 30days
Ajaba	July	July, 2017-Nov. 2017 (150days)	Nov 1 – Nov 30, 2017 30days	Nov15 – Dec15, 2017 (30days)	Dec. , 2017– Feb., 2018 (90days)	July, 2017 – Feb., 2018 8months (243 days)	Dec, 2017 – Feb, 2018 3months (90days)
Otun	July	July – Aug.,2017 (62days)	Sept – Nov, 2017 (91days)	Nov1-30,2017 (30days)	Dec , 2017– March, 2018 (121days)	July , 2017– March, 2018 9 months (274 days)	Dec 1, 2017 – March 30,2018 3 months (121days)
Ayetoro	Aug.	Aug1 – Oct 30, 2017 (90 days)	Oct – Nov, 2017 (60days)	Nov 1 – Dec 30, 2017 61days	Dec13, 2017 – May5, 2018 (140days)	Aug,2017 – May, 2018 10months (304days)	March15-April, 15,2018 (30days)
Idito	Nov	Nov 1 – Nov30, 2017 (30days)	Nov 1 – Dec 30, 2017 (61days)	Dec 1, 2017– Jan 31, 2018 (61days)	Jan 5 –June16, 2018 (160 days)	Nov , 2017– June,2018 8 months (242days)	Jan – June, 2018 6 months (181days)
Adewumi	Nov	Nov 1 – Nov30, 2017 (30days)	Nov 1 – Dec 30, 2017 (61days)	Dec 1, 2017 – Feb 28,2018 (89days)	Feb 1 – June 16,2018 (135days)	Nov 1 – June 30 8months (242days)	April – June, 2018 3months (91days)

4.4 Fruit Maturation/Fruit Colour

There were different colours showing the stages of Fruit Maturation in *Pycnanthus angolensis*. In morphological descriptions of the fruits, it was observed that fruit colour changes from one stage of immaturity to maturity and from one location to another (Table 4.5 and Plate 4.5). During maturation, fruit colour changed from light olive (10Y 5/4) to dark olive green (5GY3/4), upon attaining physiological maturity the epicarp takes to yellow 2.5Y7/8 and 5Y7/8 in Ajaba, pale olive 10Y6/4 or pale yellowish green 5GY6/4 to olive green 5GY4/4 or pale olive 10Y6/4 to light olive brown 2.5Y5/6 or olive 5Y5/6 in Gbongan, dark olive green 5GY3/4 to olive yellow 5Y6/8 to yellow at 2.5Yand 5Y7/8 in Idito, dark olive 10Y3/4 to dark olive green 5GY3/4 to olive yellow 2.5Y 6/8 and 5Y 6/8 at maturity in Adewumi, pale olive10Y6/4 to olive yellow 5Y6/8 to 2.5Y7/8, 5Y7/8 or 8/8 in Otun. Fruit colour changed from light olive 10Y5/4 to dark olive green 5GY3/4 to olive yellow 5Y6/8 or yellow 2.5Y7/8 at maturity in Ayetoro (Table 4.5). This discussion is in agreement with the Munsell colour chart (2009) where fruit colour of *Pycnanthus angolensis* changed light green to yellow.

Table 4.5 Fruit colour of *Pycnanthus angolensis* in selected locations

Location	Colour Code @ Immaturity	Reference Number	Color-code @ Immaturity	Reference Number	Colour Code@Maturity	Reference Number
Ajaba	Light Olive	10Y5/4	Dark Olive Green	5GY3/4	Yellow	2.5 7/8, 5Y7/8
Gbongan	Pale olive /	10Y6/4	Olive green	5GY4/4	Lightolive brown	2.5Y5/6, 5Y5/6
	Pale yellowish Green	5GY6/4	Pale olive	10Y6/4	Olive	5Y5/6
Idito	Darkolive-Green	5GY3/4	Olive yellow	5Y6/8	Yellow	2.5Y7/8, 5Y7/8
Adewumi	Dark olive Green	10Y3/4	Dark Olive-	5GY3/4	Olive yellow	2.5Y6/8, 5Y6/8
Otun	Pale olive	10Y6/4	Olive yellow	5Y6/8	Yellow	2.5Y 7/8, 5Y7/8 or 8/8
Ayetoro	Light Olive	10Y5/4	Dark Olive Green	5GY3/4	Olive yellow/ Yellow	52.5Y 7/8 Y6/8

Location: Munsell Colour Chart (2009).

FrM_1 ⟶ FrM_2 ⟶ FrM_3 ⟶ FrM_4

KEY:

FrM_1 = Immature

FrM_2 = Immature

FrM_3 = Immature

FrM_4 = fully mature

Plate 4.5: Different colours showing the stages of Fruit Maturation of *Pycnanthus angolensis*

4.5 Study 3: The seed and fruit morphology of *Pycnanthus angolensis*

4.5.1 Variations in Fruits of *Pycnanthus angolensis*

Fruits sourced from six seed locations were assessed to know the special fruit qualities. Provenances/locations displayed significant differences in fruits. The weight, length and width of fruit of *Pycnanthus angolensis* varied among the six selected seed locations in the study. The fruit weight ranged from 12.52g at Ayetoro to 19.86g at Ajaba, Fruit width ranged from 3.85 cm at Idito to 5.26cm at Otun and Fruit Length ranged from 2.74 cm at Idito to 4.23cm at Gbongan (Table 4.7).

4.5.1.1 Fruit Weight (FWT)

The results from Table 4.6 showed that States and locations within the state had significant effect on fruit weight of *Pycnanthus angolensis* ($P<0.0001$ Confidence interval CI). At Tukey Multiple Comparison of means among the states (Table 4.7), Ekiti, Oyo, and Osun states had significant effects on fruit weight. Fruit weights from Adewumi (12.54g) and Ayetoro (12.61g) were similar/ overlapping but statistically significantly different from that observed from Otun (15.84g) and Gbongan (14.86g) which showed statistical similar mean values but statistically significantly different values from those of Idito (18.13g) and Ajaba (19.86g). However, highest significance 19.86g was observed in Ajaba and least fruit weight 12.54g was observed in Adewumi (Table 4.8)

4.5.1.2 Fruit Length (FL)

The results from Table 4.6 showed that different States and locations within each of the states had significant effect on fruit length of *Pycnanthus angolensis* ($P<0.0001$ Confidence interval CI). At Tukey Multiple Comparison of means among the states, Ekiti and Osun states showed significant effects on fruit length (Table 4.6), however, highest significant mean value of 4.98cm in Ekiti state followed by Osun state with mean value of 4.68cm. Oyo showed no significant effect on the fruit length (4.45cm) (Table 4.7). However, in Table 4.8, fruits showed significant differences in their length, and it varied from 3.85cm to 5.25cm. Highest fruit length was significantly recorded from Otun seed location (5.25cm) followed by Gbongan seed location (5.22cm), Adewumi (5.05cm), Ayetoro (4.71cm), and Ajaba (4.13cm). The least fruit length was observed in the fruits collected from Idito kernel location (3.85cm) (Table 4.8).

4.5.1.3 Fruit Width (FW).

The results from Table 4.6 showed that States and locations within the state had significant effect on fruit width of *Pycnanthus angolensis* (P<0.0001 Confidence interval CI). At Tukey Multiple Post HOC test of means among the states, Osun and Ekiti state significantly affected fruit width and Oyo states had no significant effect on fruit width However, highest significant fruit width mean value 4.98cm was observed in Osun state while Oyo state had least significant mean value of 3.27cm (Table 4.7). However, the mean of the width of fruits observed from Ajaba was found to be statistically insignificantly different from those occurring from Ayetoro and Otun while that of Ayetoro was observed to be similar to that from Gbongan. In addition, the width of fruits from Otun were observed to be similar to those of Gbongan and statistically significant as those fruits from Ayetoro

Table 4.8 showed that highest fruit width was documented in fruits sourced from Otun seed location (4.14cm), followed by Ayetoro (4.06cm), Adewumi (3.40cm), Ajaba kernel location (3.28cm) and Gbongan (3.10cm). However, the significantly least seed width was observed in Idito seed (2.76cm).

4.5.1.4 Fruit Bunch Weight (FBW)

The results from Table 4.6 showed that States and locations within each state had significant effect on fruit bunch Weight of *Pycnanthus angolensis* (P<0.0001 Confidence interval CI). At Tukey Multiple Comparison of means among the States, FBW in Osun and Ekiti showed no statistically different result while that of Oyo differed significantly from that observed from Osun and Ekiti. However, Oyo State had significant highest mean value of 821.95g effect on FBW while Osun had no significant effect on FBW at mean value of 311.29g (Table 4.7). Within locations, however, the mean bunch weight from Otun, Gbongan, Adewumi, Ayetoro and Ajaba were observed to be statistically insignificantly different from one another. Amongst these five, only the mean bunch weight from Gbongan was not statistically significantly different from those observed from Idito.In addition, significantly higher bunch weight was observed in Idito seed location of the mean value 1181.34g, followed by Otun (668.86g), Gbongan (573.79g),

Adewumi (462.55g), and Ajaba seed location (161.41g). The least bunch weight was recorded in Ayetoro seed location (117.54g). This indicated that Idito seed location had the highest bunch weight of 1181.34g (Table 4.8).

4.5.1.5 Number of fruit per bunch

The results from Table 4.6 showed that states and locations within the state had significant effect on fruit bunch Weight of *Pycnanthus angolensis* ($P<0.0001$ Confidence interval CI). At Tukey Multiple post HOC test of means among the States, Oyo and Osun be not statistically significantly different from each other but differed notably from observations from those from Ekiti state (Table 4.7). The number of fruits per bunch observed in Gbongan was observed to differ significantly from that observed from Otun but not notably dissimilar from the other places. Furthermore, it implies that fruit per bunch from all locations were observed to be statistically insignificantly different from one another, except from Otun which showed a statistically significant result to other locations.

Considerably higher number of fruit per bunch was observed in Otun seed location (120.00 fruits), followed by Idito (66.40fruits), Gbongan (56.70 fruits), Ayetoro (32.10 fruits), and Adewumi seed location (30.90 fruits). The least Number of fruit per bunch was recorded in Ajaba seed location (21.00 fruits) (Table 4.8). This indicated that Otun seed location had the highest number of fruit per bunch. In comparison with fruit bunch weight, at lower bunch weight less number of fruits was produced in Ajaba (161.41g; 21.00 fruits).

Table 4.6 Analysis of Variance on Fruit weight, length, width, bunch weight and number of fruit per bunch of *Pycnanthus angolensis*

Fruit weight Location of distinction	Degree of freedom	Sum of Square	Mean Square	F-value	p-value
Among states	2	1013.90	506.93	121.89	< 0.0001***
Among Locations within state	3	3334.60	1111.53	267.26	< 0.0001***
Within Location	594	2470.40	4.16		
Total	599	6818.90			
Fruit length					
Among states	2	28.88	14.44	82.22	< 0.0001***
Among Locations within state	3	146.27	48.76	277.66	< 0.0001***
Within Location	594	104.31	0.18		
Total	599	279.45			
Fruit width					
Among states	2	68.65	34.32	165.38	< 0.001***
Among Locations within state	3	98.50	32.83	158.17	< 0.001***
Within Location	594	123.31	0.21		
Total	599	290.45			
Bunch weight					
Among states	2	2606051.00	1303025.00	6.36	0.0033 **
Among Locations within state	3	4953348.00	1651116.00	8.06	0.0002 ***
Within Location	54	11066207.00	204930.00		
Total	59	18625606.00			
Number of fruit per bunch					
Among states	2	14871.00	7435.50	5.87	0.0050**
Among Locations within state	3	51306.00	17101.90	13.49	< 0.0001 ***
Within Location	54	68458.00	1267.70		
Total	59	134635.00			

Significant codes: "*" 0.001 "**" 0.01 "*" 0.1**

Table 4.7 Tukey Multiple Comparisons of Means among state

Variable	State		
	Osun	Ekiti	Oyo
Fruit weight	17.36[c]	14.22[a]	15.33[b]
Fruit length	4.68[b]	4.98[c]	4.45[a]
Fruit width	3.76[b]	4.10[c]	3.27[a]
Bunch weight	311.29[a]	390.90[a]	821.95[b]
Number of fruit per bunch	38.85[a]	73.85[b]	48.65[a]

Cells with the same letter is not significantly different from each other; the order of the letter corresponds to increase in measurement according to Tukey's HSD Post HOC

Table 4.8: Tukey Multiple Comparisons of Means within location of *Pycnanthus angolensis*

Variable	Location					
	Otun	**Gbongan**	**Adewumi**	**Ayetoro**	**Idito**	**Ajaba**
Fruit weight	15.84[b]	14.86[b]	12.54[a]	12.61[a]	18.13[c]	19.86[d]
Fruit length	5.25[e]	5.22[de]	5.05[d]	4.71[c]	3.85[a]	4.13[b]
Fruit width	4.14[ef]	3.10[bf]	3.40[d]	4.06[be]	2.76[a]	3.28[c]
Bunch weight	668.86[a]	573.79[ab]	462.55[ab]	117.54[a]	1181.34[b]	161.41[a]
Number of fruit per bunch	120.00[b]	56.70[a]	30.90[a]	32.10[a]	66.40[a]	21.00[a]

Cells with the same letter is not significantly different from each other; the order of the letter corresponds to increase in measurement according to Tukey's HSD Post HOC

4.5.2 Variation in Seeds of *Pycnanthus angolensis*

4.5.2.1 Seed colour

Seed locations of *Pycnanthus angolensis* determined slight disparity in seed colour model of their seed-coat (Table 4.9). Seed sourced from Ayetoro, Idito and Gbongan corresponded to theYellowish red group (YR) colour model, Ajaba corresponded to the red (5R) and yellowish red groups (5YR) at medium range while Adewumi and Otun corresponded to red groups at medium and high range (5R-10R) and yellowish red at low range (2.5YR).

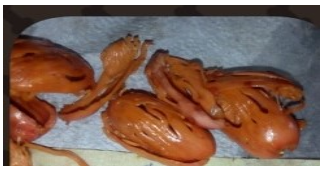
The color of *Pycnanthus angolensis* seeds is very variable. The colour of seeds varied from Reddish brown, dark reddish brown, brown to dusky red (Plate 4.7). They are dark reddish brown 2.5YR 2.5/3 and 5YR3/4 in Ajaba reddish brown 2.5YR4/4 OR 4/3, 5YR4/4, /brown 7.5YR4/4 in Gbongan, dark reddish brown 5YR3/3 and 2.5YR2.5/3 in Idito and 2.5YR and 5YR3/4 in Ayetoro, dark reddish brown 2.5YR2.5/3, 5R and 10R 3/3 and 3/4 / dusky red in Adewumi and Otun. It is difficult to distinguish between species depending on the colour of seed, because of the narrow range of colour degree between species, and most species have the same seed colour. Dark reddish brown was found in seeds collected from Ajaba, Idito, and Ayetoro, reddish brown in Gbongan and dusky red from seed location from Adewumi and Otun.

4.5.2.2 Aril colour

The colour of the aril is of high diagnostic and systematic value at different levels. The colour of the arils matched to the red group but varied from light red, light pink, pinkish white to pink. However, the arils were light red in Ajaba at 5R 7/8, and 7.5R 7/8, Gbongan 2.5R, 7.5R 6/8 and 10R 6/6, Idito7.5R 6/8 and 6/6 and Ayetoro 2.5YR 7/6, light pink/pinkish white in Adewumi at 7.5R 8/3, 2.5YR 8/2 and 5R 8/3, pink in Otun 2.5YR8/3 and 8/4 respectively (Table 4.9 and Plate 4.6). The colour is also used to distinguish between locations, light red was the the majority widespread colour in Ajaba, Gbongan, Idito and Ayetoro, for it is light pink/pinkish white in Adewumi and pink in Otun.

Table 4.9 Seed and Aril colour of *Pycnanthus angolensis* in selected locations

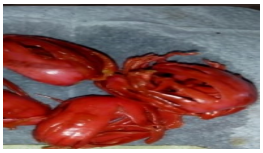
Location	SeedCode Colour	Reference Number	ArilCode Colour	Reference Number
Ajaba	Dark reddish brown	5R7/8	Light red	5R7/8
	Dark reddish brown	5YR3/4	Light red	7.5R7/8
Gbongan	Brown	7.5YR4/4	Light red	2.5YR6/6
	Reddish brown or 4/3	2.5YR 4/4	Light red	10Y6/6
	Reddish brown	5YR 4/4	Light red	7.5R7/8
Idito	Dark reddish brown	2.5YR 2.5/3	Light red	7.5R6/6
	Dark reddish brown	5YR3/3	Light red	7.5R6/8
Adewumi	Dark reddish brown	2.5YR2.5/3	Light pink	7.5R8/3
	Dusky red	5R3/4	Pinkish white	2.5YR8/2
	Dusky red	10R3/4	Light pink	5R8/3
Otun	Dark reddish brown	2.5YR 2.5/4	Pink	2.5YR 8/3
	Dusky red	5R3/3	Pink	2.5YR 8/3
	Dusky red	7.5R 3/3	Dusky red	10R 3/3
Ayetoro	Dark reddish brown	2.5YR 2.5/3	Light red	2.5YR 7/6
	Dark reddish brown	5YR3/4	Light red	



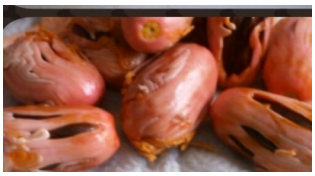
Otun Ekiti

Gbongan

Ajaba



Idito



Adewumi

Ayetoro

Plate 4.6: Variations in seed aril from selected locations of *Pycnanthus angolensis*

Ayetoro Ekiti Ajaba Otun Ekiti

Adewunmi Idito Gbongan

Plate 4.7: Variations in seed colour of *Pycnanthus angolensis* in A, B and C locations

KEY:

A= Osun state

B = Ekiti state

C = Oyo state

The weight, length and width of seeds of *Pycnanthus angolensis* varied among the six selected seed locations in the study. The seed weight ranged from 2.13g at Ayetoro to 4.35g at Ajaba, seed width ranged from 1.41cm at Ajaba to 2.41cm at Gbongan and length ranged from 2.88cm at Idito to 3.41cm at Otun (Table 4.4).

4.5.3 Seed Weight (SWT)

The results from Table 4.9 showed that states and locations within the state had significant effect on seed weight of *Pycnanthus angolensis* (P<0.0001 Confidence interval CI). At Tukey Multiple Comparison of means among the states seed, weight from Osun is statistically significantly different from Ekiti and Oyo states. However, Osun had highest significant mean value of 3.55g while least minimum seed weight 2.37g was observed in Oyo state (Table 4.10). However, the weight of seeds from Adewumi and Ayetoro were observed to statistically vary from each other while those of Idito and Gbongan were not statistically significantly different from each other. These, however, vary from those from Otun and Ajaba. Seed weights from Adewumi (2.16g) and Ayetoro (2.13g) were similar/ overlapping but statistically significantly different from seeds locations from Idito (2.57g) and Gbongan (2.75g) which showed statistical similar mean values but statistically significantly different values from those of Otun (3.10g) and Ajaba (4.35g). Maximum seed weight was observed from Ajaba with mean value of 4.35g and minimum mean value of 2.13g from Ayetoro. However, seeds from Ajaba had the highest mean seed weight of 4.35g, followed by the seeds from Otun 3.09g, Gbongan 2.75g, Idito 2.57g and Adewumi 2.16g, while Ayetoro recorded the least seed weight of 2.10g (Table 4.11). The results indicated that Ajaba had the highest seed weight 4.35g

4.5.3.1 Seed Length (SL)

The results from Table 4.9 showed that states and locations within the state had significant effect on seed length of *Pycnanthus angolensis* (P<0.0001 Confidence interval CI). At Tukey Multiple post Hoc.test of means among the states, the seed length observed in Oyo is not statistically significantly different from that observed in Osun but different from that observed in Ekiti state while that of Osun and Ekiti were not statistically significantly different. However, Ekiti had the highest significant mean value of 3.19cm on seed length

while Oyo state had no significant effect on seed length having the least mean value of 3.04cm (Table 4.10).

Furthermore, Table 4.11 showed that seed lengths from Idito and Ajaba produced statistically insignificantly different results to each other while they are both statistically insignificantly different from the seed length observed from Ayetoro. Furthermore, seed length from Gbongan and Adewumi were observed to be statistically insignificantly dissimilar from all other but statistically notably diversed from that observed from Otun. Seeds showed significant differences in their length, and it varied from 2.88cm to 3.40cm within the locations. Significantly higher SL was recorded from Otun seed location (3.40cm) compared to the other seed locations, followed by Gbongan seed location (3.34cm), Adewumi (3.20cm), and Ayetoro (2.98cm), Ajaba (2.88cm). The least SL was observed in the seeds sourced from Idito and Ajaba seed locations showed similar mean value of 2.88cm. This indicated that Otun in Ekiti State had the longest seed lenght of 3.40cm.

4.5.3.2 Seed Width (SW)

The results from Table 4.9 showed that states and locations within the state had significant effect on seed width of *Pycnanthus angolensis* ($P<0.0001$ Confidence interval CI). At Tukey Multiple Comparison of means among the states, seed width from Ekiti is statistically significantly different from Osun and Oyo states (Table 4.10). However, data from Table 4.11 Tukey multiple post HOC test showed that the observations from the different states were significantly ($P<0.05$) varied from one another. However, seed width from Ajaba was observed to be significantly ($P < 0.05$) different from those from every other location while those from Adewumi and Idito were observed to be statistically insignificantly different from each other. Seed width from Otun and Gbongan were statistically significantly special from each other. **S**eed width from Ajaba was observed to statistically significantly vary from those from every other location while those from Adewumi and Idito were observed to be statistically insignificantly different from each other. Highest seed width was recorded in seeds collected from Gbongan seed location (2.41cm), followed by Otun (2.39cm), Adewumi (2.17cm), Ayetoro (2.08cm), and Ajaba seed location (1.96cm). The considerably least seed width was observed in Idito seed

location (1.41cm). The results indicated that Gbongan and Otun seed locations had the highest seed width of 2.41cm.

Table 4.10: Analysis of Variance on seed weight, length and width of *Pycnanthus angolensis* seeds in various locations

Seed weight Per Location	**Degree of freedom**	**Sum of Square**	**Mean Square**	**F-value**	**p-value**
Among states	2	156.66	78.33	277.90	0.0001***
Among Location within States	3	183.48	61.16	216.98	0.0001***
Within Location	594	167.43	0.28		
Total	**599**	**507.57**			
Seed length per location					
Among states	2	2.17	1.09	8.19	0.0003***
Among Location within states	3	23.96	7.99	60.18	0.0001***
Within Location	594	78.83	0.13		
Total	**599**	**104.96**			
Seed width per location					
Among states	2	10.96	5.48	31.79	0.0001***
Among Location within states	3	56.58	18.86	109.37	0.0001***
Within Location	594	102.43	0.17		
Total	**599**	**169.98**			
Significant codes: "*" 0.001**	**"**" 0.01**	**"*" 0.1**			

Table 4.11 Tukey Multiple Comparisons of Means among states of *Pycnanthus angolensis* Seed

Variable	States		
	Osun	**Ekiti**	**Oyo**
seed weight	3.55^{c}	2.61^{b}	2.37^{a}
seed length	3.12^{ab}	3.19^{b}	3.04^{a}
seed width	1.92^{a}	2.25^{c}	2.06^{b}

Cells with the same letter are not significantly different from each other; the order of the letter corresponds to increase in measurement according toTukey's HSD Post HOC**.**

Table 4.12: Tukey Multiple Comparisons of Means within location of *Pycnanthus angolensis* Seed

Variable	Location					
	Otun	**Gbongan**	**Adewumi**	**Ayetoro**	**Idito**	**Ajaba**
Seed weight	3.10^{c}	2.75^{b}	2.16^{a}	2.13^{a}	2.57^{b}	4.35^{d}
Seed length	3.40^{cf}	3.34^{ef}	3.20^{e}	2.98^{bd}	2.88^{abc}	2.88^{ad}
Seed width	2.41^{e}	2.41^{e}	2.17^{bc}	2.09^{cd}	1.96^{bd}	1.42^{a}

Cells with the same letter are not significantly different from each other; the order of the letter corresponds to increase in measurement according toTukey's HSD Post HOC

4.5.3.3 Number of seeds/kg

This ranged from 230 to 470kg. However, one hundred *Pycnanthus angolensis* seeds weighed approximately 2.12g so that 212.75g contained approximately 470 seed at Ayetoro, followed by 2.16g containing 463 seeds at Adewumi, 2.75g containing 363 seeds at Gbongan, 2.57g containing 389 seeds at Idito, 3.10g contained 323 seeds at Otun while the highest mean weight 4.35g contained the least 230 seeds at Ajaba (Table 4.13).The result indicated that the more the seed weight on a tree, the lesser the seeds production and vice versa. Seed weights were categorized into three groups, heavy (>3.0), medium (>2.5) and light (< 2.5). However, seeds collected from Otun and Ajaba were regarded to be heavy (3.09g, 4.35g), medium seed weights were found from seeds collected from Gbongan and Idito (2.75g,2.57g) while light SWT were observed from seeds sourced from Adewumi and Ayetoro (2.16g , 2.10g), respectively.

4.5.3.4 Number of Striations /internal structures

Striations found in the internal structures of *P angolensis* seeds varied among the selected locations in numbers and in length. It ranged from 15 to 18 in number but varied in size with Long Stripes (LS) ranging from 2 to 4 and Short Stripes (SS) ranging from 12 to 15. However, Ayetoro had the highest number of striations 18, followed by tree locations from Ajaba, Gbongan, Otun and Idito having 16. The least number of striations 15 was found in Adewumi. Highest LS 4 was found in Gbongan followed by 3 in Ayetoro and 2 were found in Ajaba, Otun and Idito. Adewumi did not have long striations. Highest SS 15 was found in Ayetoro and Adewumi, followed by 14 LS in seeds locations from Ajaba, Otun and Idito. The least number of SS 12 was found in Gbongan (Table 4.14 and Plate 4.8). It was also observed from Tables 4.33 and 4.44 that seed with highest number of internal striations (18) had the least mean seed weight (2.11g) in Ayetoro.

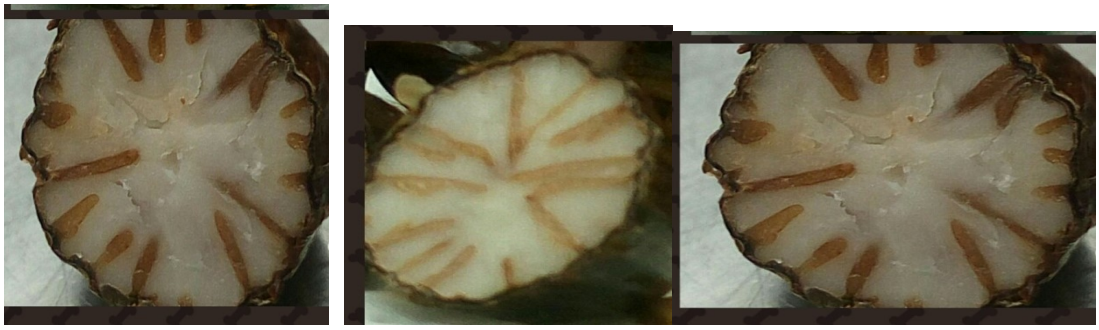
Table 4.13: Number of *Pycnanthus angolensis* seeds/kg per location

Location	Total	Mean	Number of seeds/kg
Otun	310.04	3.10	323
Gbongan	275.25	2.75	363
Adewumi	216.01	2.16	463
Ayetoro	212.75	2.12	470
Idito	256.98	2.57	389
Ajaba	435.1	4.35	230

Table 4.14: Form Distinction on Transverse Section of Selected *Pycnanthus angolensis* seed

Locations	Number of Striations	Number of Long Stripe (LS)	Number of Short Stripe (SS)
Ajaba (AJ)	16	02	14
Gbongan (GB)	16	04	12
Otun (OT)	16	02	14
Ayetoro (AY)	18	03	15
Adewumi (AD)	15	00	15
Idito (ID)	16	02	14

Adewumi **Otun** **Ayetoro**



Idito **Gbongan** **Ajaba**

Plate 4.8: Variation of striations in cross section of seeds of *Pycnanthus angolensis*

4.5.4 Effects of fruit metric characters on seed variables of *Pycnanthus angolensis*

This section assesses whether the fruit variables (weight, length and width) predict the seed parameters (weight, length and width).

4.5.4.1 Fruit weight and seed weight

The result in the Table 4.15 showed that there was an r of 0.764 and an r2 of 0.583 implying that there was a strong correlation between the self-determining variable and the dependent variable (seed weight). Also, the independent variable accounted for 58.3% of the distinction in the dependent variable. The result further showed that the model significantly predicted the dependent variable, meaning that the fruit weight significantly affected the seed weight. Further examination of the result revealed that fruit weight had a coefficient of 0.204 ($t = 28.92$; $p < 0.05$). This implied that for every distinction in the fruit weight, there was a change of 0.204 units in the seed weight.

4.5.4.2 Fruit Length and Seed Length

The result in the Table 4.15 showed that there was an r of 0.568 and an r2 of 0.323 meaning a moderate link between the FL and SL. This further implied that the fruit length accounted for 32.3% of the distinction in the seed length. The result further showed that the model significantly predicted the seed weight of length of the samples [$F (1,598) = 284.682$; $p < 0.05$]. Further examination of the result revealed that fruit length had a significant coefficient of 0.317 ($t = 16.873$; $p < 0.05$). This implied that for every unit change in fruit length, seed length changed by 0.317 units.

4.5.4.3 Fruit Width and seed Width

Table 4.15 showed that there was an r of 0.496 and an r^2 of 0.246; implying a moderate link among the fruit width and the SWD and that the FWD accounted for 24.6% of the distinction in the seed width. The result further showed that the model significantly predicted the seed width among the samples ($F = 195.597$; $df = 1,598$; $p < 0.05$). Thus, it could be concluded that fruit width significantly affected seed with among the samples. Further examination of the results revealed that fruit width had a significant coefficient of 0.314 ($t = 13.986$; $p < 0.05$), implying that for every unit change in fruit width, seed width increased by 0.314 units.

Table 4.15 Simple linear regression for effect of fruit metrics on seed metrics of *Pycnanthus angolensis*

	Unstandardized Coefficients		Standardized Coefficients	.	
	B	**Std. Error**	**Beta**	**t**	**p**
(Constant)	-0.349	0.113		-3.101	0.002
Fruit weight	0.204	0.007	0.764	28.916	0.000
r = 0.764	***r^2 = 0.583***	***F = 836.13***	***df = 1, 598***	***p = 0.001***	

	Unstandardized Coefficients		Standardized Coefficients		
	B	**Std. Error**	**Beta**	**t**	**p**
(Constant)	1.624	0.089		18.180	0.000
Fruit Length	0.317	0.019	0.568	16.873	0.000
r = 0.568	***r^2 = 0.323***	***F = 284.682***	***df = 1, 598***	***p = 0.001***	

	Unstandardized Coefficients		Standardized Coefficients		
	B	**Std. Error**	**Beta**	**t**	**p**
(Constant)	0.88	0.085		10.393	0.000
Fruit width	0.314	0.022	0.496	13.986	0.000
r = 0.496	***r^2 = 0.246***	***F = 195.597***	***df = 1,598***	***p =0.001***	

4.6 Effect of locations on *Pycnanthus angolensis* seed and seedling growth.

4.6.1 Variation in seed germination of *Pycnanthus angolensis*

4.6.1.1 Seed categorisation

Seed weights were categorized into three groups, heavy (>3.0), medium / intermediate (>2.5) and light (< 2.5). However, seeds collected from Otun and Ajaba were regarded to be heavy (3.09g, 4.35g), medium / intermediate-seed weights were found from seed collected from Gbongan and Idito (2.75g, 2.57g) while light SWT was documented from seeds sourced from Adewumi and Ayetoro (2.16g, 2.10g), respectively.

4.6.2 Seed germination

The total number of seed germinated from the selected locations ranged from 13 to 81%, the total number of seedlings ranged from 13 to 78% while the days to germination ranged from 27 to 49 days. In general, germination of *Pycnanthus angolensis* seed was 34%; 14% of light-weight seeds, Adewumi and Ayetoro as did 39%, 81% of intermediate-weight (Gbongan and Idito) and 38%, 13% of heavy-weight seeds (Otun and Ajaba). Germination began on the 44th day and finished on 49th day for light-weight seeds, on the 29th day and 41st day of intermediate- seed weight and on the 27th day and 36th day for heavy- seed weight. However, the highest germination percentage was recorded for seeds from Idito (81%) followed by those from Gbongan (39%), Ajaba (38%), Ayetoro (34%), and Adewumi (14%) while the least was recorded from Otun (Table 4.16).

4.6.3 Percentage survival of seedlings among locations of *Pycnanthus angolensis*

Percentage survival of seedlings after transplanting ranged from 11 to 53%. Among the locations, the highest percentage survival of seedlings was obtained from seeds in Idito at 56% of intermediate-weight, followed by Gbongan at 31% of intermediate-weight, Ayetoro at 23% of light-weight seeds, Ajaba at 15% of heavy-weight seeds, Otun at 13% of heavy-weight seeds while Adewumi had the least percentage survival of seedlings at 12% of light-weight seeds (Table 4.16).

Table 4.16 Variation in seed germination of *Pycnanthus angolensis* in selected locations

Locations	Number of seeds planted	Total germination (%)	Seedling survival	Germination Speed (days^{-1})	Percentage Survival After Transplanting (PSAT) (%)
Ajaba	100	38	18	27	15
Gbongan	100	39	32	29	31
Otun	100	13	13	36	12
Ayetoro	100	34	27	44	23
Adewumi	100	14	14	49	11
Idito	100	81	78	41	56

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4.6.4 Effect of seed locations on seedlings growth variables of *Pycnanthus angolensis*

4.6.4.1 Shoot height

Shoot height from the six different locations were statistically significantly different from one another. These measurements were also observed to change significantly over the observation times. However, there was no noteworthy difference in the effect of location on seedlings with respect to the observation times at 95 % confidence level (Table 4.17). From Table 4.18, no major effect was observed in shoot height of seedlings from Ayetoro with the mean values of 8.62cm and Otun 8.36cm. For shoot height, the marginal mean of shoot seedlings from Otun and Ayetoro were observed to be non-significant. These shoot height values differed significantly from values observed from Idito (19.10cm), Adewumi (8.62cm), Gbongan (17.21cm) and Ajaba (11.86cm) while the highest shoot height (19.10cm) was observed in Idito. However, the shoot heights from Idito (19.10 cm), Adewumi had 14.16cm, Gbongan 17.21cm, Ayetoro had.62cm, Ajaba with the mean value of 11.86cm and Otun with the mean value of 8.36cm were statistically different. In furtherance, shoot height from Idito with the mean value of 19.10cm was observed to be the maximum while shoot height from Otun with the mean value of 8.36cm was the least.

From Table 4.19, though there was progressive increase in shoot height from the first week of observation through to the twelfth week, it was observed that the increase recorded in shoot height for the first seven weeks with the mean value of 11.06cm, 11.85cm, 12.09cm, 12.09cm, 12.28cm, 12.76cm, 13.24cm, respectively were not significantly different. At the eighth week, there were major differences in shoot height with 13.77cm compared with the shoot in the seventh with the mean value of 13.24cm while this increment continued insignificantly until the twelfth week.

4.6.4.2 Number of nodes

From Table 4.17, observed NN from the different locations were statistically significant different. This number was identified to vary significantly across the twelve week of assessment. Furthermore, there were large effects in the interaction of location of seed collection with time. That is, the number of nodes presented by the seedlings was influenced by both the seed location and the time.

Number of nodes for Ayetoro with 4.33 and Otun 4.71 were not statistically significant dissimilar from one another but the NN for seedlings from Adewumi had the mean value of 7.50; Idito with mean value of 7.77 and Ajaba with the mean value of 6.85 were also found not to be statistically significantly different from one another. However, these results were statistically significantly different from those observed from Gbongan with (10.18). From Table 4.18, Gbongan had the utmost number of nodes with 10.18 while seedlings from Ayetoro presented the least number of nodes of 4.33. Also, the result from Table 4.19 showed that the number of nodes for the seedlings increased insignificantly for the first three weeks with 3.90, 4.66, and 5.18, respectively. At the fourth week, there were significant changes in the number of nodes with the mean value of 5.56 recorded for the variables. This increase continued by changing significantly at the sixth week with the mean value of 6.77. There was no significant difference from NN at seventh and eighth week with the mean values of 7.26 and 7.96) but this increment continued insignificantly until the twelfth week.

4.6.4.3 Number of leaves

From Table 4.17, the NL of the seedlings from the six locations was statistically significantly different from one another. Also, this distinction was observed to significantly vary with time. The seed location was observed to be influenced by time as this showed a significant result (that is, "location * time). Table 4.18 also showed that the NL for Ayetoro 2.52 and Otun with 3.18 were not significantly dissimilar from each other. The NL from Idito seedlings (5.23), Adewumi (5.67) and Ajaba (5.01) were also not significantly different from one another. This was however different for seedlings from Gbongan. Also, Gbongan had the maximum NL 7.80 while Idito had the least NL 5.23 (Table 4.18). Conclusively, NN and NL produced by seedlings from Ayetoro and Otun were observed to be the same; similarly, seedlings from Idito, Adewumi and Ajaba were observed to produce insignificantly different NN and NL. Gbongan had the significantly highest NN and NL from others (10.18 and 7.80), respectively (Table 4.18). Furthermore, Table 4.19 showed that the NL for the seedlings significantly varied at the seventh week at 5.10 compared to the number of leaves observed at the first weeks with 2.94, 3.29, 3.66, 3.92, 4.28 and 4.86 respectively. Also, the number of leaves recorded at the eighth week at 5.64 varied significantly from the number of leaves observed at the seventh week at

4.86. This increase continued insignificantly till the eleventh week at 6.57 until another significant increase was observed on the twelfth week at 6.97. All seedlings were observed to have a steady growth.

Table 4.17: Analysis of variance for *Pycnanthus angolensis* seedling growth location assessment

Shoot height Location Variation	**Sum of Square**	**Degree of freedom**	**Mean Square**	**F**	**p-value**	**Partial Eta Sq.**
Location	11467.41	5	2293.48	123.46	0.001	0.498*
Time	1342.40	11	122.04	6.57	0.001	0.104*
Location*Time	1264.87	55	23.00	1.24	0.123	0.099ns
Error	11573.16	623	18.58			
Total	**148282.64**	**695**				
Number of nodes						
Location	2722.35	5	544.47	77.03	0.001	0.382*
Time	2096.21	11	190.57	26.96	0.001	0.323*
Location*Time	535.17	55	9.73	1.38	0.041	0.108*
Error	4403.46	623	7.07			
Total	**42992.00**	**695**				
Number of leaves						
Location	2089.19	5	417.84	98.26	0.001	0.441*
Time	1080.63	11	98.24	23.10	0.001	0.290*
Location*Tim	577.01	55	10.49	2.47	0.001	0.179*
Error	2649.21	623	4.25			
Total	**23198.00**	**695**				

Significant *; ns = Not significant

Table 4.18: Effect of locations on *Pycnanthus angolensis* seedling growth

Variable	Location					
	Idito	**Ayetoro**	**Adewumi**	**Gbongan**	**Ajaba**	**Otun**
Shoot height	19.10^{e}	8.62^{a}	14.16^{c}	17.21^{d}	11.86^{b}	8.36^{a}
Number of nodes	7.77^{b}	4.33^{a}	7.50^{b}	10.18^{c}	6.85^{b}	4.71^{a}
Number of leaves	5.23^{b}	2.52^{a}	5.67^{b}	7.80^{c}	5.01^{b}	3.18^{a}

Cells with the same letter are not significantly different from each other; the order of the letter corresponds to increase in measurement according toTukey's HSD Post HOC

Table 4.19: Assessment of *Pycnanthus angolensis* Seedling growth

Variable	Time (in weeks)											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Shoot height	11.06^{a}	11.85^{ab}	12.09^{ab}	12.09^{ab}	12.28^{ab}	12.76^{abc}	13.24^{abcd}	13.77^{bcd}	14.01^{bcd}	14.43^{bcd}	15.19^{cd}	15.82^{d}
Number of nodes	3.90^{a}	4.66^{ab}	5.18^{abc}	5.56^{bc}	6.167^{bcd}	6.77^{cd}	7.26^{def}	7.96^{efg}	8.1^{efgh}	8.43^{fgh}	9.02^{gh}	9.61^{h}
Number of leaves	2.94^{a}	3.29^{ab}	3.66^{abc}	3.92^{abcd}	4.28^{bcd}	4.86^{cde}	5.10^{de}	5.56^{ef}	5.64^{ef}	6.01^{efg}	6.57^{fg}	6.97^{g}

Cells with the same letter is not significantly different from each other the order of the letter corresponds to increase in measurement according1 to Tukey's HSD Post HOC

4.7. *In vitro* propagation Techniques of *Pycnanthus angolensis*

4.7.1 Effects of hormonal Treatments, treatment levels, seed location and time of assessment for growth variables of regenerated *Pycnanthus angolensis*

4.7.1.1 Root length

The Analysis of variance result for RL is given, in Table 4.20; four main factors were considered (Treatment, treatment level, location and time). The result from the analysis showed that the treatments had significantly different effect from one another; the RL variable measurements on plantlets from the different locations were significantly different from one another; the treatment levels also showed a significantly different result compared to one another; and the measurements across time was equally significantly different. The interaction between the administered treatments (hormonal combinations) and the treatment levels, treatments and location of seed and treatment and time of observation were also observed to show significantly different RL values at 95% confidence level. Furthermore, interactions between treatment levels and location of seed, treatment level and time of observation and seed location and time of observation were observed to be insignificantly different for the RL.

The Post HOC test Table 4.21, for treatment levels for the root length considered in this study showed that, treatment level 0 and treatment level 3 were observed not to be significantly dissimilar from every other while treatment level 1 and treatment level 2 were observed not to be notably dissimilar from all other for the RL variable. Treatment level 4 for the RL was found to be significantly different from all other levels, having the least observed mean value of 0.006cm but level 1 had significant highest value 0.151cm on root length.This indicated that lower concentration of growth regulator has good effect on root elongation than higher concentration. From the post HOC test Table 4.22 of the location of kernel, it was found that idito, Ajaba and Adewumi produced RL with mean value of 0.035cm, 0.023cm, and 0.047 cm, which were insignificantly dissimilar from one another. However, RL ranged from 0.023 to 0.213 cm in Ajaba and Ayetoro. However, Ayetoro had the highest value of root length of 0.213 cm while Ajaba had the least value of root length of 0.023cm. This implies that embryonic explants location from Ayetoro had potential for elongation of root. This could be traced to genetic makeup of the embryos.

The post HOC test Table 4.23 for treatment types, showed results which were statistically insignificantly different for treatment types D, E, F, G, and H supplemented with 3mg/lKIN, 0, 1, 2, and 3mg/l BAP for the RL. Treatment types A, B and C supplemented with 0, 1 and 2mg/l KIN, however, were observed to produce statistically significant result from all the other treatment types. The growth assessment on the root length from Table 4.24 post HOC test for time of observation, no difference was observed for the RL and SH variables across the entire weeks of measurement weeks 2, 3, 4, 5 and 7 were similar but significantly different from weeks 3 and 6. However, at week 6, highest significant mean RL of 0.119cm as compared to week 4 with the least mean RL of 0.066cm.

Table 4.20: Analysis of Variance for the effects of treatment, level of hormones, locations/seed locations and time on root length of zygotic embryo of *Pycnanthus angolensis*

Location of Variation	Sum of Square	Degree Of freedom	Mean Square	F	p-value
Treatment	52.389	7	7.484	26.185	0.001*
Level	21.62	4	5.405	18.911	0.001*
Location	31.37	5	6.274	21.951	0.001*
Time	3.463	5	0.693	2.423	0.033*
Treatment vs Treatment Level	54.316	28	1.94	6.787	0.001*
Treatment vs Location of seed	84.676	35	2.419	8.465	0.001*
Treatment vs Time	22.99	35	0.657	2.298	0.001*
Treatment Level vs Location of seed	39.514	20	1.976	6.912	0.001ns
Treatment Level vs Time	7.926	20	0.396	1.387	0.117ns
Location of seed vs Time	8.128	25	0.325	1.138	0.289*
Treatment vs Treatment Level vs Location of seed	183.476	140	1.311	4.585	0.001*
Treatment vs Treatment Level vs Time	59.292	140	0.424	1.482	0.001*
Treatment vs Location of seed vs Time	83.187	175	0.475	1.663	0.001ns
Treatment Level vs Location ofseed vs Time	32.556	100	0.326	1.139	0.164ns
Treatment vs Treatment Level vs Location of seed vs Time	201.253	700	0.288	1.006	0.453*
Error	1644.864	5755	0.286		
Total	**2530.753**	**7194**			

Table 4.21: Effect of treatment levels on growth variables of *Pycnanthus angolensis*

Variable	Treatment Level(mg/l)				
	0	**1**	**2**	**3**	**4**
Root length (RL)	0.065^{b}	0.151^{c}	0.150^{c}	0.093^{b}	0.006^{a}
Shoot height (SH)	0.070^{b}	0.123^{c}	0.067^{b}	0.043^{ab}	0.004^{a}
Number of leave (NL)	0.063^{bc}	0.085^{c}	0.088^{c}	0.037^{ab}	0.001^{a}
Number of node (NN)	0.056^{b}	0.106^{c}	0.106^{c}	0.036^{ab}	0.004^{a}
Number of root (NR)	0.063^{b}	0.101^{b}	0.100^{b}	0.108^{b}	0.004^{a}

Cells with the same letter is not significantly different from each other; the order of the letter corresponds to increase in measurement according to Tukey's HSD Post HOC

Table 4.22: Effect of location of seed on embryonic plantlet variables of *Pycnanthus angolensis*

	Location of Seed					
Variable	**Ayetoro**	**Gbongan**	**Otun**	**Idito**	**Ajaba**	**Adewumi**
Root length (RL)	0.213^{c}	0.118^{b}	0.122^{b}	0.035^{a}	0.023^{a}	0.047^{a}
Shoot height (SH)	0.106^{bc}	0.061^{ab}	0.125^{c}	0.021^{a}	0.024^{a}	0.032^{a}
Number of leaves (NL)	0.080^{ab}	0.048^{ab}	0.094^{b}	0.027^{a}	0.027^{a}	0.051^{ab}
Number of node (NN)	0.099^{c}	0.058^{abc}	0.095^{bc}	0.026^{a}	0.041^{ab}	0.051^{abc}
Number of root (NR)	0.154^{c}	0.086^{ab}	0.097^{bc}	0.032^{a}	0.027^{a}	0.015^{ab}

Cells with the same letter is not significantly different from each other; the order of the letter corresponds to increase in measurement according toTukey's HSD Post HOC

Table 4.23: Effect of treatment types on growth of *Pycnanthus angolensis* using embryo

Variable	Treatment type							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
Root length (RL)	0.287^{d}	0.159^{c}	0.10^{bc}	0.066^{ab}	0.059^{ab}	0.019^{a}	0.014^{a}	0.041^{ab}
Shoot height (SH)	0.230^{c}	0.043^{ab}	0.091^{b}	0.062^{ab}	0.028^{ab}	0.000^{a}	0.011^{a}	0.027^{ab}
Number of leave(NL)	0.158^{d}	0.041^{ab}	0.110^{cd}	0.078^{bc}	$0.026^{a\,b}$	0.002^{a}	0.013^{ab}	0.009^{a}
Number of node (NN)	0.184^{c}	0.045^{ab}	0.099^{b}	0.074^{ab}	0.042^{ab}	0.013^{a}	0.010^{a}	0.027^{a}
Number of root (NR)	0.156^{a}	0.125^{bc}	0.087^{abc}	0.093^{abc}	0.052^{ab}	0.041^{a}	0.019^{a}	0.027^{a}

Table 4.24: Growth assessment of *Pycnanthus angolensis* for seven weeks from Embryo

Variable	Time of Observation (in weeks)					
	2	3	4	5	6	7
Root length (RL)	0.072^{a}	0.077^{a}	0.066^{a}	0.108^{a}	0.119^{a}	0.116^{a}
Shoot height (SH)	0.059^{a}	0.045^{a}	0.043^{a}	0.076^{a}	0.093^{a}	0.052^{a}
Number of leave (NL)	0.042^{ab}	0.030^{a}	0.051^{ab}	0.089^{b}	0.053^{ab}	0.062^{ab}
Number of node (NN)	0.051^{ab}	0.027^{a}	0.056^{ab}	0.080^{ab}	0.093^{b}	0.064^{ab}
Number of root (NR)	0.052^{a}	0.050^{a}	0.072^{a}	0.134^{b}	0.060^{a}	0.083^{ab}

Cells with the same letter is not significantly different from each other; the order of the letter corresponds to increase in measurement according to Tukey's HSD Post HOC

4.7.1.2 Shoot height (SH)

From the Table 4.21, at the Post HOC test for treatment levels, treatment level 0 and treatment level 2 were ascertained not to be significantly different from each other while treatment level 2 with mean value of 0.067cm and treatment level 3 with mean value of 0.043cm, levels 3 with mean value of 0.043 and 4 0.004cm were found not to be significantly different from each other for the SH variable but treatment level 1 had the highest significant mean value of 0.123cm while treatment level 4 with mean value of 0.004cm produced least SH. From the post HOC test Table 4.22 of the location of kernel, it was found that Idito, Ajaba and Adewumi produced SH with mean values of 0.021, 0.024, and 0.032 cm which were insignificantly different from one another. However, Otun had the highest value of shoot height at 0.125cm while Ajaba had the least value of shoot height at 0.021cm.

The post HOC test table 4.23 for treatment type showed results which were insignificantly ($P<0.05$) different for treatment types D, E, F, G, and H containing 3mg/lKIN, 0, 1, 2, and 3mg/l BAP) for the SH. Treatment types A, B and C supplemented with 0, 1 and 2mg/l KIN, however, were observed to produce statistically significant result from all the other treatment types. However, treatment type A supplemented with 0mg/l KIN (Kinetin) produced the highest growth shoot height (0.230cm) compared to other treatment types. The growth assessment on the shoot height from Table 4.24 post HOC test for time of observation, no difference was observed for the SH variables across the entire weeks of measurement. From the Analysis of Variance table 4.25 for SH, Treatment, treatment level and location (or seed location) were observed to produce significantly different results for SH observations while there was no significantly different result for the time of observation for SH. Furthermore, the interaction between treatment and treatment level, treatment and seed location, treatment level and seed location were observed to be significantly different from one another while the interaction between treatment and time, treatment level and time of observation and seed location and time of observation were not significantly different at the 95% confidence level.

Table 4.25: Analysis of Variance for the effects of treatment, level of hormones, locations/ seed locations and time on shoot height of zygotic embryo of *Pycnanthus angolensis*.

Location of Distinction	Sum of Square	Degree of freedom	Mean Square	F	p-value
Treatment	34.3	7	4.9	20.798	0.001*
Level	10.74	4	2.685	11.396	0.001*
Location	11.913	5	2.383	10.113	0.001*
Time	2.317	5	0.463	1.967	0.08ns
Treatment vs Treatment Level	45.334	28	1.619	6.872	0.001*
Treatment vs Location of seed	44.611	35	1.275	5.41	0.001*
Treatment vs Time	8.734	35	0.25	1.059	0.374ns
Treatment Level vs Location of seed	27.295	20	1.365	5.793	0.001*
Treatment Level vs Time	7.041	20	0.352	1.494	0.072ns
Location of seed vs Time	4.182	25	0.167	0.71	0.852ns
Treatment vs Treatment Level vs Location of seed	116.723	140	0.834	3.539	0.001*
Treatment vs Treatment Level vs Time	41.814	140	0.299	1.268	0.019ns
Treatment vs Location of seed vs Time	37.787	175	0.216	0.916	0.776ns
Treatment Level vs Location of seed vs Time	13.694	100	0.137	0.581	1ns
Treatment vs Treatment Level vs Location of seed vs Time	135.337	700	0.193	0.821	1ns
Error	1355.866	5755	0.236		
Total	**1897.59**	**7194**			

Significant * Not Significant = ns

4.7.1.3: Number of Leaves (NL)

Similarly, at the Post HOC test Table 4.21 for treatment levels on the variable NL showed that treatment level 4 (4mg/l) had 0.001 which was not appreciably special from treatment level 3 containing 3mg/l with mean value of 0.037. Furthermore, treatment level 0 (0mg/l) and treatment level 3 (3mg/l) were found not to be statistically dissimilar while treatment levels 1 (1mg/l) and 2 (2mg/l) showed no significant difference between one another, meaning that level 1 and 2 supplemented with 1mg/l and 2mg/l are the same but level 2 supplemented with 2mg/l had significant highest value 0.088 on number of leaves. From the post HOC test table 4.22 of the location of seed, it was found that idito, Ajaba and Adewumi produced NL with mean value of 0.027, 0.027, and 0.051 which were insignificantly different from one another. On the number of leave production, Otun had the highest leaf production of 0.094 while Ajaba had the least value of leaf production at 0.027.

The post HOC test table 4.23 for treatment type showed results which were statistically insignificantly different for treatment type D, E, F, G, and H containing 3mg/lKIN, 0, 1, 2, and 3mg/l BAP) for theNL. Treatment types A, B and C containing 0, 1 and 2mg/l KIN, however, were observed to produce statistically significant result from all the other treatment types.The highest treatment effect with mean 0.158 was observed when the seeds were treated with treatment A containing 0mg/LKIN followed by treatment C containing 2mg/LKIN, which was not significantly different in effect to observed effect of treatment A containing 0mg/LKIN. From the post HOC test table 4.24 for time of observation, a statistically different result was found to occur at the fifth week of measurement with mean value of 0.089. From the analysis of variance for NL means table 4.26, there was significant difference in the observed NL values for the treatment, treatment levels, location (or location of seed) and time. Similarly, the interaction between treatment and treatment levels, treatment and location of seed, treatment level and location of seed were ascertained to be significantly different. However, the interaction of treatment and time, treatment level and time and location of seed and time were ascertained not to be significantly dissimilar.

Table 4.26: Analysis of Variance for the effects of treatment, level of hormones, locations/ seed locations and time on number of leaves of zygotic embryo of *Pycnanthus angolensis*

Location of Distinction	Sum of Square	Degree of freedom	Mean Square	F	p-value
Treatment	19.701	7	2.814	13.596	0.001*
Level	7.522	4	1.881	9.084	0.001*
Location	4.484	5	0.897	4.332	0.001*
Time	2.378	5	0.476	2.297	0.043ns
Treatment vs Treatment Level	27.154	28	0.97	4.685	0.001*
Treatment vs Location of seed	32.957	35	0.942	4.549	0.001*
Treatment vs Time	8.89	35	0.254	1.227	0.168ns
Treatment Level vs Location of seed	11.269	20	0.563	2.722	0.001*
Treatment Level vs Time	4.364	20	0.218	1.054	0.393ns
Location of seed vs Time	4.825	25	0.193	0.932	0.56ns
Treatment vs Treatment Level vs Location of seed	91.18	140	0.651	3.146	0.001*
Treatment vs Treatment Level vs Time	38.181	140	0.273	1.317	0.008ns
Treatment vs Location of seed vs Time	37.392	175	0.214	1.032	0.372ns
Treatment Level vs Location of seed vs Time	20.754	100	0.208	1.003	0.474ns
Treatment vs Treatment Level vs Location of seed vs Time	152.311	700	0.218	1.0561	0.184ns
Error	1191.313	5755	0.207		
Total	**1653.775**	**7194**			

Significant * Not significant = ns

4.7.1.4 Number of Nodes (NN)

The Post HOC test, Table 4.21 for treatment levels the variable NN showed that treatment level 4 with mean value of 0.004 was not significantly different from treatment level 3 with mean value of 0.036. However, treatment level 0 with mean value of 0.004 and treatment level 3 with mean value of 0.036 were found not to be statistically different while treatment levels 2 and 3 showed no significant difference with one another, meaning that level 2 and 3 are the same having similar effect on number of nodes. Result from the post HOC test table 4.22 on NN showed that it ranged from 0.026 to 0.099 at Idito and Ayetoro. Invariably, explants from Ayetoro had the highest explant germination rate compared to other locations but not significantly different from explants from Gbongan, Otun and Adewumi. However, Ayetoro had the highest production of nodes 0.099 while Idito had the least value of number of nodes of 0.026.

The post HOC test Table 4.23 for treatment type showed results which were statistically insignificantly different for treatment types D, E, F, G, and H containing 3mg/lKIN, 0, 1, 2, and 3mg/l BAP for the RL. Treatment types A, B and C supplimented with 0, 1 and 2mg/l KIN, however, were observed to produce statistically significant result from all the other treatment types. In furtherance, treatment A supplimented with 0mg/lKIN was observed to produce the highest effect with mean value of 0.184 for NN variable compared to other treatment types. From the post HOC test Table 4.24 for time of observation, a statistically different result was found to occur at the fifth week of measurement while a statistically significant result was observed at the sixth week with mean value of 0.093. From the analysis of variance for NN means, Table 4.27; there is significant difference for NN values for the treatment, treatment level, location and time. The interaction of the main effects (that is, treatment, treatment level, location and time) all showed significant difference except the interaction between treatment level and time and location of kernel and time at the 95% confidence level**.**

Tables 4.27: Analysis of Variance for the effects of treatment, level of hormones, locations/ seed locations and time on number of nodes of zygotic embryo of *Pycnanthus angolensis*

Location of Distinction	Sum of Square	Degree of freedom	Mean Square	F	p-value
Treatment	21.018	7	3.003	13.26	0.001*
Level	11.44	4	2.86	12.631	0.001*
Location	5.184	5	1.037	4.579	0.001*
Time	3.238	5	0.648	2.86	0.014*
Treatment vs Treatment Level	38.913	28	1.39	6.138	0.001*
Treatment vs Location of seed	31.169	35	0.891	3.933	0.001*
Treatment vs Time	17.857	35	0.51	2.253	0.000*
Treatment Level vs Location of seed	18.129	20	0.906	4.003	0.001*
Treatment Level vs Time	5.361	20	0.268	1.184	0.257ns
Location of seed vs Time	4.161	25	0.166	0.735	0.826ns
Treatment vs Treatment Level vs Location of seed	119.512	140	0.854	3.77	0.001*
Treatment vs Treatment Level vs Time	38.592	140	0.276	1.217	0.043*
Treatment vs Location of seed vs Time	48.15	175	0.275	1.215	0.03*
Treatment Level vs Location of seed vs Time	22.608	100	0.226	0.998	0.486ns
Treatment vs Treatment Level vs Location of seed vs Time	174.927	700	0.25	1.104	0.038*
Error	1303.102	5755	0.226		
Total	**1863.936**	**7194**			

Significant * Not Significant = ns

4.7.1.5 Number of root (NR)

From the post Hoc., Table 4.21, treatment levels 0, 1, 2 with mean value of 0.063, 0.101, 0.100, respectively and treatment level 3 with mean value of 0.108 were ascertained not to be significantly dissimilar from every other but treatment level 3 had significant highest 0.108 on NR produced compared to level 0 with mean value of 0.063.

From the post Hoc, Table 4.22, highest NR (0.154) was observed in Ayetoro while Ajaba had the least value of 0.027 number of root. However, Ayetoro was found to produce best growth form traits of RL, NN and NR, Otun had the highest leaf production for photosynthesis. Ajaba was found to produce poor growth form traits of RL, NN, SH, NL and NR on *in vitro* regeneration of *Pycnanthus angolensis*. The post HOC test Table 4.23 for treatment type showed results which were statistically insignificantly different for treatment types D, E, F, G, and H containing 3mg/lKIN, 0, 1, 2, and 3mg/l BAP) for the NR. Treatment types A, B and C supplemented with 0, 1 and 2mg/l KIN, however, were observed to produce statistically significant result from all the other treatment types.

From the post HOC test Table 4.24 for time of observation, fifth week was observed to give a statistically significant high result for NR with mean value of 0.134**.** From the analysis of variance Table 4.28, all the main effects were found to be significant. Similarly, all the two way interaction effects were found to be significantly different except for treatment level and time of observation.

Table 4.28: Analysis of Variance for the effects of treatment, level of hormones, locations/ seed locations, time and their interactions on number of root of zygotic embryo of *Pycnanthus angolensis*

Location of Distinction	Sum of Square	Degree of freedom	Mean Square	F	p-value
Treatment	15.019	7	2.146	7.777	0.001*
Level	10.848	4	2.712	9.83	0.001*
Location	13.631	5	2.726	9.882	0.001*
Time	5.979	5	1.196	4.334	0.001*
Treatment vs Treatment Level	41.408	28	1.479	5.36	0.001*
Treatment vs Location of seed	49.153	35	1.404	5.09	0.001*
Treatment vs Time	31.23	35	0.892	3.234	0.001*
Treatment Level vs Location of seed	29.683	20	1.484	5.38	0.001*
Treatment Level vs Time	4.795	20	0.24	0.869	0.628ns
Location of seed vs Time	13.479	25	0.539	1.954	0.003*
Treatment vs Treatment Level vs Location of seed	142.973	140	1.021	3.702	0.001*
Treatment vs Treatment Level vs Time	79.253	140	0.566	2.052	0.001*
Treatment vs Location of seed vs Time	79.747	175	0.456	1.652	0.001*
Treatment Level vs Location of seed vs Time	23.311	100	0.233	0.845	0.865ns
Treatment vs Treatment Level vs Location of seed vs Time	210.613	700	0.301	1.091	0.059ns
Error	1587.707	5755	0.276		
Total	**2340.607**	**7194**			

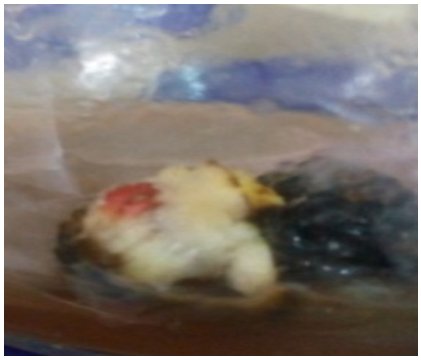
Significant * Not Significant = ns

Plate 4.9: Rooted plantlet from zygotic embryonic explants of *Pycnanthus angolensis*

Ajaba

Ayetoro

Ayetoro



Gbongan

Ayetoro

Plate 4.10: Performance of zygotic embryo of *Pycnanthus angolensis* from different locations

4.7.2 Callus induction in embyogenesis of *Pycnanthus angolensis*

It is worthy to note that zygotic embryo induced callus at different hormones and hormonal concentrations. Treatment media A produced 2 to 3 calli, B produced 1-3, C_0,C_1, D and F1 produced 2 to 3 calli. This indicated that callus was stimulated in MS incorporated with NAA alone at 2.0 and 3.0 mg/l, 2.0mg/l KIN singly and combined with 1.0 to 3.0 mg/l NAA, Kinetin with 1.0 mg/l NAA, 3.0mg/l KIN combined with NAA @ 2.0-3.0 mg/l and Benzyl amino purine @ 1.0 mg/l combined with 1.0 mg/l of NAA (No data).

In overall, a total of 14 explants induced callus at the bottom or cut edges of explants that showed white, brown, greenish yellow colour (Plate 4.8). Highest number of callus was observed in MS incorporated with 1.0 KIN combined with 1.0 to 3.0mg/l NAA and the minimum number of callus formation was found in MS incorporated with Kinetin (2.0mg/l) and 1.0 mg/lBAP combined with (1.0-3.0 mg/l NAA (Plate 4.8). Callus also occurred in all the selected locations for embryo explants but Ayetoro in Ekiti State had the maximum callus formation. Complete plantlet also possessed callus (Plate 4.9)

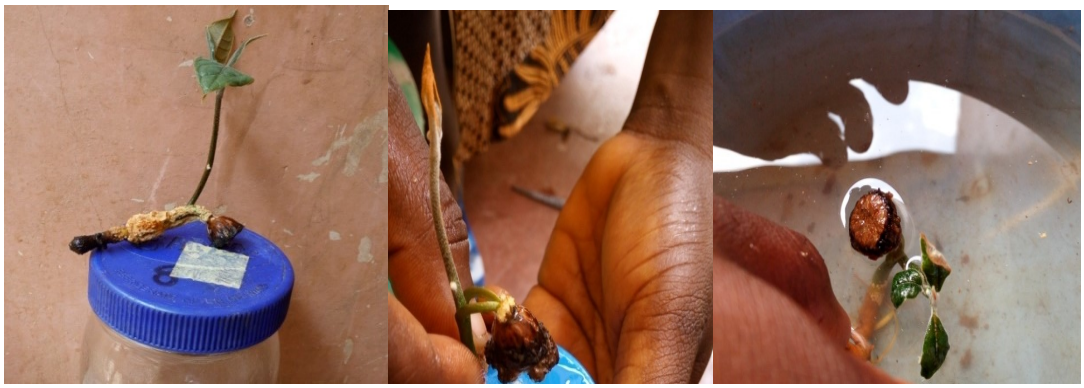
Plate 4.11: Callus formed from embryonic explant of *Pycnanthus angolensis*

Plate 4.12: Complete plantlet with callus formation of *Pycnanthus angolensis*

4.7.3: Acclimatization:

Complete plantlets were detached from the media, washed under running water to get rid of remains, hardened off inside the growth room in Nylon pots (8cm in diameter) filled with top soil for two weeks, covered with see-through polythene bags. After two weeks, pots were transferred and positioned in a greenhouse. One week later, the covers were removed gradually (Plate 4.13).

A total of twenty-eight plantlets were acclimatized. *In-vitro* regenerated plantlets from the following six locations thrived well *ex vitro* and finally transferred to the greenhouse. In GB: treatment C_2 had 4 plantlets, treatment B_3-1, And AY: : treatment A_1 had 3 plantlets, treatment C_1-2, and OT: A_1-2, A_0- 1, F_0-2, F_1- 1, D_0-2, AJ: treatment C_2- 1, D_0- 2, AD: treatment A_2 had 2 plantlets, treatment C_1 had 3 plantlets, while treatment D_3 had 2plantlets and ID had no surviving plantlet (0) with 4-25% plantlet survival after acclimatization.



a & b: Removal of plantlet from the medium c: Washing of plantlet from agar

d: Aclimatisation of plantlet with Nylon e: Plantlet exposed to screen house condition

Plate 4.13 Different acclimatization stages of *Pycnanthus angolensis* plantlets

4.8 Effects of Hormonal influence on nodal explants growth of *Pycnanthus angolensis*

4.8.1 Percentage of callus induction in nodal explants

In the current investigation, the nodal explants of *Pycnanthus angolensis* were employed for callus initiation. Medium incorporated with different concentrations of NAA alone (0mg/l – 3.0mg/l) combined with Kinetin, 0 – 3.0mg/l BAP modified with 10mg/l IBA to regenerate callus from the nodal explants of *Pycnanthus angolensis.* Maximum callus stimulation rate 30% was recorded in the treatments A(0 KIN+ 1.0 NAA+10 IBA and F(1.0 BAP +1.0 NAA +10 IBA), followed by B, C, E (20%) while D and G had the lowest of callus induction (15%). This result shows that Auxin and BAP at equal proportion NAA modified with 10mg/l IBA formed best callus.

4.8.2 Colour of callus formed

Colour of callus produced from nodal explants of *Pycnanthus angolensis* varied from brown, chocolate, brownish green, green, white and black based on the combination of growth regulators and concentrations (levels) (Table 4.29 and Plate 4.11). Die back also occurred in treatments A_3, E_1, F_3, and G_3-H_3 due to higher concentrations and combinations of hormones and browning. Callus formation occurred at different positions on the nodal explants from the base (Plate 4.15), axillary only, all over the explants (Plate 4.17), both at the tip and base (Plate4.16). Callus initiation first started along the cut-edge of the explant and margin, callus formation at both ends of cutting of nodal explants (Plate 4.16) and subsequently, the callus growth spread all over the explants (Plate 4.17).

4.8.3 Days of sprouting (Days)

Callus initiation from nodal explants was noticed at 12 - 97days after inoculation. It varies differently depending on the treatments. In treatment A, callus initiation was noticed from 14-29 days, treatment B initiated callus between 13-92 days, treatment C initiated callus between 17-66 days, treatment D initiated callus between 26 - 66 days, treatment E initiated callus between 23-63 days, treatment F initiated callus between 14-97 days, treatment G initiated callus between 12-97 days and treatment H did not support

callus induction at all (Table 4.29). This result indicated that higher concentration of hormones had longer days to initiation and vice versa. This also showed that small/ low concentrations of hormones stimulate earlier than higher concentration of growth hormones. Callus form was seen at the end of the first fortnight as cream to brown callus mass which, in later stages, turned into morphogenic callus (Table 4.29)

Table 4.29: Morphogenic Response of Callus Formed from Nodal Regeneration of *Pycnanthus angolensis*

Treatment/ Level with callus	Colour of callus formation	Position of callus formation	Number of explants with callus formation	Total number of explants per treatment with callus formation/%
A_0	Brownish green	Axillary	1	
A_1	i. Chocolate ii. Brown	Base	3	6(30)
A_2	i. Wet&White	Stem and base near media.	2	
	ii. Brown	All over the stem with white patches		
A_3	Die Back		0	
B_0	Brown	Base	1	
B_1	Greenish	Base near the media	1	4(20)
B_2	i. Wet white ii. Greenish	Auxiliary Point Base	2	
B_3	No callus formation		0	
C_0	Wet brown	Base	1	
C_1	Brown	Base near the media	1	4(20)
C_2	Chocolate & glossy	Base	1	
C_3	Brown	Base	1	
D_0	i. Brown/ Chocolate/ Black	Base	2	
	ii. Small brown	Base		
D_1	No response		0	3(15)
D_2	No response		0	
D_3	White with green patches	Base	1	
E_0	i. Brown	Both at tip and base all over the stem/ explant	2	
	ii. White			
E_1	Die back due to browning		0	4(20)
E_2	Brownish white	Round the explant	1	
E_3	Brown	Base	1	
F_0	Greenish	Base with elongation	1	
F_1	i. White ii. Brown &wet iii. White	Tip &base Base Thread like all over	3	6(30)
F_2	i. Brown ii. Black shinny/glossy	Base Base	2	
F_3	Die back			
G_0	i. Brown (smooth) ii. Whitish	Base Base	2	
G_1	Brownish	Base round the explant	1	5(25)
G_2	i. Brown with white patches ii. Whitish	Base all over	2	
G_3	Die back		0	
H_0	Die back		0	
H_1	Die back		0	0
H_2	Die back		0	
H_3	No response		0	

* Out of 20 explants inoculated per treatment (A-H)

Greenish Callus **Whitish Callus**

Plate 4.14: Different colours of callus formation from nodal explants of *Pycnanthus angolensis*

Callus formation at the tip **Callus formation at the base**

Plate 4.15: Different positions of callus formation from nodal explants of *Pycnanthus angolensis*

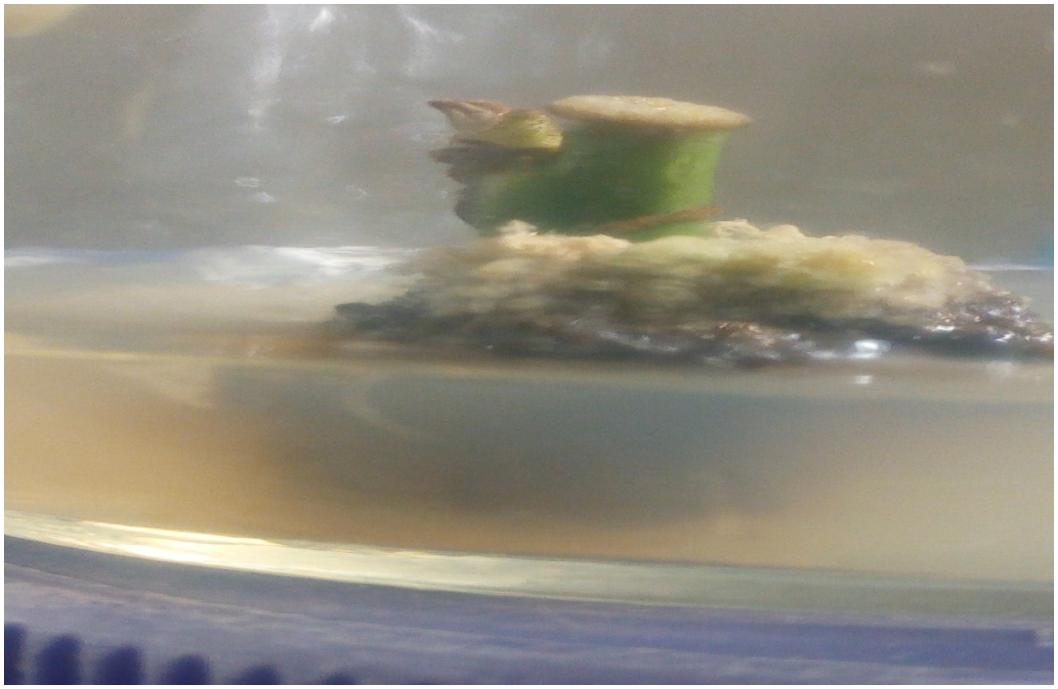


Plate 4.16: Callus formation at both ends of cutting of nodal explants of *Pycnanthus angolensis*

Plate 4.17: *Pycnanthus angolensis* Nodal explants showing callus induction of shoot on NAA 2.0mg/l + IBA 10.0 mg/l medium (Treatment F_1)

4.8.4 Shoot initiation

In our study, a total of 20% shoot was induced in media containing treatment A_1 supplemented with 1.0NAA+10 IBA, A_2 supplemented with 2.0 NAA+10 IBA, B_0 supplemented with 1.0 KIN+10 IBA, E_3 supplemented with 3.0 NAA+10 IBA and F_0 supplemented with 1.0 BAP + 10 IBA at 10-23 days but callus proliferation was very prominent (Table 4.30). This indicated that auxins alone produced more shoots than cytokinins (Benzyl amino purine and Kinetin) at 1 mg/l. Medium incorporated with 2 mg/l NAA +10mg/l IBA had early shooting while late shoot induction occurred in medium incorporated with BAP (1.0mg/l BAP+ 1.0mg/l NAA+10mg/l IBA). This could be traced to the effect of BAP and NAA at equal concentrations.

After shoot induction, number of leaves or shoots were 2 on the media supplemented with A_1 containing 1.0 NAA+10 IBA, and treatment A_2 supplemented with 2.0 NAA+10 IBA, but no leaf in media supplemented with B_0 containing 1.0 KIN+10 IBA, E_3 containing 3.0 NAA+10 IBA and F_0 containing 1.0 BAP+10 IBA (Table 4.30). This result indicated that at equal amount / concentration of BAP and KIN with 10.0 mg/l of IBA/NAA did not support leaf production.

Table 4.30: Effects of cytokinin and auxin on shoot multiplication from Nodal explants of *Pycnanthus angolensis*

Treatment	IBA	No of explants	Observations (no of explants formed, shoot buds)	Shoot induction (%)	Days of shooting
A0	0	5	-	0	
A1	10.0	5	1	20	14
A2	10.0	5	1	20	10
A3	10.0	5	-	0	0
B0	10.0	5	1	20	13
B1	10.0	5	-	0	0
B2	10.0	5	-	0	0
B3	10.0	5	-	0	0
C0	10.0	5	-	0	0
C1	10.0	5	-	0	0
C2	10.0	5	-	0	0
C3	10.0	5	-	0	0
D0	10.0	5	-	0	0
D1	10.0	5	-	0	0
D2	10.0	5	-	0	0
D3	10.0	5	-	0	0
E0	10.0	5	-	0	0
E1	10.0	5	-	0	0
E2	10.0	5	-	0	0
E3	10.0	5	1	20	14
F0	10.0	5	1	20	23
F1	10.0	5	-	0	0
F2	10.0	5	-	0	0
F3	10.0	5	-	0	0
G0	10.0	5	-	0	0
G1	10.0	5	-	0	0
G2	10.0	5	-	0	0
G3	10.0	5	-	0	0
H0	10.0	5	-	0	0
H1	10.0	5	-	0	0
H2	10.0	5	-	0	0
H3	10.0	5	-	0	0

Table 4.31: Growth related parameters of *in vitro* Propagation of *Pycnanthus angolensis* through nodal explants.

Treatment/ Treatment level	Size of shoot (cm)	Presence of root (cm)	Nature and number of leaf	Number of explant per treatment for callus formation	Days of sprouting
A_0	No shoot (callus)	No root	-	1	29
A_1	0.5	No root	Two leaves	3	14
A_2	0.2	No root	Two leaves	2	18
A_3	No shoot	No root	-	0	0
B_0	0.6	No root	-	1	13
B_1	No shoot (callus)	No root	-	1	97
B_2	No shoot(callus)	No root	-	2	92
B3	No shoot	No root	-	0	0
C0	No shoot (callus)	No root	-	1	66
C1	No shoot (callus)	No root	-	1	17
C2	No shoot	No root	-	1	20
C3	No shoot(callus)	No root	-	1	33
D0	No shoot (callus)	No root	-	2	66
D1	No shoot(callus	No root	-	0	29
D2	No shoot	No root	-	0	0
D3	No shoot(callus)	No root	-	1	26
E0	No shoot (callus)	No root	-	2	23
E1	No shoot(callus	No root	-	0	63
E2	No shoot	No root	-	1	63
E3	No shoot	No root	-	1	26
F0	0.3	No root	-	1	14
F1	0.2	No root	-	3	26
F2	No shoot	No root	-	1	28
F3	No shoot (callus formed)	No root	-	1	97
G0	No shoot(callus	No root		2	21
G1	0.7	No root	-	1	12
G2	No shoot (callus)	No root	-	2	97
G3	No shoot	No root	-	0	0
H_0	No shoot	No root	-	0	0
H_1	0	0	0	0	0
H_2	No shoot	No shoot	0	0	0
H_3	No shoot	No root	0	0	0

0/- = No response

Table 4.32 Callus induction in nodal explants of *Pycnanthus angolensis* at various treatment levels

Treatment	IBA	Number of explants	Observations (no of explants formed, shoot buds)	Callus responded (%)
A0	0	5	1	20
A1	10.0	5	3	60
A2	10.0	5	2	40
A3	10.0	5	-	-
B0	10.0	5	1	20
B1	10.0	5	1	20
B2	10.0	5	2	40
B3	10.0	5	-	-
C0	10.0	5	1	20
C1	10.0	5	1	20
C2	10.0	5	1	20
C3	10.0	5	1	20
D0	10.0	5	2	40
D1	10.0	5	-	-
D2	10.0	5	-	-
D3	10.0	5	1	20
E0	10.0	5	2	40
E1	10.0	5	-	-
E2	10.0	5	1	20
E3	10.0	5	1	20
F0	10.0	5	1	20
F1	10.0	5	3	60
F2	10.0	5	1	20
F3	10.0	5	1	20
G0	10.0	5	2	40
G1	10.0	5	1	20
G2	10.0	5	2	40
G3	10.0	5	-	-
H0	10.0	5	-	-
H1	10.0	5	-	-
H2	10.0	5	-	-
H3	10.0	5	-	-

Where:

K=Kinetin, BAP (B) = Benzyl Amino Purine, NAA= Naphthalene Acetic Acid

A, B, C, and D = 4 levels of Kinetin (0-4 mg/l)

E, F, G, and H = 4 levels of BAP (0-4 mg/l)

KN= Kinetin and NAA, BN= BAP and NAA

CHAPTER FIVE
DISCUSSION

5.1 Population frequency of *Pycnanthus angolensis*

Morphological variations in fruit and seed qualities among the natural population are helpful in assortment programme for heritable improvement of forest species (Kaushik *et al*., 2007). For screening the naturally obtainable inherited variations to select the most excellent planting material for attaining higher yield, location variation tests are very much necessary (Bhat and Chauhan, 2002). Such tests can yield important information that may be helpful for marketable planters, nursery growers, foresters and tree breeders. Fruit and seed variability can be associated to the hereditary potential of a genotype (Pavithra *et al*., 2013). Understanding intra and inter population distinction for reproductive traits would be fundamental steps for domestication. Information generated through such studies help in further selection and multiplication of high yielding genotypes.

5.1.1 Abundance and distribution

Pycnanthus angolensis was spread in all the study sites of the considered area, although it was very uncommon in Ajaba and Idito. It was prominent that the community and abundance of this plant were slightly reduced with the alteration in territory type within the study area. It appeared with a high distribution frequency in Gbongan, Otun, Adewumi, and Ayetoro, whereas the species showed with low distribution occurrence in Idito. *Pycnanthus angolensis* had 10.5% distribution occurrence in the abandoned or fallow land (protected area) of Osun state. It was either occasionally or rarely available in Ekiti state. It was very rare in the highly grazed and disturbed sites of Oyo state and this concurs with Adekunle *et al.* (2010).

The current study established that the embattled plant mostly grow on moist habitat. This result displayed similarity with Orwa *et al*. (2009) who reported that *Pycnanthus angolensis* was established in the moist lowland rainforests

5.1.2 Fruiting Trees

The tree status varied across the states from juvenile to maturity. A total of thirty-nine (39) trees of *Pycnanthus angolensis* existed as juvenile and nineteen (19) as mature trees (flowering and fruiting). However, tree status varied in ratios of 30:6 in Osun state, 9:7 in Ekiti state and 0:6 in Oyo state respectively. The highest 30 juvenile trees were found in Osun state, the least juvenile 9 in Ekiti state and no juvenile was found in Oyo state. The origin with principal quantity of fruiting trees (7) was found in Ekiti state while the lowest fruiting trees (6) was recorded from Oyo and Osun states respectively.This indicated that Osun State had more juvenile trees than Ekiti and the origin with highest number of fruiting trees (7) mature was Ekiti state. The large distinction as shown by the percentage frequency of occurrence of 39 juvenile trees and 19 fruiting trees probably is an indication of the huge distinction that exists in the wild (Muok *et al*., 2000).

5.2 Flower and fruiting duration of *Pycnanthus angolensis* in Selected States

Germination, leafing, flowering, fruiting and growth are the periodic phenomena in plants from which the most important events of the phenology in flowering are the timing and interval.

5.2.1 Floral duration of *Pycnanthus angolensis*

The reproductive units (flowers) are hairy, thick and scented, with inflorescence (panicles) rust in colour while the individual flowers are tightly packed pending the enlargement of stamens. Onocha and Otunla (2010) had similar result. The reproductive units are unisexual flowers (androecium and gynoecium are separate) formed with small, stalkless 3-lobed enclosed dim chocolate hairs. The inflorescences are basipetal (from the base to the apex) (Plate 4.2) while the male reproductive organs are fused together with no unique filaments. This result matched with Xu *et al*. (2010), but they are in clusters of inflorescence. The floral duration /timing are referred to as the periods of the budding stage were experiential to be extended to permit the assembly of genetic resources for enrichment and hybridization in and among the locations (Ujor, 1984). Floral duration is a factor of location which takes place at different times between September-June.The results concur with Lok (1983). Phase of flowering in *Pycnanthus angolensis* is prolonged

as in *Irvingia* (22-80 days) (Ujor, 1984); *Terminalia ivorensis* (40-110 days) and *Triplochiton scleroxylon* (40-90 days) (Oni *et al*., 1991; Oni, 1985). Nevertheless, flowering in Osun occurred in September up till January, that of Ekiti started flowering in November and ended in April while Oyo flowering begins in November and ended in June. Bawa *et al.* (2003) explained that flowering timing varied greatly in cold regions. Plants from the same area like Gbongan and Ajaba did not have the similar phenological prototype. Identical result was accounted by Gomez (1993) that vegetation from the same area does not have the similar phenological guide. Disparity in the flowering life cycle procedures can be from days to year according to Opler *et al*. (1980).

Flowers of *Pycnanthus angolensis* were seen mostly in October and January as small clustered flowers in rusted colour, especially in December. This colour is observed by Agyare (2009). In contrary, single flowers were difficult to identify and this is similar to Mapongmetsem (2007). Falling of inflorescent in Ajaba might be as a result of geo-coordinate on latitude. Late falling of inflorescent might be due to position on which the trees were found on longitude, and altitude. The variation varied from one place to another. Flowering during studying periods specifies seasonal events of flowering and fruiting life cycles in *Pycnanthus angolensis* as well as in sources.

5.2.2 Fruiting duration of *Pycnanthus angolensis* in selected states

Pycnanthus angolensis ripens for a long period of time (8-10 months) and this is similar to the finding of Perez *et al.* (2005) except that the fruiting period did not endure into the next flowering season, which begins around October as claimed by Perez *et al.* (2005). Fruiting lasted for 241 days in Gbongan, 243 days in Ajaba, 274 days in Otun Ekiti, 304 days in Ayetoro Ekiti, 242 days in both Idito and Adewumi. Fruit maturation and ripening in *Pycnanthus angolensis* requires eight to ten months and these events occur in the rainy seasons at which time there is seed dispersal. Fruiting tends to be seasonal as those plots with more fruiting trees were found to have fewer fruiting trees the following fruiting season. Given the fact that *Pycnanthus* seeds are highly recalcitrant, fruit ripening during the rainy season could be a dispersal adaptation for maintenance of the species in its environment.

5.3 Morphological variations in Fruits of *Pycnanthus angolensis*

Variations in nature are responsible for creating provenances, clones, races and ecotypes (Zobel and Talbert, 1984). These distinctions are important location for a tree breeder to improve a species. Distinctions can be successfully utilized for adaptability of a species e.g. drought resistance or selection of a suitable genotype for growth or fruit quality and so on (Sundaram *et al*., 2003). The genetic gain can be realized by making kernel collections from phenotypically and genotypically superior trees or stands.

5.3.1 Fruit Maturation/Fruit Colour

Colour of the shell in fruits is an essential structure to recognize maturity clearly for nearly all fruit varieties. Speedy change in shell colour from green to yellow is identified during the maturity stage of several fruits as a result once harvesting and marketing fruits, surface colour can be used for maturity index, quality index as well as fruit damage index (Amarasinghe and Sonnadara, 2009). It is a high-quality to be used in deciding the maturity of fruits from the day of harvest (Amarasinghe and Sonnadara, 2009). There were different colours showing the stages of fruit maturation in the stages of *Pycnanthus angolensis*. Morphological descriptions of the fruits was observed that fruit colour changed from one stage of immaturity to maturity and from one location to another fruits collected from Ajaba, Adewumi, Otun, Ayetoro and Gbongan matched to the yellow group (Y) colour pattern which varied from light olive, pale olive and dark olive while Idito matched to the green (G) group which varied into dark olive green at immaturity.

At maturity all fruits from the six locations matched to the yellow group but there were distinction in the colour potency between these locations.Yellow colour in Ajaba and Idito, light olive brown/ olive in Gbongan, olive yellow in Otun and Ayetoro. This distinction in the colour potency could be traced to ecological and evolutionary (Bums and Dalen 2002). Fruit colour is considered as an indicator of seed maturation mainly in forestry by several researchers (Srimathi, 1997). Willan (1985) also indicated that fruit colours serve as a device for collection of good quality seeds in forestry.

5.3.2 Fruit weight

States and locations within the state displayed significant effect on fruit weigth of *Pycnanthus angolensis* (P<0.0001 Confidence interval CI) and at Tukey Multiple Comparison of means among the states. State displayed significance differences on fruit weight in which Osun state produced highest fruit weight while Ekiti state had the least.This could be traced to edaphic and climatic conditions of the habitats. The finding in this study is similar to Friis (1992) and Willan (1985). However, fruit location from Ajaba had the highest mean fruit weight while Adewumi recorded the least fruit weight. This may be accredited to hereditary differences resulted from alteration of diverse locations to various ecological conditions and soil types (Elmagboul *et al*., 2014).

This explains the plain trends that the superior the fruit weight the better the seed weight. The results are supported by Mkonda *et al*. (2003) who reported strong correlation between seed weight and fruit weight in *Strychnos cocculoides* because the sink strength of fruits depends on the numbers and sizes of the seed they contain. Fruit weight could therefore be of an important consideration in breeding programs for good fruit production. Ajaba and Adewumi were constantly dissimilar from other populations' most likely showing that they are distinctive ecotypes. The other four populations' were usually in-between which may imply that they are hereditarily similar.

Fruit weight observed from Adewumi and Ayetoro were similar but statistically significantly different from that observed from Otun and Gbongan which showed statistical similar mean values but statistically significantly different values from those of Idito and Ajaba. However, Ayetoro (Ekiti state) and Adewumi (Oyo state), Otun (Ekiti state) and Gbongan (Osun state) had overlapping which means that they have the same genetic component. It is known that, fruit traits are seriously controlled by environment, cultural practices and gene effects (Kasvanga *et al*. 2007).

5.3.3 Fruit Length

Among states, Ekiti state significantly affected fruit length with mean 4.98cm while Oyo state had the least 3.27cm fruit length. Within locations among states, fruits showed significant differences in their length, and it varied from 3.85cm to 5.25cm. Significantly higher fruit length was recorded from Otun seed location (5.25cm) contrast to the other seed locations, followed by Gbongan seed location (5.22cm), Adewumi (5.05cm), Ayetoro (4.71cm),), and Ajaba (4.13cm). The least fruit length (3.85cm) was evidence in Idito seed location. This could be due to adjustment to unusual locations and different ecological circumstances and soil types (Elmagboul *et al*., 2014).

5.3.4 Fruit Width

Result showed that highest fruit width (4.14cm) in Otun seed location followed by Ayetoro (4.06cm), Adewumi (3.40cm), Ajaba (3.28cm), and Gbongan seed location (3.10cm). The significantly least seed width (2.76cm) was observed in Idito. These results indicated that Otun Ekiti had the highest fruit width. Fruit widths locationd from Gbongan and Ayetoro, Otun and Ayetoro including Otun and Gbongan were not significantly different from each other due to overlapping. This implies that locations with overlapping were hereditarily similar (Shankar, 2006).

5.3.5 Bunch Weight

Provenances displayed that states and locations within the state had significant effect on fruit bunch Weight of *Pycnanthus angolensis* ($P<0.0001$ Confidence interval CI). At Tukey Multiple post Hoc test of means among the states, the Bunch weight from Osun and Ekiti are statistically insignificantly dissimilar from every other whereas these are statistically significantly special from that of Oyo. In addition, the mean bunch weight from Otun, Gbongan, Adewumi, Ayetoro and Ajaba were observed to be statistically insignificantly different from one another. Amongst these five, only the mean bunch weight from Gbongan was not statistically significantly different from those observed from Idito. This could be traced to environmental conditions, soil and geographical position of tree (Elmagboul *et al*., 2014). Bunch weight from Idito and Ajaba including Ayetoro were significantly dissimilar from each other and this could be attributed to environmental conditions, soil and geographical position of tree (Ginwal *et al*., 2005).

5.3.6 Number of fruit per tree bunch

Locations displayed significant differences (P<0.0001) in fruit per bunch. The fruit per bunch from Oyo and Osun states were not statistically considerably dissimilar from each other but differed a lot from observations from those from Ekiti. Higher number of fruit per bunch was recorded in Otun seed location (120.00 fruits), followed by Idito (66.40 fruits), Gbongan (56.70 fruits), Ayetoro (32.10 fruits), and Adewumi seed location (30.90 fruits). The least number of fruit per tree bunch was recorded in Ajaba seed location (21.00 fruits). This indicated that Otun seed location had the highest number of fruit per bunch. In comparison with fruit bunch weight, at lower bunch weight less number of fruits was produced in Ajaba.This could be attributed to latitudinal position of the tree.

Numbers of fruit per bunch locationd from Otun and Adewumi, Ayetoro, Ajaba, including Gbongan were not considerably dissimilar from each other. This implies that locations with overlapping were genetically similar. The number of fruits per tree and the fruit quality has to be the target for planters to select elite trees, and research has to encourage the efforts of tree improvement and conservation of genetic relocations of this species. It was also observed from the studies that the more the fruit weigth, the lesser the fruit/seed production and this could be attributed to local environmental factors. This is similar to Ague *et al*. (2016) report that fruit sizes and seed productions significantly differed between trees, suggesting those differences in conditions among trees and their interaction with the local environment affect fruit and seed production

5.4 Seed morphology

Information on form distinction within seed qualities along with the locations of a species has been reported to be valuable for tree development programs (Singh *et al*., 2010). Seed coat is an exceptional trait that affects plants in nearly all cases and with varieties. Researchers have made-up that seed coat colour is the main issue in the quality of the seed and seed coat colour distinction to seed quality distinctions. Variability revisions are required in support of better output and upcoming proliferation work.

5.4.1 Seed weight

States and locations within the state displayed significant effect on kernel weight of *Pycnanthus angolensis* (P<0.0001 Confidence interval CI) and at Tukey Multiple post Hoc. Test of means among the states. This could be traced to edaphic and climatic conditions of the habitats The findings in this study is similar to Friis (1992) who reported that distinction in weight and dimensions of seeds may result from the diversity in edapho-climatic conditions of the habitats combined with genetic variability. However, seeds locations from Ajaba had the highest mean seed weight while Ayetoro recorded the least seed weight.

The distinction in seed qualities for individual trees show clear trends that the higher the fruit weight the higher the seed weight. The results are supported by Mkonda *et al*. (2003) who reported strong relationship between seed weight and fruit weight in *Strychnos cocculoides* because the sink strength of fruits depends on the numbers and sizes of the seed they contain. Fruit weight could therefore be of an important consideration in breeding programs for good fruit production. Ajaba and Ayetoro were constantly special to other locations because they have exceptional ecotypes. The other four communities were generally intermediate/ overlapping which may imply that they are genetically similar. However, fruit and seed qualities are greatly influenced by environment; cultural practices and additive gene make up (Kasvanga *et al*. 2007).

5.4.2 Seed Length

Seeds showed significant differences in their length, and it varied from 2.74cm to 4.23cm. Significantly higher fruit length was recorded from Gbongan seed location (4.23cm) compared to the other seed locations, followed by Otun seed location (4.14cm), Ayetoro (4.06cm), Adewumi (3.79cm), and Ajaba (3.29cm). The least fruit length was recorded in the fruits collected from Idito seeds location (2.74cm). This may possibly be as a result of genetic make up in order to adapt to different locations and ecological setting. Otun and Gbongan, Ayetoro, Idito and Ajaba were not significantly different from each other which may implies that they are genetically similar. It may also due to different environmental conditions.

5.4.3 Seed Width

Provenances displayed that states and locations within the state had significant effect on seed width of *Pycnanthus angolensis* (P<0.0001 Confidence interval CI). At Tukey Multiple post Hoc. test of means among the states Osun-Ekiti, Oyo-Ekiti and Oyo-Osun states had significant effects on seed width (Table 4.30) had no significant effect on the seed width.This may be as a result of the seed arrangement on mother plants and diverse ecological setting (Singh *et al*., 2010). Distinction among seed locations with respect to seed traits (length, width and weight) have earlier been reported in many species such as *F. albida*, *Acacia karroo*, *Pinus roxbrughii*, *Dalbergia melanoxylon* and *Celtis australis*. Seed width from Ajaba was observed to statistically significantly vary from those from every other location while those from Adewumi and Idito were observed to be statistically insignificantly different from each other. Seed width from Otun and Gbongan were also observed to be statistically significantly different from each other.

However, highest seed width was evident in seeds sourced from Gbongan seed location (2.41cm) and Otun (2.41cm), followed by Adewumi (2.17cm), Ayetoro (2.08cm), and Ajaba seed location (1.96cm). The significantly minimum kernel width was recorded in Idito seed location (1.41cm). The results indicated that Gbongan kernel location had the highest seed width. The significant differences in various seed form qualities of *Pycnanthus angolensis* locations is pinpointing the chance of selecting phenotypically advanced plant within the species for advance enhancement and genetic conditions (Ginwal *et al*., 2005).

Environmental conditions contribute to changing of the seed dimension as the plants develop in their natural state and hence, populations can experience selection stress under seed traits. The analysis of variance showed considerable variations in fruit/seed metrics viz. length, diameter/ width and n = 100 test weights. Data in seed characters showed enormous disparities with selected locations. This distinction in seed qualities can be influence of their natural where they grow with temperature. However, it was established that seed locations with high length and width possessed superior seed weight. Viable seeds are constantly with good sinks (Srimathi, 2001). Hence seed weight can be used for

selection of superior location. Selection of seed locations and improving seed production depend seed size and weight (Srivastava, 1995).

Seed locations from Otun, Gbongan and Ajaba were found to be advanced for seed and fruit traits and they stand out for other seed locations. Arjunan *et al*. (1994) reported similar result in *Pongamia pinnata.* The intercharacter correlations were significant for fruits. The greater the dimensions (length and diameter), the greater is the weight, both in case of fruits and seeds. Interestingly, diameter as compared to length was more strongly correlated with weight in fruits as well as seeds. This is in conformity with the results reported in other species (Shankar, 2006)

The parameter also showed that the size of the seeds depended on the size of the fruits, with large fruits having large seeds and the small fruits with small seeds.This could be as a result of evolutionary effects on the plant. This is similar to the finding of Elmagboul *et al*. (2014) reported on seed weight, length, width and thickness between or within plant species are due to evolutionary rejoinder of plants to capitalize on the potential fitness by producing a better number of seeds.

5.4.4 Number of seeds/kg

This ranged from 230 to 470 seeds per kg. This result contradicts Orwa *et al*. (2009) who reported 500 seeds per kilogram. This result also indicated that the more FWT, the lesser the seed making and vice versa. The correlation between number of seeds/Kg. and FWT reveals the superiority or the unquality of the seed location and this may be due to true biological and environmental factors, which encourages the species to oppose the unfavorable situations of the seed locations, and/or depends on the hereditary control of the species (Idah *et al*., 2015) (Table 4.13).

5.4.5 Aril Colour

The colour of the aril is of high diagnostic and systematic value at different levels. The colour of the arils matched to the red group but varied from light red, light pink, pinkish white to pink. However, the arils were light red in Ajaba at 5R 7/8, and 7.5R 7/8, Gbongan 2.5R, 7.5R 6/8 and 10R 6/6, Idito7.5R 6/8 and 6/6 and Ayetoro 2.5YR 7/6, light pink/pinkish white in Adewumi at 7.5R 8/3, 2.5YR 8/2 and 5R 8/3, pink in Otun

2.5YR8/3 and 8/4 respectively (Table 4.6). The colour is also used to distinguish between locations, of these light red was the most common color in Ajaba, Gbongan, Idito and Ayetoro, for it is light pink/pinkish white in Adewumi and pink in Otun. This result concur to Kitamura *et al*. (2011) who accounted that red was the majority colour of nutmeg arils followed by orange, yellow, and pink. Correspondingly, within a study site, red arils were a good number in Myristicaceae followed by orange in a particular place.

5.5 Morphological variation in seed and seedlings growth of *Pycnanthus angolensis*

Plants are mostly outcrossing, hence seedling in offspring that separate with reverence to parental qualities thus affording the opppotunity for selection. Since most seed collections for wood or plantation organization are obtained from naturally happening trees, the estimate of the supremacy of seed growth from diverse locations is of principal significance (Akinyele, 2007). Every obtainable *Pycnanthus angolensis* trees are supposed to grow by usual rejuvenation. An attempt to domesticate it was carried out. Germination and early seedlings behavior from diverse locations of *Pycnanthus angolensis* at garden phase will help estimate the fitness of certain locations of *Pycnanthus angolensis*.

In this study, variations were recorded in seed germination percentage and shoot height, number of nodes and leaves among seed locations. The seed characters expressed in germination and seedling development varied during the study period indicating the genotype-environmental-interaction. This could be linked to the differences in the hereditary composition of different seed collection locations and environmental factors during seed development. The days to germination ranged from 27 to 49 days which contradicts the reports of Onocha and Otunla (2010) that the duration of germination of *Pycnanthus angolensis* ranged 16–36 days. Distinction in *Pycnanthus angolensis* seed locations with respect to their form characters of seed and seedlings could be due to seed coat, climatic conditions and soil. Though there was progressive increase in shoot height from the first week of observation through to the twelfth week of shoot heights. It means that as the plants become grown-up, distinction in height amid locations tend to increase and this contradict the report of Charity *et al*. (2015). There was major distinction in leaf number at all stages of growth (Table 4.24). This result is in line with Singh *et al*. (2010)

who accounted important distinction in the NL per plant in *Quercus glauca*. All seedlings were observed to have a steady growth.

However, seedlings from Idito produced the highest growth pattern in shoot height compared with those from other regions followed by those from Gbongan and this could be traced to genetic makeup, seed location and environment. This result is similar to seedlings from Ayetoro and Otun that had the least growth pattern. Seedlings from Gbongan produced the highest number of nodes as well as number of leaves pattern compared with those from other regions followed by those from Adewumi. Seedlings from Ayetoro and Otun had the least number of nodes pattern as well as number of leaves. Seedlings distinction to environment has been accounted in *Celtis australis* and *Magnolia officinalis* (Shu *et al*., 2012). These studies are needed for higher yield and upcoming propagation work.

5.6 *In vitro* propagation techniques of *Pycnanthus angolensis*

In this sudy, there were major differences among the growth hormones, locations and explants (Table 4.20). In this study, it has become necessary to add special cytokinins, viz., BAP, Kinetin, for regeneration of *Pycnanthus angolensis* in which KIN-free MS media (Treatment A (0mg/l KIN+ NAA) produced the highest explant germination rate of 1.53% and this could be due to the presence of NAA. This result is contrary to report by Gulati and Jaiwal (1992).

The optimization of MS medium by supplementing with right auxin and cytokinins combination and concentration is sure way of prompt regeneration of plantlets in culture. This revealed that the BAP decreased the NR, NN, SH, RL and other growth variables. This is similar to Modarres and. Jami (2003) who reported decreased number of root and nodes in addition of BAP on potato culture. The maximum value of plantlets was suitable to BAP-free MS consequently that increasing BAP levels brought about a major decrease in height and quality of plantlets. This result agreed with Arab *et al*. (2014) on proliferation of hybrid of almond xPeach rootstock. However, by increasing the hormone level, the height and quality of micro-shoots were significantly decreased, respectively. In this plant, shoot regeneration was established in the single use of naphthalene acetic acid

(NAA) which is found in exceptional cases. Maximum shoot regeneration was observed in 1.0 mg/l NAA.

5.6.1 Acclimatization

Hazarika (2003) opined that transferring plants derived from *in vitro* conditions, to the free-living (*ex-vitro*) is considered as a problem that affects the success of such technique. About 4-25% of plantlets were effectively established in the greenhouse.

5.6.2 Callus induction in embyogenesis of *Pycnanthus angolensis*

It is worthy to note that zygotic embryo induced callus at different hormones and hormonal concentrations. Treatment media A(2-3), B (1-3), C0,C1, D(2-3) and F1. This indicated that callus was stimulated in MS supplemented with naphthalene acetic acid alone at 2.0 and 3.0 mg/l, 2.0mg/l KIN singly and in combination with 1.0-3.0 mg/l NAA. In overall, a total of 14 explants induced callus at the cut edges of the explants used with white, brown, greenish yellow colour (Plate 4.8). Highest number of callus was observed in MS supplemented with combined 1.0 KIN with 1.0-3.0mg/l NAA and the minimum number of callus formation was found in MS supplemented with combined Kinetin (2.0mg/l) and 1.0 mg/lBAP with 1.0-3.0 mg/l NAA (Plate 4.8). Callus also occurred in all the selected locations for embryo explants but Ayetoro in Ekiti State had the maximum callus formation. Complete plantlet also possessed callus (Plate 4.9)

5.6.3 Nodal Explants and Indirect Regeneration

5.6.3.1 Root induction

Rooting of woody plants like *P.angolensis* is more difficult than herbaceous in tissue culture and that was reason for no root development in the nodal explants used. This is similar to Nemeth (1986). The result in this study showed that shoots on any media could not produce roots and this result could be traced to the role of roots for conduction of nutrient and water. Schiefelbein *et al*. (1997) reported similar result.

5.6.3.2 Callus formation

Callusing near cut end and on the whole explants was observed with MS used singly and or in combination with IBA and NAA. Nodal explants collected from 120 days old seedlings produced adventitious shoots through indirect regeneration. Callus initiation from nodal explants was noticed at 12–97 days after inoculation. Different concentrations of auxin (NAA, 0–3.0mg/l) singly or combined with cytokinin (KIN 0–3.0mg/l) were used for callus induction. Better callusing response (60.0%) was observed on medium 1.0 BAP with 10.0 mg/l and1.0 mg/l NAA modified with 10.0 mg/l. This means that for optimum growth callus to be highly induced low concentration of BAP and NAA must be in combination with 10.0 mg/l IBA. Concentrations of IBA and NAA used alone and in combination failed to stimulate rooting. So far, *in vitro* micropropagation of *Pycnanthus angolensis* using nodal explants has not been attempted. Currently an efficient protocol to micropropagate the economically important medicinal tree species *Pycnanthus angolensis* has been evolved. The results which we obtained in this research will make the propagation of this species much easier.

5.6.3.3 Initiation of shoot from nodal explants

Initiation of shoot from nodal explants was noticed at 10–23 days after inoculation. The optimization of MS medium by supplementing with right auxin and cytokinins combination and concentration is sure way of prompt regeneration of plantlets in culture. Optimum growth medium that will support the culture of *Pycnanthus* nodal explants for early shooting was evident on 2.0 mg/l NAA +10mg/l IBA and late shoot initiation was noticeable on BAP of 1.0 mg/l + 1.0 mg/l of NAA+ 10mg/l IBA. This could be as a result of the equal application of BAP and NAA. This indicated that auxins alone (NAA, IBA) produced more shoots than cytokinins (BAP and KIN) at 1.0 mg/l. This result is contrary to report in other species, such as *Citrus aurantifolia* and *Capsicum frutescens* (Balaraju *et al*., 2008) that cumulative effect of the KIN and BAP resulted into successful *in vitro* shoot multiplication. It is worthy to note that out of 32 treatments, 5 treatments supported shoot induction while callus proliferation was prominent.

After shoot induction number of leaves or shoots were (2) on the media supplemented with the A_1 (1.0NAA+10 IBA), and A_2 (2.0NAA+10 IBA). This indicated that cumulative effect of combination and concentration of auxins support leaf production contrary to Balaraju *et al*. (2008) on *Vitex agnus-castus.* Other observation made in this study revealed that increase in concentration between cytokinin(KIN and BAP) and auxin (NAA and IBA) tends to produce no leaf in media supplemented with little or no cytokinin and high combination of auxins B_0 (1.0 KIN+10 IBA), E_3 (3.0NAA+10 IBA) and F_0 (1.0 BAP+10 IBA) (Table 4.32). These results are in consonant with Feng *et al*. (2001) on *Aloe barbensis*.

CHAPTER SIX
SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Implication for conservation

The large variation as shown by the percentage frequency of occurence of 39 juvenile trees and 19 fruiting trees could be a sign of the huge distinction that occurs in their natural habitat (Muok *et al*., 2000). Fruit set in the *Pycnanthus angolensis* largely depends on timely pollination and its related factors like pollinators and environmental conditions and on the success in fertilization. The life of individual flower is very short and many flowers apparently are not pollinated during their respective period. Hence, by studying the elaborated informations on various aspects breeders can overcome the bottlenecks and hindrances in *Pycnanthus* breeding. Fruit production in *Pycnanthus angolensis* experienced annual peaks (once in a year). An increased understanding of the flowering and fruiting phenologies and associated communities of this species will hopefully contribute to its future successful cultivation in rain forest areas.

Comparisons between corresponding traits of fruits and seeds were significant. This implied that for every distinction in the fruit characters, there was a change in the seed characters. The major differences in different seed form qualities of *Pycnanthus angolensis* locations are necessary for further improvement work. A kernel location from Gbongan was found to be superior for seed and fruit traits. Notwithstanding, the fruit bunch weight and number of fruit per bunch were weakly compaired, the more the bunch weight the lesser the number of fruit per bunch. The Bunch Weight and number of fruits per bunch of *Pycnanthus angolensis* varied between locations. There were different colours showing the stages of fruit maturation in the developmental stages of *Pycnanthus angolensis*.

Pycnanthus angolensis flowered between middle of September–june. Nevertheless, flowering starts between September-January in Osun, November-April in Ekiti state while Oyo flowered between November-June. *Pycnanthus angolensis* refused to flower at the same time in all the locations. Among six different seed locations, Ajaba, Otun, and

Gbongan locations were found to be superior with respect to fruit weight, length and width. However, Adewumi and Ayetoro seed locations recorded the lowest or least values for the seed length. The statistical analysis revealed that seed seed locations differed significantly for all the seed traits. Among the six different seed locations, seeds collected from Gbongan and Otun had the longest and widest seed trait.

Ajaba had the heaviest fruit weight of all the locations while lightest fruits were found in Adewumi and Ayetoro. Gbongan and Otun had the longest fruit lengths. Gbongan and Otun had the longest fruit lengths and Gbongan, Otun and Ayetoro had the widest fruit width. The days to germination ranged from 27 to 49 days. *Pycnanthus angolensis* is best at Idito and Gbongan because of good seedling qualities and displayed utmost steady growth pattern. Low seedling morphological qualities were observed in Ayetoro and Otun. Distinction in seed seedling traits trailed a steady trend in Gbongan with highest number of nodes as well as number of leaves pattern compared with those from other regions followed by those from Adewumi. Seedlings from Ayetoro and Otun had the least number of nodes pattern as well as number of leaves.

To the best of my knowledge *in vitro* micropropagation techniques using embryo, nodal and leaf of *Pycnanthus angolensis* have not been hitherto reported. However, Treatment A (0mg/lKIN) was observed to produce the highest effect for NN variable compared to other treatment types while treatment A(0mg/LKIN) was also observed to produce the highest effect for NR variable compared to other treatment types. Going by the foregoing, treatment A (0mg/LKIN) is observed to give a relatively better growth performance compared with other treatments for each of the variables considered,Treatment A supplemented with 0mg/l KIN+ NAA is observed to produce the highest explant germination rate of 1.53% and this ccould be due to the presence of NAA.

Results showed that in all media or treatments, where the embryos were inoculated, root formation was achieved. Root primordial emerged from the embryo on first week of culture on hormone free medium. In this experiment, embryo explant showed different responses to increasing BAP and KIN in the culture medium. In embryo explants, root formation decreased as the BAP concentration increased whereas, embryo rooting percentage decreased by increasing BAP concentration/levels in culture medium.

However, Treatment A which was Kinetin free (0mg/LKIN) produced maximum number of root (NR) and root length (RL) compared to other treatment types. Plantlets were obtained from embryonic explants of *Pycnanthus angolensis.* Accordingly, the present study evolved with an effective protocol to regenerate the callus from the nodal explants of *Pycnanthus angolensis*.

A total of 20% shoot was induced from nodal explants in treatments A_1, A_2, B_0, E_3 and F_0 at 10-23 days and early shooting on 2.0 mg/l NAA +10mg/l IBA. This could be traced to the effect of auxins. Late shoot induction on 1.0mg/l BAP+1.0mg/l NAA+ 10mg/l IBA. This could be traced to the combination of equal amount of BAP and NAA. After shoot induction, number of leaves or shoots were (2) on the media supplemented with the A_1 (1.0NAA+10 IBA), A_2 (2.0NAA+10 IBA), but no leaf in media supplemented with B_0 ($_{1.0}$ KIN+10 IBA), E_3 (3.0NAA+10 IBA) and F_0 (1.0 BAP+10 IBA) as in table 4.62

6.2 Conclusion

In this study, *Pycnanthus angolensis* has a broad form and biological diversity and as such a very good selection can be made for conservation and improvement programmes. The information provided by this study has provided some useful hints on biological study of *Pycnanthus angolensis.* It has been shown that flowering and fruiting periods are long and varied within and among study areas and can help the tree breeders collect genetic materials for their research. This will enable the forest manager collects enough seeds during the fruiting period. The species is easily propagated through seeds. *Pycnanthus angolensis* amenability to *in-vitro* regeneration will make possible the establishment of large scale plantation.

6.3 Recommendations

Upon the outcomes of this study the following recommendations could be suggested that highest community distribution of *Pycnanthus angolensis* is Osun State. Flowering period of *Pycnanthus angolensis* is between September to November and Fruiting period from middle of July to June of another year. *Pycnanthus angolensis* has four different maturation colours which must be known by the collectors or breeders and the colours start from brown, green, greenish-yellow/ olive and lastly to yellow/orange. Collection for

the best fruit traits should be taken from Gbongan at yellow colour of maturation and propagules from Gbongan in Osun State could be considered in initiating improvement programme as well as establishing orchards for seed collection activities for *Pycnanthus angolensis.*

Seeds from Ayetoro were observed to give a characteristic better growth compared with seeds from other locations for emryogenesis. Breeding programme for improved form traits in this species should be through the crossing of Ayetoro and Gbongan. Optimum culture medium for shoot initiation from embryo explants of *Pycnanthus angolensis* is MS supplemented with auxin alone at 1 mg/l of NAA while optimum culture medium for rooting from embryogenesis should be MS + 3 mg/l of NAA. Optimum culture medium for callus induction in embryogenesis of *Pycnanthus angolensis* is MS + 3 mg/l of NAA

In vitro regeneration through embryo and nodal is possible but nodal shooting should be subjected for further research to solve rooting problem. Optimum culture medium for shoot initiation from *Pycnanthus angolensis* nodal explants should be MS supplemented with NAA (1.0 - 2.0mg/l) incorporated along with 10.0mg/l IBA in the absence of Cytokinin.

6.4 Contribution to Knowledge

Natural distribution of *P.angolensis* in rainforest parts of Nigeria was determined along with flower and fruiting phenology. *Pycnanthus angolensis* amenability to seed raising and *in vitro* regeneration will make possible the establishment of large scale plantation. The study provided baseline protocol for surface sterilization of nodal cuttings collected from the nursery or wild to eliminate contaminants.The study provided baseline information on fruit and seed morphological variations in *Pycnanthus angolensis.*

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