**INTELLIGENT PLATFORM MANAGEMENT CONTROLLER**

**ACCRA, GHANA**

**SOFTWARE ENGINEERING.**

**PHARMACY MANAGEMENT SYSTEM FOR PHARMACY COUNCIL, GHANA**

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**ABSTRACT**

Introduction

■ Centralized system that provides wide range of Pharmaceutical wholesale and distributors for retailors to choose from and work with.

PMIS basically deals with sufficient quantity of approved retails shops, drugs and

consumable materials for patients to choose from.

■ This will also enhance the efficiency of clinical work, ease the patients

convenience and process drug prescriptions effectively through odering from the convenience of their homes.

■ The system will help removing maintenance of drugs and consumables in

the pharmacy unit.

■ The system will ensure availability time wasting, saving resources, allow easy

access to doctors and pharmacists to interact with, as well as bring on more security on the data

compared to the old based system.

4

Importance of PMIS

■ A good PMIS provides the necessary information to make

sound decisions in the pharmaceutical sector.

■ Effective pharmaceutical management requires policy

makers, program managers and health care providers to

monitor information related to patient adherence, drug

resistance, availability of medicines and laboratory supplies,

patient safety, product registration, product quality, financing

and program management etc.

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**Abbreviations**:

* CTAB – cetyltrimethyl ammonium bromide
* EDTA – ethylenediamine tetraacetic acid
* AMD-adult male nutmeg
* AFN-adult female nutmeg
* SDS – sodium dodecyl sulphate
* PCR- polymerase chain reaction
* RAPD- Random amplified polymorphic DNA
* DNA- Deoxyribonucleic Acid

CHAPTER ONE

# INTRODUCTION

Perfume, often described as an olfactory masterpiece, has held a unique and enduring allure for humanity throughout history. Beyond its aromatic appeal, it serves as an expression of identity, a reflection of individuality, and a vehicle for sensory delight. In today's fast-paced world, where convenience and personalization are paramount, the perfume industry finds itself at the intersection of tradition and technology. It is in this dynamic context that the concept of a Perfume Ordering System emerges, offering a modern solution to age-old desires.

The perfume industry, while steeped in tradition, has undergone a transformation in recent years, driven by evolving consumer preferences and digital innovation. Fragrance enthusiasts no longer confine their exploration to the aisles of brick-and-mortar stores; they seek a diverse array of scents that resonate with their unique personalities and sensibilities. They crave the ability to discover, sample, and acquire perfumes with ease, all within the digital realm.

The Perfume Ordering System is designed to cater to these evolving demands, seamlessly merging the artistry of perfumery with the convenience of e-commerce. It is a digital gateway to a world of fragrances, offering customers an immersive experience where they can explore a curated selection of scents, receive personalized recommendations, and effortlessly place orders from the comfort of their homes. Simultaneously, it empowers perfume retailers, from boutique artisanal perfumeries to established brands, to expand their market reach, optimize inventory management, and engage with customers on a deeper level.

This comprehensive system addresses various facets of the perfume industry, from the intricacies of fragrance classification to the intricacies of secure online transactions. It integrates cutting-edge technology to deliver a user-friendly interface, robust security measures, and seamless payment and shipping solutions. Moreover, it takes into account the legal and regulatory landscape surrounding e-commerce, ensuring compliance with taxation, data privacy, and consumer protection laws.

As the fragrance landscape continues to evolve, the Perfume Ordering System stands as a testament to the industry's adaptability. It seeks to redefine how perfumes are discovered, experienced, and acquired, fostering a symbiotic relationship between fragrance connoisseurs and the creators who craft these olfactory masterpieces. In this digital age, it is poised to usher in a new era of fragrance exploration, where the art of perfumery meets the efficiency of modern technology, all while preserving the timeless enchantment that perfumes bring to our lives.

## Background Study

The Perfume Ordering System study emerges from the confluence of two compelling forces: the enduring allure of fragrances and the transformative potential of digital technology. Perfumes, revered for centuries as olfactory art forms, continue to captivate individuals worldwide. Their ability to evoke emotions, memories, and personal identity has made them an integral part of human culture. Concurrently, the rise of e-commerce and technological advancements has reshaped the way consumers discover, engage with, and purchase products.

A perfumery, Fragrance\_by\_Caly, traditionally reliant on in-person interactions at physical stores, has witnessed a significant paradigm shift. Consumers increasingly seek personalized, convenient, and online avenues to explore and acquire fragrances that resonate with their unique tastes. This shift necessitates an innovative approach to perfume wholesaling and retailing, one that bridges the gap between the tangible elegance of scents and the virtual realm of digital commerce.

The Perfume Ordering System for Fragrance\_by\_Caly, as the subject of this study, embodies this innovation. It will strive to create a dynamic and interactive online platform that caters to the evolving needs and expectations of perfume enthusiasts. It will embrace technology to curate a diverse collection of perfumes, offer personalized recommendations, and provide a secure and user-friendly interface for seamless transactions. By doing so, it will not only address the demands of modern consumers but also empowers perfume retailers/users to adapt and thrive in the digital landscape.

This study aims to delve deep into the conceptualization, development, and implementation of the Perfume Ordering System for Fragrance\_by\_Caly. It will explore various facets, from market analysis and user experience design to security considerations and legal compliance. Through this comprehensive examination, the study seeks to shed light on how technology can enhance the perfume shopping experience, foster customer loyalty, and optimize operations for retailers at Fragrance\_by\_Caly perfumery.

Ultimately, the Perfume Ordering System for Fragrance\_by\_Caly represents a pivotal juncture in the intersection of tradition and technology within the perfume industry. As the study unfolds, it will reveal how this innovative approach can redefine the way perfumes are discovered, ordered, and cherished by fragrance aficionados, while also revitalizing the business strategies of perfume retailers in the ever-evolving landscape of modern commerce.

## Problem Statement

In the realm of the fragrance industry, characterized by its rich history and profound sensory experiences, a persistent challenge has emerged. While Fragrance\_by\_Caly perfumery continue to provide perfumes that enchant individuals with their evocative scents and personal significance, the traditional modes of purchasing and experiencing fragrances have begun to clash with the expectations of the modern, digitally-driven consumer. This misalignment presents a pressing problem: how to harmonize the timeless allure of perfumes with the convenience and personalization that contemporary consumers demand.

But the main problem facing the Fragrance by Caly Perfumery is:

1. Limited Accessibility: The physical constraints of visiting perfume stores restrict access for consumers, particularly those living in remote areas or with mobility challenges. This limitation impedes the discovery of unique fragrances and inhibits market growth.
2. Personalization Gap: Traditional perfume wholesale/retail at Fragrance\_by\_Caly often struggle to provide tailored fragrance recommendations, leaving consumers overwhelmed by the abundance of choices. This lack of personalization hinders customer satisfaction and the discovery of scents that truly resonate.
3. Inefficient Transactions: Manual order processing, limited payment options, and complex checkout processes in physical stores deter potential buyers. The inefficiencies in transactions leads to lost sales and decreased customer retention.
4. Inventory Management: Fragrance\_by\_Caly faces the ongoing challenge of managing inventory effectively, ensuring that popular fragrances are in stock while minimizing overstocking of less sought-after scents. Inefficient inventory management leads to financial losses and customer frustration.
5. E-commerce Security: As the fragrance industry transitions to e-commerce, security concerns related to online transactions, customer data protection, and fraud prevention have become increasingly pertinent. Failure to address these issues can result in reputational damage and legal consequences.
6. Regulatory Compliance: The complex landscape of e-commerce regulations, encompassing taxation, data privacy, and consumer protection, poses a substantial burden for perfume retailers. Failure to navigate these regulations can result in financial penalties and legal complications.

## Objectives

Specifically, the study sought to develop a system with:

Increased accessibility and reduced physical constraints to perfume stores to access for consumers, particularly those living in remote areas or with mobility challenges. This limitation impedes the discovery of unique fragrances and inhibits market growth.

Personalization Gap: Traditional perfume retailers often struggle to provide tailored fragrance recommendations, leaving consumers overwhelmed by the abundance of choices. This lack of personalization hinders customer satisfaction and the discovery of scents that truly resonate.

Inefficient Transactions: Manual order processing, limited payment options, and complex checkout processes in physical stores can deter potential buyers. The inefficiencies in transactions can lead to lost sales and decreased customer retention.

Inventory Management: Perfume retailers face the ongoing challenge of managing inventory effectively, ensuring that popular fragrances are in stock while minimizing overstocking of less sought-after scents. Inefficient inventory management can lead to financial losses and customer frustration.

E-commerce Security: As the fragrance industry transitions to e-commerce, security concerns related to online transactions, customer data protection, and fraud prevention have become increasingly pertinent. Failure to address these issues can result in reputational damage and legal consequences.

Regulatory Compliance: The complex landscape of e-commerce regulations, encompassing taxation, data privacy, and consumer protection, poses a substantial burden for perfume retailers. Failure to navigate these regulations can result in financial penalties and legal complications.

## Significance of the study

Addressing these challenges requires the development of a Perfume Ordering System—an innovative, user-centric, and secure platform that combines the sensory allure of fragrances with the convenience of digital commerce. This system must facilitate the discovery of unique scents, offer personalized recommendations, streamline transactions, and ensure robust security and regulatory compliance.

By bridging the gap between tradition and technology, this Perfume Ordering System aims to redefine the perfume shopping experience, empowering both consumers and retailers in the ever-evolving landscape of the fragrance industry. It seeks to provide an elegant and efficient solution that not only preserves the essence of perfumery but also propels it into the future, where fragrances are accessible, personalized, and secure, meeting the diverse needs of today's discerning consumers.

## Scope of the study

The perfume ordering system will encompass the following key features and functionalities:

1. User registration and login.
2. Perfume catalog with detailed product descriptions, images, and pricing.
3. Search and filter options for easy product discovery.
4. Shopping cart and secure checkout process.
5. Multiple payment options, including credit card, digital wallets, and cash on delivery.
6. Order tracking and status updates.
7. Customer reviews and ratings.
8. Integration with payment gateways and shipping services.
9. Customer support chat and inquiry submission.

## Limitations of the study

One limitation of this study is limited access to up-to-date data on the effectiveness of their existing system as personnel are reluctant in giving out credible information for security reasons which is a major drawback of implementing a pharmacy automation system.

Another limitation to this study is time. Developing this system is very complex and require significant technical expertise. This complexity can cause delays in the project timeline. Aso Testing and validating before is it implemented is very time consuming.

User Acceptance: Some pharmacy stuffs are resistant to change from the old system to a new system. This requires extensive education and training on the importance of this new system and its effective use.

Meeting of supervisor is somehow a limitation as there is no much meeting times. This is because he has other task and student projects to supervise as this causes delays in implementing the system.

## Organization Study.

Organizational study will be done at the preliminary stage and information were obtained from a Pharmacovigilance and also a Regulatory Affairs Specialist about the importance of this centralized system and all features included to the pharmaceutical industry in collaboration with pharmacists, technicians and other healthcare professionals.

Analysis on the current process and system is done and benefits to the current system, carefully considered along with cost and feasibility of implementation.

Once this is done detailed implementation plan will be developed including timelines and training requirement. This also, involved all other stakeholders in the planning process to ensure their buy-in and support for the project.

In all, organization study is done to help the pharmacy council manage every process, reduce errors and enhance patience safety.

## 

CHAPTER TWO

# LITERATURE REVIEW

## Introduction

According to the American Pharmacist Association (APhA) a pharmacy “is a facility that is **licensed** to dispense prescription medications to patience and provide medication-related advice. The APhA also notes that, pharmacies may also sell over the counter medications, health and wellness products and medical supplies. (https://www.pharmacist.com)

The role of pharmacists in a pharmacy is essential. According to the National Community of Pharmacists Association (NCPA), pharmacists are responsible for ensuring the safe use of medications, monitoring drug interactions and side effects and counselling patients on how to properly take their medications. Pharmacists may also offer immunizations, medication therapy, management services and other healthcare related issues. (<https://ncpa.org>)

Pharmacies must adhere to strict regulations and guidelines set forth by state and federal agencies. The Ghana Food and Drugs Authority (FDA) regulates the safety and effectiveness of prescription and over-the-counter medications. State board of pharmacy oversee the licensing and regulation of pharmacies and pharmacists.

Automation system refers to the use of technology and software to automate various processes in a wide range of industries such as manufacturing, transportation, finance and healthcare.

Automation in this context refers to the use of technology and software to automate various processes in a pharmacy, such as dispensing medications, managing inventory and processing orders. This can include automation dispensing systems which uses barcoding technology to help pharmacists fill prescriptions more efficiently and accurately. This can help improve patient safety, reduce errors, and increase productivity.

### Aim of the project

* The aim of undertaking this project is to improve on the efficiency and accuracy of pharmacy councils operations. A system that automates the various processes such as managing various pharmacy units under it, prescription processing, dispensing and record keeping.
* It is also aimed to provide real time data on drug availability, usage patterns and education on adverse reactions of various medications to enable better decision making. It will also enhance patience safety by reducing risk of medical errors and improving medication adherence.
* Additionally, it will facilitate regulatory compliance from wholesale units to retail and down to the final consumer/patient and also enable seamless communication between pharmacies, healthcare providers, and patients.
* Also, this is projected to aim at providing a centralized platform to bring together medical/pharmaceutical expertise and patients information to address their needs. This will help in the following reasons:

1. **Streamlined communication**: A dashboard provides a centralized platform for doctors and patients to communicate efficiently. It enables secure messaging, appointment scheduling, and sharing of medical records and test results. This streamlines the communication process, reduces the need for unnecessary in-person visits, and allows for timely and convenient interactions.
2. **Access to medical information**: The dashboard can give patients easy access to their medical information, including lab results, diagnoses, treatment plans, and medication history. By having a comprehensive overview of their health records, patients can actively participate in their care, make informed decisions, and take ownership of their well-being.
3. **Remote monitoring and Home medicine**: With a dashboard, pharmacists and other health professionals can remotely monitor patients' vital signs, track their progress, and provide virtual consultations. This is particularly beneficial for patients with chronic conditions or those who live in remote areas. It also provides options to book for home medications for physical visits, which improve accessibility to healthcare, and enable proactive management of health conditions.

* Lastly it is aimed at regulating the practices of pharmacies in the country, including licensing and accrediting pharmacies and pharmacists at ease without the old manual ways. It will help enforce compliance with regulations, as well as improve the quality of pharmacy services provided to patience.

### Reasons For Literature Review

Recently, the Pharmacy Council cau­tioned pharmaceu­tical wholesalers in the country against selling their prod­ucts to unlicensed drug retailers and peddlers or they risk sanctions.

According to the Council, the menace of drug peddling and van peddling has become a major healthcare challenge due to the difficulty in tracing the sources of some of the drugs, and medica­tions peddled by these unlicensed individuals not captured by their data.

The Pharmacy Council hav­ing difficulty in dealing with illegal drug peddling is mainly due to the use of an ineffective automation system and therefore some unlicensed retailers source their medicines from some wholesales whose main motive is just about their money.

Since the number of unlicensed retails and peddlers is becoming alarming, then there must be a working and an effective automation system where all pharmacy units can be managed and monitored in accordance to all regulations of the council to help deal with the canker instead of just issuing cautions to wholesalers under this act which might not be effective.

Therefore, conducting a literature review will allow the Pharmacy Council of Ghana to stay updated and on the latest advancements and technologies in pharmacy automation systems. It provides insights into the available options, features, and capabilities of these systems. This knowledge helps the Council to manage and make informed decisions when it comes to selecting or recommending automation systems for all pharmacy units in Ghana.

Also, Pharmacy automation systems directly impact patient safety and the quality of pharmaceutical services or considerations. By conducting a literature review, the Pharmacy Council can identify safety concerns, best practices, and guidelines associated with automation systems. It helps them understand potential risks, such as medication errors, system failures, or data security issues. This knowledge is essential for developing regulations, standards, and guidelines that ensure the safe and effective use of pharmacy automation systems in Ghana.

Identification of regulatory considerations: Pharmacy automation systems will be subjected to regulatory requirements and standards. Conducting a literature review will help the Pharmacy Council identify relevant regulations, guidelines, and policies from other countries or international bodies. It will assist them in understanding the regulatory landscape and considering the necessary regulatory framework for the adoption and use of automation systems in Ghana. This is to ensure compliance with quality standards, data privacy regulations, and other legal requirements. This knowledge can guide the Council in developing guidelines or recommendations for pharmacies in Ghana to effectively integrate automation systems with their existing infrastructure.

Cost-effectiveness and resource allocation: A literature review will provide insights into the cost-effectiveness and return on investment of pharmacy automation systems. By examining studies and experiences from similar settings, the Pharmacy Council can assess the economic impact, benefits, and potential challenges associated with implementing automation systems in pharmacies. This knowledge helps in resource allocation decisions and allows the Council to recommend cost-effective solutions that improve pharmacy efficiency and patient care

In summary, a literature review for pharmacy automation systems by the Pharmacy Council in Ghana is essential to gain knowledge of current technologies, make evidence-based decisions, address safety and quality considerations, ensure seamless integration, identify regulatory requirements, and make informed choices regarding cost-effectiveness and resource allocation. It provides a solid foundation for developing guidelines, regulations, and recommendations that support the adoption and effective use of pharmacy automation systems across the country.

Evidence-based decision-making: A literature review will provide scientific evidence and research findings related to pharmacy automation systems. By reviewing published studies, reports, and relevant literature, the Pharmacy Council can evaluate the effectiveness, safety, and impact of these systems on patient care and pharmacy operations. This evidence-based approach ensures that decisions regarding the adoption, implementation, and regulation of pharmacy automation systems are based on credible and reliable information.



**Plate 1:** Pharmacy Council, addressing pharmaceutical stakeholders about sales to unlicensed retailers and peddlers.

### Information About the Topic

Pharmacy automation system has expanded in to different categories that increase efficiency and accuracy since 1980s. Since around the 1980s, pharmacists have used early forms of automation system such as, digital tablets counters, to lower the risk and labor costs for pharmacists. However, these techniques have been expensive and therefore inaccessible to independent pharmacists.

Now, this automation system has become user friendly and a necessity in our part of the world today. Certain tasks performed in the pharmacy time consuming and prone to human errors. Pharmacy automation system accurately, efficiently and conveniently performs tasks, so patience can get their correct medications and services.

Pharmacy automation system is the electronic process of distributing, sorting, packaging and counting prescription medications. In this study, it helps many different purposes including improving efficiency, minimize labour cost, providing services for appointments, conveniency in purchasing me at the comfort of their homes.

When automation became popular, pull counting was one of the only techniques. Now there are many more accessible automations machines one can choose from.

## Conceptual / Theoretical Framework

Takes orders or appointments from the system

**Pharmacist/Doctors**

**Administrator/Technician**

a

Manages data

Provides Services

Accept/Decline/provide services to users & Stocks Medications

**Courier**

**Retailers**

**PHARMACY AUTHOMATION SYSTEM**

Takes order

Takes order

Orders/Book Appointments

Take Orders

**User/Customer**

**Wholesalers**

Receives order / Appointment services

Accept/Decline services to retailers & Stocks Medications

### Administrator/Technician

* + Changes or give permissions
  + Change Data
  + Request and Feedbacks
  + Classifies information
  + Create items

### Pharmacist / Doctor

## Empirical Framework

### Journal, Conference, Precedent, Forums, Seminar.

## Summary

CHAPTER THREE

# LITERATURE REVIEW

## **Origin, Taxonomy and Production of Nutmeg**

New Guinea and surrounding islands are regarded as the center of distribution of genus Myristica. *M. fragrance* probably originated in Moluccas islands Indonesia. The major nutmeg growing countries are Indonesia and Grenada. Myristicaceae is generally considered to be a primitive family. This has been collaborated based on its distribution, habitat, the presence of aromatic tissues, simple leaves dioecy, scaleriform vessels in the wood (Josy, 1980).

The ethnobotany of nutmeg (*Myristica fragrans* Houtt), Myristicaceae was studied in the provinces of Maluku and of Central and East Java. The utility of nutmeg as a spice has been known since ancient times in Indonesia, and nutmeg was probably introduced into Europe during the twelfth century (Muchtaridi *et al.,* 2011). The Portuguese then discovered the nutmeg tree on the Banda island of Indonesia (spice Island) in 1512. Beginning the 17th century, the Dutch controlled the spice Island and monopolized the spice trade until the British obtained nutmeg seedlings from the Banda Island at the end of the 18th century (Donald, 2009).

Joseph (1980), reported that nutmeg plant, *M. fragrans* Houtt, is a member of the small primitive family Myristicaceae, taxonomically placed between the Annonaceae and Lauraceae. The family Myristicaceae contains only 18 genera and about 300 species. *Myristica* is the largest genus for which has been listed 72 species, spreading from India and Sri Lanka eastwards through Malaysia to North-Eastern Australia, Taiwan and the Pacific, including the Solomon Islands, Fiji and Samoa (Purseglove *et al*., 1981). Most of the species in the genus *Myristica* are tropical evergreen trees found growing mainly in the lowland tropical rain forest, but some mountain species also occur. Genetically the somatic number of chromosomes in Myristica Fragrans is 2n=42,  
and the basic chromosome number for the genus is suggested to be 7 (Purseglove, et al., 1981)

Grenada produces over 23% of the world's nutmeg, which is second to Indonesia which produces 73% of the world's nutmeg. Nutmeg in Grenada is grown by both large and small scale farmers.

## Botany

The tree itself is about 25 feet high which does not bloom till it is nine years old, when it fruits; it continues to do so without attention. *M fragrans* has four parts - the skin, the fruit, the seed and the mace. Fruit is a pendulous, succulent pericarp, the mace arillus covering the hard endocarp, and a wrinkled kernel with ruminated endosperm. When the arillus is fresh it is a brilliant scarlet, when dry it is more of a horny, brittle texture, and a yellowish brown colour. The seed of nutmeg is firm, fleshy, whitish, traversed by reddish brown veins and abounding in oil (Kritika *et al.,* 2014).

Fruits are oval or pyriform, drooping, yellow, smooth, 6-9 cm long with a longitudinal ridge and a fleshy husk. When ripe, the husk splits into 2 halves revealing a purplish-brown, shiny seed surrounded by a leathery red or crimson network of tissue. The shiny, brown seed inside, or the kernel of the seed is the Nutmeg. The brown seed has a red cover that makes another spice called mace. Bark contains watery pink or red sap. There are both male and female type trees, both required for pollination and fruit set. The seedling reveal their sex at first flowering (Orwa *et al.,* 2009)

The root system is shallow but extensive, one tap root and a spreading mat of lateral feeder roots branchings. This mat may extend beyond the spread of the stem branches and has measured as much as 3.5 - 5 m from the stem base (Muller *et al*., 1980).

The stem may be a single woody trunk or multi-stemmed. The single stemmed trunk is cylindrical sometimes furrowed and showing very slight decrease in girth from ground level to 1/3 up the plant. In older mature plants, the bark is rough with a dark brownish grey colour and showing longitudinal fissures, whilst the trunk bark of younger plants is smooth with a light-brownish grey colour.

Profusely spreading lateral branches arise from the main stem with a slight whorled, spiral arrangement. The arrangement affords maximum leaf display for photosynthesis. On wounding both stem and branches reddish sap is produced.

Leaves are simple, persistent, alternate, glabrous and exstipulate, elliptic or oblong lanceolate with an apex acute or slightly acuminate and an acute base tapering into a short petiole slightly flattened adaxially and about 1-1.5 cm long. The lamina, 5-15 cm long and 2-7 cm broad, is coriaceous, medium to dark green above and shining, light silvery-green or subglaucous beneath. In young leaves the upper surface is yellowish green.

The typical tree is dioecious, with male and female flowers on different trees. On occasion, both male and female flowers may occur on the same tree and even rare hermorphrodite flowers may be encountered (Guido, 2011). Flowers tend to occur on the outermost branches. The inflorescences with male and female flowers are structurally similar and are axillary and glabrous, bearing flowers in umbellate cymes with the male 1-10 usually outnumbering the female 1-3. The pedicels, 1-1.5 cm long, are pale green with a minute caducous bracteole at the base of the flower (Guido, 2011).

Lawrence (1978) reported that, the flowers of nutmeg are creamish-yellow in appearance, waxy and fleshy, fragrant and may measure up to 1 cm in length. Petals are absent so the dominant calyx is bellshaped, nectiferous at the base, with 3-reflexed triangular lobes. The female flowers, up to 1 cm long, exhibit a puberulous, superior, sessile, one celled ovary about 7 mm long and topped by a very short, white two lipped stigma. The male flowers consist of an androecium 1 cm long, glabrous, a 2 mm stalk, 8-12 stamens, with anthers adnate to a central column and attached to each other by their Fruit.



**Plate 1:** Nutmeg seed (left) and dried mace (right).

## **Pollination and Fruit Setting**

According to Cruickshank (1973), Deinum wrote that pollination was effected by a moth. Peryl concluded that *M. fragrans* may be able to produce seeds without pollination, while Flach is of the view that cross pollination is obligatory and the team of Duncan and Ferguson suggested a cross-pollination mechanism.

Cruickshank (1973) reported on a bagging experiment carried out on a marcotted flowering tree in Grenada. Although the results seem to suggest that the stimulation of pollination may be necessary for fruit set, the issue of pollination still requires further investigation.

## Propagation of Nutmeg

According to Orwa *et al.* (2009), nutmeg can grow on rich volcanic soils in lowland tropical rain forests. Also the plant requires a warm and humid tropical climate, an altitude of 4500m, a temperature of about 25-30 C and a rainfall pattern of 2000-3500 mm.

Guido (2011), proposed that only two methods have been predominantly used for propagating nutmeg plants. These are the seedling method and the vegetative method.

### The Seedling Method

#### From Volunteer Plants

Traditionally, small farmers have used "volunteer plants" as seedlings for planting. These seedlings have their origin from fallen seeds that have germinated and grown in and around the parent plant. The farmers may use seedlings at two stages of development, the young undeclared plants, plants which have not flowered, or the more mature declared plants, which have flowered and thus the sex could be identified. In the latter instance plants that produced female flowers and then fruits will be selected.

#### From Government Agricultural Stations

Nutmegs are usually propagated by fresh seeds with their testa still attached. Seeds where the kernel rattle in the shell and old seeds will not germinate. In shaded nurseries the selected seeds are sown 2.5 - 5 cm deep and 30 cm apart in boxes or well prepared moistened nursery beds. Germination takes about one month or more. In Ghana seedling of nutmeg may be obtained from CSIR/PGRRI for growth.

### The Vegetative Method

Following the ravishes of hurricane lanes in 1955, which completely or partially destroyed most of the nutmeg tree population nationwide, investigation into the vegetative propagation of nutmeg was initiated. Two methods emerged and were established in commercial approach-grafting and marcotting (Nicols and Cruickshank, 1964; Cruickshank, 1973).

#### Approach grafting

In India, successful **approach grafting** has been done on root stocks of *M. fragrans , M. malabarica* and *M. beddomei* (Sundararaju and Varadharajan, 1956; KAU, 2001). Those on *M. beddomei* and *M. malabarica* developed into low spreading trees. The trial on approach grafting at KAU registered about 95% success (KAU, 2001) while, at HRS, Kanyakumari revealed 82.2% success (Thangaselvabai *et al*., 2010). Haldankar *et al*. (1999), reported that the approach grafts can be prepared throughout the year and maximum percentage of graft success was recorded on *M. malabarica* , (30-100%), and in M. fragrans it was 40-90 %

#### Marcotting/air-layering

In **marcotting or air-layering**, vigorous healthy branches, 1.2-1.5 cm in diameter are chosen from selected female trees. The branch is split in the middle longitudinally for 5 cm at a distance of 90 cm from the terminal growth. A bamboo is placed on the back of the split and tied firmly at both ends with plastic tape. A portion of the split branch 6-12 mm long is then removed on the lower side of the split with secateurs. The cut end is lifted and a splint of hard wood is inserted to keep the split open. The section is then dusted with rooting stimulant such as Seradix L15. Moist peat moss, sawdust or coconut coir dust are applied around the split, extending above and below the incision for 5 and 10 cm respectively. Such a medium is kept in place by polyethene sheeting, tied around the branch and secured with plastic tape. Roots occur after 4 - 18 months. Once rooting is adequate the plant is severed from the tree and potted, after removal of polyethene sheeting. The plants are kept in closed concrete bins covered with clear plastic and watered thrice daily for a period of 6-8 weeks. The plants are then hardened off by lifting the bin covers until they are fully exposed. This is done for a period of two to three months. The plants are then stored under 70% shade for a further 8-10 weeks before planting in the field (Guido, 2011).

#### Top working

The sex of nutmeg tree can be identified only 7-8 years after planting, when they begin to flower. Generally, male and female trees are produced in a 1:1 ratio. Since a single male tree is sufficient for every 20 female, trees for pollination, the rest of the unproductive male trees can be made productive by converting them into female trees by top working. Trials on topping of male trees indicated that cutting the trees above the first tier during August was found to be the best with regard to sprout production and reducing the time for sprouting. Successful graft union was obtained by wedge grafting during March with scion shoots having mature leaf and full green stem and stock having two months growth (Rema *et al* ., 2000; Rema *et al* ., 2009).

#### Epicotyl grafting

This has been found to be the most successful method (Krishnamoorthy and Mathew, 1985; Krishnamoorthy and Rema,1987; Haldankar et al ., 1999) . At CPCRI, the epicotyl grafting using *M. beddomei* as rootstock resulted in 48 % survival of grafts (Mathew and Joseph, 1982). A grafting success of 80 per cent was obtained during the month of August when *M. fragrans* was used as the rootstock (Krishnamoorthy, 1987). Nageswari *et al*.(2010), recorded 54% success when two leaved root stock were grafted with orthotropic scion.

## Planting and aftercare

Pits of 90cmᶾ are filled with top soil and compost or well decomposed farmyard manure and nutmeg plants are planted at a spacing of 7.5-8 m. Ten percent of males may be retained for pollination and the remaining male trees may be either removed or converted to females by top working. Grafted plants are to be planted in pits of 75cmᶾ size and at a spacing of 5 x 5 m. A male graft has to be planted for every 20 female grafts in the field. Nutmeg is a shade loving plant. Young as well as grown up plants require an amount of shade. Locations with permanent natural shade will be the optimum. For open space, artificial shade is provided by growing certain tree species such as, *Glyricidia sp*. Dadap, banana, *Acacia sp* and Subabool. Lopping of branches may be done at later stages to regulate shade. Regular weeding and mulching is done as an after care activity mainly to keep the field clean and conserve moisture. Cover crops such as *Mimosa sp.* and *Stylosanthes sp*. may also be cultivated for suppressing weed growth. Application of herbicide mixture (gramaxone and fernoxone) check weeds up to six months. Four years old plants require 20 liters of water per plant thrice in a week and the quantity of water is to be increased at later stages of growth. Dripping of 8-10 litres of water/day/plant was found to enhance the yield by 18- 20% (Thangaselvabai *et al.,* 2011).

## Importance of Nutmeg

According to Agbogidi *et al.*, (2013), apart from its sweet, spicy, nutty taste, nutmeg is known to be an essential constituent used in the kitchen. It also has both medicinal and therapeutic values such as serving as a tonic for the heart by helping the cardiovascular system through blood circulation and stimulates the heart functions.

It contains anti-oxidant properties that are very useful for the smooth functioning of the body. These anti-oxidants prevent free radical formation which are capable of triggering unwanted reactions in the body that may lead to many formation of compound which may be cancerous (Anamika M, 2017).

Nutmeg oil is also a good herb for the kidney, helping in dissolving kidney stones as well as relief infections and detoxify toxins in the body (Kadhim *et al.,* 2013).

## Side Effects of Nutmeg

Weiss (1960), reported that a loss of reflexes do occur when more than ample doses of nutmeg oil is administered and also causes dilatation of the pupils, unsteadiness of gait, subsequent sleepiness and slow respiration.

## Sex determination in dioecious plants

Dioecism, is when the male and female reproductive organ are on separate plants (of the same species) and mature female flowers have no trace of stamens and mature male flowers lack any evidence of female structures (Wannan and Quinn 1991).

In the plant kingdom, however, dioecy is found in only approximately 4% of the angiosperms (Yampolsky and Yampolsky, 1922), although that frequency may be an underestimate because of incomplete information on many tropical species where the occurrence of dioecy appears to be higher than in temperate zones (Bawa, 1980). Dioecism has arisen independently in different families and genera (Westergaard, 1958), and several distinct genetic mechanisms regulating dioecy have been found in different plant species (Irish and Nelson, 1989; Durand and Durand, 1990). Michelmore *et al*. (1991) described an application of genetic marker systems based on direct analysis of the genomic DNA and being used widely for genetic mapping, disease diagnostics and evolutionary studies, and reported that they could prove very useful in the study of sexual determination in dioecious plants.

In Ghana, according to PGRRI, (2014), the attempt to improve plants uses the phenotype of a plant for a specific trait as a tool for selection. This application uses external plant characteristics as a marker, called morphological markers (i.e. plant height, stem width and girth etc (Van Wezel and Rodgers, 1996; Estoup *et al*., 2002). These markers depend on visual observation and measurement to identify, classify, and characterize the genetic evolution of different species or populations. The seed testing department of the Plant Genetic Resources Research Institute of Ghana has a unique way of also determining the sexes of nutmeg seedlings and even matured ones. A special device called genetic tester is used which works with the principle that when suspended on top of the leaves of the nutmeg plant, the genetic tester moves in a circular motion to indicate that the seedling is a female whilst when it moves in a pendulum motion indicates that the seedling is a male. These morphological and chemical characters showed only limited success (Sheeja *et* *al*., 2006).

## Molecular markers

A genetic marker is a DNA sequence with a known location on a chromosome and associated with a particular gene or trait. It can be described as a variation, which may arise due to mutation or alteration in the genomic loci that can be observed. A genetic marker may be a short DNA sequence, such as a sequence surrounding a single base-pair change (single nucleotide polymorphism, SNP), or a long one, such as mini and micro satellites. Recent years have witnessed a great interest in molecular markers, as they reveal polymorphism at the DNA level, they also play an important role in plant and animal genetics studies. The main aim of the breeder is to select plants with superior genetic potential as parents for the next generation. The first attempt to improve plants used the phenotype of a plant for a specific trait as a tool for selection. This application uses external plant characteristics as a marker that called morphological markers (i.e. plant height, stem width and girth etc (Van Wezel and Rodgers, 1996; Estoup *et al*., 2002). The morphological markers depend on visual observation and measurement to identify, classify, and characterize the genetic evolution of different species or populations. The conclusions reached through applying morphological markers are often not completely accurate when they used for the evaluation of plant genetics, because these markers are based on subjective judgments and descriptions. Another type of markers which uses cytological markers that includes several criteria such as chromosome karyotypes, bandings, repeats, translocations, deletions, and inversions is also used to investigate the genetic resources of plants (Yang *et al*., 2013). The chromosome mutations lead to genetic variation (Bitgood and Shoffner, 1990) These mutations were used as markers to identify a certain location of the gene on a specific chromosome. In plants, cytological markers allow to investigate their genetic diversity by comparing the chromosome number and the structure between cultivated species and their wild ancestors (Becak *et al*., 1973). Cytological markers are still widely used in elucidating the origin and classification of species Jonker J *et al*., (1982) because of their good properties, rapid economic and straightforward technique. The third type of markers is biochemical markers, such as the blood type and isozymes. These markers represent biochemical traits that could be analyzed by protein electrophoresis. The differences in the amino acid composition of isozymes and soluble proteins were used to investigate the genetic variation within species and phylogenetic relationships between species (Buvanendran V and Finney, 1967) The application of these markers was limited because the proteins and isozymes are not genetic materials. They are products of gene expression, so they could be affected by environmental factors (Drinkwater and Hetzel, 1991). Thus, the direction of researchers are converted to the molecular markers. The molecular markers are based on the nucleotide sequence mutations within the individual’s genome; they are the most reliable markers available. The most popular markers for sex determination in plants include RAPD (Random Amplified Polymorphic DNA), SCAR (Sequence-characterised Amplified Region), AFLP (Amplified Fragment Length Polymorphism), RFLP (Restriction Fragment Length Polymorphism) and microsatellites. The selection criteria could be based on cost, technical labor, level of polymorphism, reproducibility, locus specificity and genomic abundance (Yang *et al*., 2013).

### RAPD markers

Random amplified polymorphic DNA (RAPD) is a PCR based technique for identifying genetic variation. It involves the use of a single arbitrary primer in a PCR reaction, resulting in the amplification of many discrete DNA products. The technique was developed independently by two different laboratories and called as RAPD and AP-PCR (Arbitrary primed PCR) respectively. This procedure detects nucleotide sequence polymorphisms in a DNA amplification-based assay using only a single primer of arbitrary nucleotide sequence. In this reaction, a single species of primer binds to the genomic DNA at two different sites on opposite strands of the DNA template. If these priming sites are within an amplifiable distance of each other, a discrete DNA product is produced through thermocylic amplification. The polymorphisms between individuals result from sequence differences in one or both of the primer binding sites, and are visible as the presence or absence of a particular RAPD band. Such polymorphisms thus behave as dominant genetic markers (Williams et. al., 1990; Welsh and McClelland, 1990)

#### Advantages RAPD Analysis

Small amount of DNA is required (about 10ng per reaction) during RAPD analysis and it can be automated which makes it possible to work with population that is not accessible RFLP. It is fast and efficient in analysis since it involves no blotting or hybridization steps. RAPDs has high-density genetic mapping as in many plant species such as apple (Hemmat et al., 1994).

#### Limitations of RAPD Marker Analysis

Nearly all RAPD markers are dominant, i.e. it is not possible to distinguish whether a DNA segment is amplified from a locus that is heterozygous (1 copy) or homozygous (2 copies). Co- dominant RAPD markers, observed as different-sized DNA segments amplified from the same locus, are detected only rarely. Mismatches between the primer and the template may result in the total absence of PCR product as well as in a merely decreased amount of the product. Thus, the RAPD results can be difficult to interpret. Problems with reproducibility (sensitive to changes in the quality of DNA, PCR components and PCR conditions). (Liu et al., 1994).

# CHAPTER FOUR

# MATERIALS AND METHODS

## Experimental site

The experiment was conducted at the Agricultural Biotechnology Laboratory at the Faculty of Agriculture, Kwame Nkrumah University of Science and Technology (KNUST) Kumasi-Ghana.

## Source of plant material

Twenty seedlings of nutmeg in total were obtained from CSIR/PGRRI – Bunso in the Eastern Region of Ghana with leaves from two matured plants each of known sexes as controls. The samples were transported to the plant biotechnology lab, KNUST in silica gel. The twenty seedlings were kept at the lath house, KNUST. The labeled leaves for the control were then stored in the -20˚C freezer.

Ten leave samples were collected from the nutmeg seedlings of unknown sexes from the lath house and sealed in 10 different tagged ziplock bags for differentiation and the leaves from matured known plants also in different ziplock bag. The leaf were first sterilized with 70% ethanol to get rid of microbes. After sterilization. 6 leaf discs were pinched from each nutmeg leaf sample into a 2.0 ml eppendorf tubes. The eppendorf tubes were labeled from N1-N10 for the seedlings with unknown sexes (N1-N5 for males and N6-N10 for females) whilst that of the controls were labeled as AMN (Adult Male Nutmeg) and AFN (Adult Female Nutmeg) with no replications making a total of 12 tubes thus, 2 tubes being the already known matured male and female nutmeg leaves. The pinched leafs in the eppendorf tubes were used for the DNA extraction.

## Total Genomic DNA Extraction from the nutmeg seedlings

Total genomic DNA was extracted from the leaf samples of the nutmeg seedlings using CTAB (cetyltrimetylammonium bromide) protocol (Takrama, 2000 CRIG) with some modification (The leaves were grinded using sterilized sea sand with mortar and pestle) (Appendix 1).

## DNA quality confirmation test

0.8% agarose gel containing 9µl (0.009%) ethidium bromide was prepared. 10µl of the extracted total genomic DNA was pipetted into tubes 2µl of loading buffer (6 Bromophenol blue) was added. The samples were then loaded into the wells on the agarose gel, and the whole gel submerged in 1x TAE buffer (Appendix two). The sample was then run in 75volts for 35 minutes. The resulting bands were then photographed under Ultra Violet light.

## RAPD primer sequences

Agarose gel was used for the electrophoresis. 2% agarose gel was used for running for the genomic and the PCR samples. 5 RAPD primers were used and already known male and female as positive controls. The primers are;

**Table 1:** OPE RAPD primer sequences

|  |  |  |
| --- | --- | --- |
| OLIGO NAME | SEQUENCE 5 – 3 | SIZE |
| OPE 4 | GTG ACA TGC C | 10 |
| OPE 5 | TCA GGG AGG T | 10 |
| OPE 8 | TCA CCA CGG T | 10 |
| OPE 11 | GAG TCT CAG G | 10 |
| OPE 20 | AAC CGT GAC C | 10 |

## RAPD-PCR Amplification

Polymerase chain reaction were performed in 20µl PCR tube volumes with the Accupower mix comprising of 1x PCR buffer.

**Table 2: The cocktail for the PCR mix and concentrations.**

|  |  |  |
| --- | --- | --- |
|  | **CONCENTRATION** | **CONCENTRATION**  **X 10 Samples** |
| **Buffer** | 7.5 | 75 µl |
| **Primer** | 0.9 | 9 µl |
| **DNA Tag Polymerase** | 0.12 | 1.2 µl |
| **MgCl₂** | 0.03 | 0.3 µl |
| **Template DNA** | 1.5 |  |
| **Water** | 4.95 | 49.5 µl |
| **TOTAL** | 15.00 |  |

PCR amplification of the extracted DNA was done using RAPD primer from the Agricultural Biotechnology Laboratory at the Crop and Soil Sciences Department, KNUST. The PCR proceeded for 35 cycles and an extended expansion (720C for 1min). Thirty five cycles were done for all the 5 samples. General cycling conditions preceded as follows.

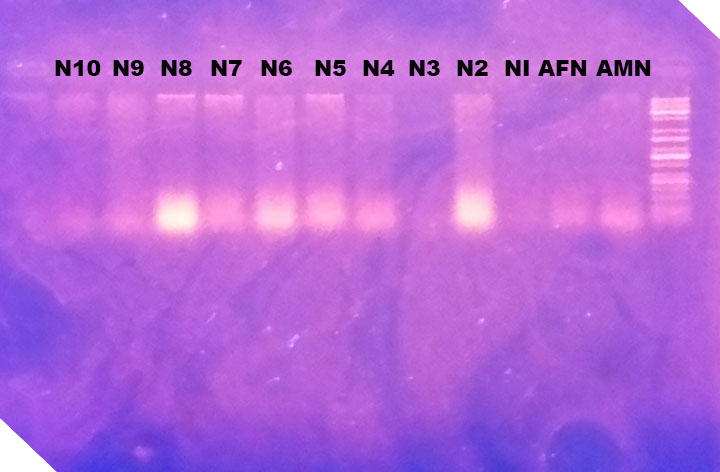
* Pre-denaturation at 94 0C for 5 minutes
* Denaturation at 94 0C for 30 minutes
* Annealing at 64 0C for 30 seconds
* Extension at 72 0C for 30 seconds
* Final extension at 72 0C for 7 minutes

The amplification products were resolved and separated in 2% agarose gel running at 100 volts for 1hour 30minutes and the DNA amplification bands were visualized by staining the gel with Ethidium bromide. The resultant bands after the gel electrophoresis process were visualized under the UV light using UV transilluminator. The visualization was done with the aid of googles and camera. The results were analysed based on the presence and absence of bands in the wells of the seedling samples on the gel being compared to the control.

# CHAPTER 4

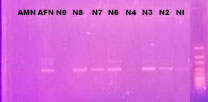
# RESULTS

## Genomic DNA test



**Plate 2:** Results of total Genomic DNA purity test after 1% agarose gel electrophoresis for samples AMN-N10

## Electrophoresis of PCR products



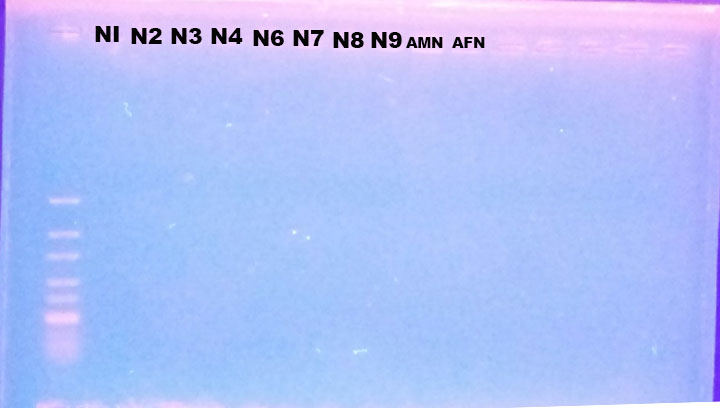
**Plate 3:** Separated PCR products of extracted genomic DNA on an agarose gel with primer OPE 5.

The last band appearing after the N2 and N1 serves as a ladder which helps to check for molecular weight.

**Table 3:** DNA bands showing suspected male and female nutmeg seedlings.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| AFN  1 | AMN  0 | N1  1 | N2  1 | N3  1 | N4  0 | N6  1 | N7  1 | N8  1 | N9  0 |

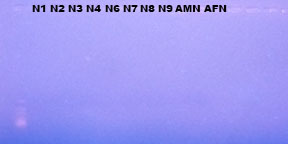
AFN and AMN represent the DNA bands from the already known adult female nutmeg and adult male nutmeg respectively and the numbers N1-N10 represent the DNA bands from the nutmeg seedlings of unknown sexes. One represent presence of DNA bands whilst zero represent absence of DNA bands as indicated in table **4.2.1** above.



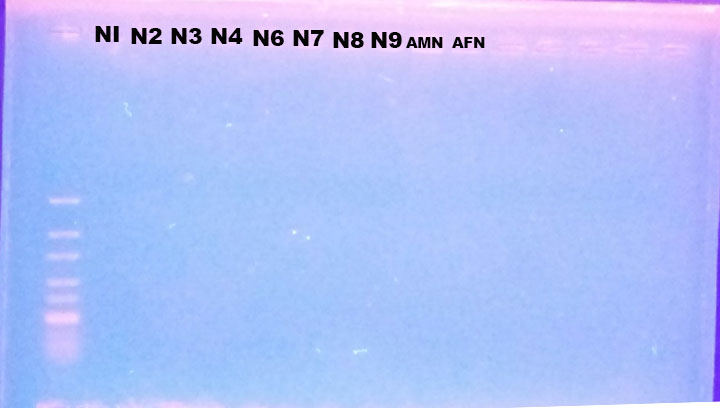
**Plate 4:** Separated PCR products of extracted genomic DNA on an agarose gel with primer OPE 4.



**Plate 5:** Separated PCR products of extracted genomic DNA on an agarose gel with primer OPE8.



**Plate 6:** Separated PCR products of extracted genomic DNA on an agarose gel with primer OPE 11.



**Plate 7:** Separated PCR products of extracted genomic DNA on an agarose gel with primer OPE 20.

The first band appearing before the AFN and AMN (Plate 4.2.2, 4.2.3, 4.2.4 and 4.2.5) serves as a ladder which helps to check for molecular weight. None of the primers (OPE 1, 4, 8, 9, 10, 11, 14, 16 and 20) showed band except OPE 5.

# CHAPTER 5

# DISCUSSION

Leaves were the main source of DNA isolation. Many metabolites in leaves interfere with isolation of clean DNA. From the results above, in Plates 4.1.1, there were DNA present in samples N1 and N3 but the bands were not very clear. According to Prittila *et al*. (2001), polyphenols and polysaccharides can bind to nucleic acids during DNA isolation and affects its distinctness. Also as reported by Doyle and Doyle (1990), the quantity of DNA in old leaves is always less as compared with the newly sprouting leaves in the sense that, there is high percentage of polyphenols and other secondary metabolites such as protein and tannins in nutmeg as the plant ages making the isolated DNA less in terms of quantity. Leaf age was reported to affect the properties of extracted DNA, which was inferred to be related with the accumulation of defense compounds during the leaf development and also because of its thick cell wall (Lefort and Douglas, 1999 and Moreira and Oliveira, 2011).

Of all the primers used, only primer OPE 5 in plate 4.1.2 above gave clearer and distinct bands and showed amplification because it was able to amplify a sequence, bind and amplify the female sex. The rest of the primers (OPE 1, OPE 4, OPE 8, OPE 9, OPE 1O, OPE 11, OPE 14, OPE 16, OPE 20) did not bind at all. This could be due to Polysaccharide contamination in isolated DNA which is known to inhibit enzymatic reactions, such as Taq DNA polymerase amplifications (Pandey et al., 1996) and restriction endonuclease cleavage (Raina and Chandlee 1996; Abdulova et al., 2002). It could also be that the primers were unable to anneal to the conserved regions of the nutmeg DNA (Verma et al., 2007). Or amplification was hindered due to unfavorable PCR condition for the primers to locate and bind to the annealing site as reported by Polymeropoulos *et al*. (1992). For primer OPE 5, there was DNA band present in AFN and absent in AMN indicating that primer OPE 5 is a suspected female specific marker as indicated in table 4.1.1 above. This work is consistent with work done by Ganeshaiah *et al*., (2010), where they indicated that only female bands after amplification of *Myristica fragrans* Houtt. with RAPD primers appeared.

# CHAPTER 6

# CONCLUSION AND RECOMMENDATION

## Conclusion

Age and quality of leaf sample interfered with DNA extract and quality.

Primer OPE 5 is a female specific primer because it was the primer that was able to amplify a sequence linked to the female sex and it was the only primer that gave distinct and clearer bands.

## Recommendation

The work should be repeated with different or the same RAPD markers due to low reproducibility of RAPD markers.

Sea sand should be used in the grinding rather than liquid nitrogen in extracting genomic DNA in nutmeg.

Fairly young leaves should be used in future DNA extractions for nutmeg.

The nutmeg seedlings should be planted on the field to full bearing to confirm the results obtained.

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# APPENDICES

NOTE: As part of Precautionary measures, it’s necessary to have all materials before proceeding. Put on gloves at all times and pipette tips should be changed when pipetting new solutions. Tubes should be balanced in centrifuge before spinning.

**Materials/Equipment used for experiment**

* Silica gel
* Eppendorf tubes
* Deep freezer
* Pipette
* Nutmeg leaf disc
* Sea sand Mortar
* Pestel
* Centrifuge
* UV transilluminator
* 200 µl PCR tubes

**Chemicals used for experiment**

* 70% ethanol
* 0.08% agarose gel containing 9 µl (0.009%) ethidium bromide
* Bromophenol blue
* Cetyltrimetylammonium bromide

## APPENDIX ONE

CTAB METHOD FOR EXTRACTION OF DNA FROM LEAVES (Dr. Takrama, 2000 CRIG).

PROTOCOL

1. Grind 20ml of fresh samples in 2.0ml microtubes to fine powder (CTAB buffer can be added) with sea sand rather than liquid Nitrogen.

2. Add 800µl of 2% CTAB with 0.1% of mercaptoethanol.

3. Incubate in a sand bath at 65˚C for 30minutes with intermittent vortexing.

4. Cool samples at room temperature and add equal volume (800µl) of chloroform isoamyl alcohol (24:1). Mix by several inversion of the tube and centrifuge at 14000rpm for 15minutes.

5. Transfer the aqueous phase of the sample into a clean 1.5ml tube.

6. Precipitate nucleic acids by adding two thirds volume of ice cold isopropanol (400µl) and shake gently. Keep on ice for 30minutes. Precipitation can be enhanced by storing at

-20C for 8 hours or overnight.

7. Centrifuge at 14000 rpm for 5 minute to pellet nucleic acids.

8. Decant the isopropanol and wash pellets with 500µl of washing buffer on a rocking surface for 15minutes and centrifuge at 6000rpm for 4minutes.

9. Decant washing buffer and wash pellet in 400µl (8%) ethanol then centrifuge at 6000rpm for 4 minutes.

10. Decant ethanol and dry pellets in vacuum or at 37C for 10 minutes or until the smell of ethanol is no longer detectable.

11. Suspend DNA in 50 µl 1X TE buffer and centrifuge at high speed for 30 seconds to remove all insolubles.

12. Prepare 1.0 % or 0.8 % Agarose gel with (3 µl) 0.003% ethidium bromide or (5 µl)

0.005% gel red solution.

13. Pipette 5 µl sample and add 1 µl loading buffer (X6 Bromophenol blue)

14. Load the samples in the wells on the gel submerged in 1X TAE buffer.

15. Run ample at (90 to 120) V for forty five minutes (45minutes).

16. Photograph under UV light

## APPENDIX TWO

PREPARATION OF GEL

1. 1.5g Agarose gel was weighed in a beaker and 1X TAE buffer was added to the beaker containing the agarose.

2. It was then swirl and placed in a microwave for 3 minutes, after that 0.3μl of Ethidium bromide was added to it

3. Electrophoresis combs were then set into the electrophoresis trough

4. 1 X TAE buffer was poured into the electrophoresis tank till the “max fill” line.

5. The gel was then poured into the electrophoresis trough and allowed to solidify.

6. After solidification the combs were then removed to create the well for loading of the gel

## APPENDIX THREE

GENERAL PRIMER CONDITIONS

|  |  |  |
| --- | --- | --- |
| Denaturation | 94 | 30 seconds |
| Annealing | 52 | 30 seconds |
| Extension | 72 | 1 minute |
| Final extension | 72 | 7 minute |