COLLECTION AND CHARACTERIZATION OF UNIQUE GENOTYPES OF NUTMEG (*Myristica fragrans* Houtt.)

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THESIS

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DEPARTMENT OF PLANTATION CROPS AND SPICES
COLLEGE OF HORTICULTURE,
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KERALA, INDIA
2016

DECLARATION

I, hereby declare that the thesis entitled "**COLLECTION AND CHARACTERIZATION OF UNIQUE GENOTYPES OF NUTMEG (*Myristica fragrans* Houtt.)"** is a bonafide record of research work done by me during the course of research and the thesis has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Date: (2014-12-130)

CERTIFICATE

Certified that this thesis entitled **"COLLECTION AND CHARACTERIZATION OF UNIQUE GENOTYPES OF NUTMEG (*Myristica fragrans* Houtt.)"** is a record of research work done independently by **Ms. Priyanka S. Chandran (2014-12-130)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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EXTERNAL EXAMINER

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Vellanikkara PRIYANKA S. CHANDRAN

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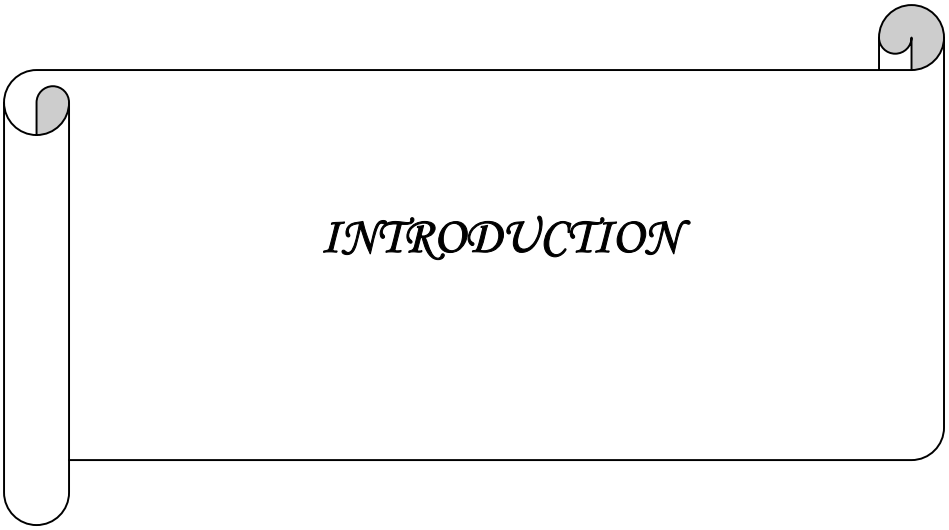

INTRODUCTION

1. INTRODUCTION

Nutmeg (*Myristica fragrans* Houtt.) is an evergreen aromatic tree spice and a member of the primitive family, Myristicaceae. About 300 species are observed in the Myristicaceae family under 18 genera and among them the commercially cultivated species is *Myristica fragrans.* Nutmeg is a highly remunerative tree spice yielding two distinct spices of commerce; nutmeg and mace. Kernel in nutmeg is the shelled seed and mace is the coloured aromatic covering of the shelled seed. Mace is commonly red in colour and in rare cases yellow coloured. Both mace and nutmeg have immense applications; as a spice in flavouring food and as a medicine for different stomachic disorders. In addition to this, the rind of nutmeg is gaining importance in market due to value added products like nutmeg syrup, nutmeg jelly and candy. Major hallucinogenic constituents in both mace and kernel are myristicin and elemicine, with significantly higher myristicin (5.92%) and elemicine (3.14%) in mace oil than nutmeg oil. Sabinene and safrole are also present in the volatile oils of nutmeg and mace.

Nutmeg is believed to be a native of Spice Island (Banda islands of Eastern Indonesia) and is now under cultivation in tropical countries. Area and production of this twin spice in India is markedly showing a log phase over the past few years. Area wise, production wise and productivity wise, Kerala occupies the pioneer position. India produced about 21,120 tonnes of nutmeg from an area of 14,400 ha during 2015-16 (Spices Board, 2016).

The tree is dioecious in general and monoecious in exceptional cases. The major constraint in its cultivation is the difficulty in sex determination at seedling stage and lack of improved varieties. Kerala has got tremendous diversity in nutmeg. There are many farmers cultivating elite nutmeg varieties possessing distinct features. These distinct / unique types which may or may not be economically sound, definitely makes a significant addition to the nutmeg gene pool and can also be used

in crop improvement programmes. Being cross pollinated, high amount of variability has been reported in the crop with respect to tree, fruit, nut and mace characters (Vikram, 2016). There are several land races developed by local farmers which enjoy only very limited distribution and can be lost in a single episode of habitat destruction.

Collection of germplasm with special attributes is a prioritized area in the conservation and utilization of plant genetic resources. These unique accessions could be evaluated and used in crop improvement programmes. A collection containing unique and desirable set of genes as opposed to just a random sample of genes is always advantageous (Gustavsson *et al.*, 2011). Exploration for collection of desired germplasm to strengthen the gene pool of a particular crop is the quickest though simplest instrument (Singh and Bhatt, 2012). The continued deportation of locally adapted landraces by elite cultivars has resulted in genetic erosion of primary gene pools. The identification and assessment of unique landrace gene pools to define their structure in relation to locally used elite germplasm allows for the development of breeding strategies for genetic conservation (Laghetti *et al.*, 2008; Cowling *et al.*, 2009).

It was in this background the present study entitled “Collection and characterization of unique genotypes of nutmeg (*Myristica fragrans* Houtt.) was taken up with the objective of collecting and characterizing the unique nutmeg accessions based on morphological and biochemical parameters.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Relevant literature on morphological and biochemical studies on nutmeg are reviewed and presented in this chapter under various subtitles. Wherever literature on nutmeg is lacking, relevant research works on other tree spices are also presented.

2.1 Unique germplasm collection

Unique germplasm can be defined as a germplasm resource which contains unique traits / genes that can be utilized for further crop improvement. Exploration for collection of desired germplasm to strengthen the gene pool of a particular crop is the quickest though simplest instrument. It should be an integral part of any plant germplasm augmentation programme (Singh and Bhatt, 2012). Long term intimate co-evolution between plants and man has resulted in domestication of plants (Harlan, 1995). However, the continued deportation of locally adapted landraces by elite cultivars has resulted in genetic erosion of primary gene pools and hence supports an *ex situ* conservation of genetic resources (Harlan, 1971).The identification and assessment of unique landrace gene pools to define their structure in relation to locally used elite germplasm allows for the development of breeding strategies for genetic conservation (Laghetti *et al.,* 2008; Cowling *et al.,* 2009). Genetic resources are the fundamental material for any crop improvement programme.

2.1.1 Unique germplasm in horticultural crops

Singh and Bhatt (2012) screened and evaluated seventy one accessions of faba bean for various morphological and yield attributes, and identified a unique line with four pods per node, which is not a commonly seen feature. This unique trait can be used in crop improvement programme they suggested. Likewise, a unique faba bean line with pods arising from collar region recorded 95.5 to 121 g seed yield per plant,

which is an indicative measure of its production potential. Another salt resistant faba bean line was reported by the same authors. Identification of lines resistant to abiotic stress is of great importance in breeding programmes.

Saxena *et al.* (2010) observed that green podded pigeon pea was on higher consumer preference and charged a higher price than striped ones and any other colours. Singh (2012) explored perennial and vegetable type pigeon pea germplasm from Vaishali district of Bihar. The perennial pigeon pea often possessed quality traits for both vegetable and grain purpose and suited well for high rainfall conditions. So this germplasm they opinioned can be exploited as a donor in developing varieties suited for high rainfall conditions suitable to Eastern states.

Escribano *et al*. (2012) reported distinct Spanish melon of Madrid provenance for their sensorial fruit attributes and quality. The study concluded that on the basis of genetic distances, Villaconejos (Madrid) melon group differed significantly from Group Indorous, Flexuosus and Cantalupensis, thus highlighting their genetic uniqueness. Villaconejos landrace accessions possessed a distinctly black epidermis when compared with other green and yellow market types.

In a study on landraces of cowpea, Hegde and Mishra (2009) concluded that landraces of cowpea are important source of variability which can act as a potential gene source for unique traits. The characters included in the study were number of pods/ plant, pods/peduncle, seed index, grain yield/plant and also resistance to biotic and abiotic stresses. The highest grain yield/plant was in the landrace DWDCC016 and it was superior over the standard cultivars. In the same way, number of pods per peduncle was maximum in DWDCC016 (1-6) when compared to standard cultivar (1-3). Thus the valuable unique traits like pods/peduncle and seed index from land race DWDCC016 can be incorporated in order to enhance the yield.

Jiang *et al.* (2009) identified local tea plant germplasm from Anhui province in China, which was unique in terms of its quality due to the agro climatic parameters of the region.

Niral *et al*. (2013) reported a unique coconut accession from Vaibhavwadi, Maharashtra for its large sized fruit .The morpho-molecular studies revealed that they had 60% similarity with Borneo Tall cultivars characterized by low husk to nut weight ratio and also have close proximity to the South East Asian Coconut accessions.

Niral *et al.* (2014) developed IND 414 - Chowghat yellow dwarf, a distinct indigenous yellow dwarf coconut line from the original population of Chowghat orange dwarf through selection and it has been conserved at CPCRI National gene bank. Chowghat yellow dwarf is characterized with erect leaves, large sized nuts with higher content of tender nut water (290 ml) with average TSS of about 6.7°brix and higher nut yield with excellent adaptability. In normal case, Malayan yellow dwarfs were having drooping leaves and straight spindle. Chowghat yellow dwarf had yellow coloured oval shaped fruits in the range of 12-20 per bunch.

Jerard *et al*. (2014) reported Andaman horned cocos, a unique coconut germplasm with distinct horny nuts. As this unique coconut has female flowers with multiple ovaries, the fruits possessed horn like appendages. They also confirmed the inheritance of this unique trait in the second generation palms. These palms belong to tall type with green coloured nuts and fruit setting percentage of 30.

2.1.2 Unique germplasm in spices

In green chilli, a unique accession (IC541402) with no pungency named '*inippumilagai'* and a highly pungent type (IC541403) named 'Hindustan *lavang*' have been reported by Abraham *et al.* (2008). In general, chillies behave like an annual crop and in some cases biennial in nature, but a perennial chilli germplasm is

not at all a common phenomenon. Singh *et al.* (2012) reported a unique perennial chilli germplasm with successful fruiting for six years. They concluded that the distinct chilli line was photo and thermo insensitive.

Kuriachen *et al*. (1992) identified two variants in clove, one non flowering and other with flower buds about 30% larger than normal. Non flowering clove variant had profuse branching pattern along with smaller leaves than normal ones. The other type showed early and regular flowering than normal ones. However clove bud yield and essential oil yield were lower than normal cloves. Zanzibar clove with more anthocyanin, king clove with extra bold flower bud and dwarf clove with short and spreading growth habit were some distinct variants in clove (Krishnamoorthy and Rema, 1994).

Krishnamoorthy *et al.* (1988) reported that the quality of cinnamon oil was highly influenced by the colour of young leaf flushes. Plants with purple pigmented leaf flush yielded 29% more bark oil than the normal green flushed leaves. Two seedling variants of allspice was identified by Mathew *et al.* (1999) that showed distinct morphological variation with respect to canopy shape, branching habit, internodal length, leaf characters and plant height (dwarf/semi-dwarf).

Krishnamoorthy and Parthasarthy (2010) reported four unique black pepper germplasm namely INGR 03091 with field tolerance to foot rot disease, INGR 370011 with bold berries along with high oleoresin content, INGR 06026 with high caryophyllene content and INGR 8100, a novel spike variant with 100% proliferating spike. Sasikumar *et al.* (2013) reported a unique black pepper accession with high dry recovery (46%) coupled with bold black berries – The Agali black pepper. Average dry recovery in black pepper is in the range of 29-38 per cent, Agali black pepper had outscored this record significantly. As it is a novel gene source for increasing the bulk

density and dry weight, it may be utilized in crop improvement programmes. In addition to this distinct feature, the accession was relatively tolerant to diseases.

Exploration and collection in black pepper resulted in few unique accessions namely Arakulamunda (good fruit set and high yield), Karimunda accession with long spike (8cm), another Karimunda type (good fruit set and bold berries) and Nedumchola with smallest leaf and spike (IISR, 2015)

Madhusoodanan *et al.* (1994) observed variations in morphological characters in cardamom like, branching of inflorescence, fruit (capsule) size, shape, leaf and plant pubescence and retention of green colour. Good variability exists in cardamom with regard to quality characters such as essential oil content and the quantity of 1, 8-cineole and alpha-terperyl acetate in essential oil (Zachariah *et al.,* 1998). In a study conducted by Prasanth and Venugopal (2009), twenty three accessions of cardamom with varied branching pattern were assessed and characterized for about 16 plant characters, panicle and capsule characters and the coefficient of variation was greatest for yield per plant (77.94%) followed by branches per panicle (49.40%) and minimum for dry recovery per cent (7.73%). Two accessions, IC 547214 and IC 349544 were identified as promising with desirable yield contributing characters; Accession IC 349553 possessed extensive branching at the terminal end of the panicle and Accession IC 547180 was unique in retaining the green colour of the capsules even after drying

2.1.3 Unique germplasm in nutmeg

There exists tremendous variability in all the major nutmeg growing areas in the world. *Myristica fatua* var. *magnifica*, an endemic species restricted in distribution to swampy forests of some parts of Kerala, Karnataka and Tamil Nadu, was subjected for proximate analyses of the kernel and mace. The chemical

composition was then compared with that of the more common nutmeg (*M. fragrans*). The study concluded that nonvolatile oil content was about half that of nutmeg, whereas the starch, protein and ash contents were higher than those of nutmeg (Bhat and Kaveriappa, 1998). Kumar *et al.* (2002) reported that seeds of *Myristica malabarica* will remain viable for only one week under natural conditions.

Nitta (1993) reported that nutmeg trees in Molucass island bear different types of fruit, which are identified by a suffix; *pala tidore* which has pear shaped fruits and *pala bali* which has large globoid fruit. The genetic diversity of nutmeg species in North Moluccas was reported by Das *et al*. (2012). The accessions marked wide range of variation for fruit shape, mature fruit colour, seed shape as well as mace weight. Based on the phenotypic markers, accessions were formed into four clusters at 70 per cent similarity level. Sasikumar *et al.* (2014) reported a distinct nutmeg type with small fruits and thin shell with profuse bearing habit.

Rahul *et al.* (2014) and Miniraj (2015) have reported the occurrence of a precious unique nutmeg with yellow mace in Kottayam district. Unlike the normal red mace colour, here the fresh aril is yellow coloured weighing 3- 4g with 42% dry recovery of mace. The average yield of a mature eight year old tree was 2000-3000 fruits per year as reported by Rahul *et al.* (2014). Double seeded nutmeg types have been identified from central parts of Kerala and it was reported that double seededness and multiple splitting are generally associated with monoecious types (Miniraj *et al.,* 2015; Vikram, 2016).

Though nutmeg is dioecious in nature, monoecious trees are reported very rarely. Krishnamoorthy *et al.* (2012) found that the seedling progenies of monoecious and female trees are cultivated in Karnataka. An intensive survey conducted in Karnataka identified hundred year old trees, that are derived from the monoecious mother trees and farmers claimed that second or third generation seedling trees of monoecious ones are predominantly females with sporadic male trees. Several

workers reported the existence of monoecious nutmeg trees in states of Kerala and Karnataka (Miniraj *et al.,* 2012; Sasikumar *et al.,* 2015; Rema *et al.,* 2015).

Kakkappara-2, a narrow leaved thick mace type nutmeg tree with open canopy structure was spotted by Sasikumar *et al.* (2014). The same authors reported two other distinct types namely Kakkapara-1 with high yield ,bold nuts and closed erect canopy shape ; and Kakkappara -3 with broad leaves, high yield and late bearing nature.

2.2 Morphological variability in nutmeg

Nutmeg is a spreading, normally dioecious evergreen tree with dark green leaves, yellow flowers without petals and large yellowish fruit, whose dried seed is the nutmeg and dried aril is the mace as reported by Weiss (2002). The tree reaches a height of 10-20 m with oblong-ovate leaves (Shanmugavelu and Rao, 1977). Vergheese *et al*. (1990) reported the leaf dimensions of nutmeg as leaf length of 5 - 15cm and leaf width of 2-7 cm. Weiss (2002) observed that leaves of nutmeg are shiny glabrous with medium to dark green in colour and leaf shape varied from elliptic to oblong- lanceolate. Vikram (2016) found significant variability in mature leaf shape of nutmeg and concluded that elliptic leaf shape was more prominent followed by lanceolate, obovate, ovate and oblong leaf shapes. Leaf colour also varied from light green to dark green shades, but green leaf colour was observed in most of the accessions.

According to Joshi (1946), the nutmeg inflorescence was considered to be an axillary raceme. Nair and Bahl (1956) observed racemose inflorescence in male nutmeg and cymose inflorescence in female nutmeg. Female flowers are larger than the male flowers and the calyx tube more oval in shape along with nectar production in both male and female flowers. Gamble (1967) described that number of flowers per inflorescence are fewer in females and in males , it varied from 3 to 5.The year round flowering of nutmeg tree with peaks in certain months has been mentioned by

Flach and Cruickshank (1969). Rendle (1971) found into a central column and may become expanded above into a disc.

Nazeem and Nair (1980) conducted a study on growth and flowering behaviour in nutmeg trees. The study revealed that, nutmeg put forth its growth in flushes, six times per year and the recorded maximum growth was seen in months of September, May and June. Natural flushing in the tree was followed by flowering with its peak time in the month of July and October in Kerala. Joseph (1980) reported that in nutmeg, female trees exhibit racemose inflorescence and male trees, branched inflorescence. Vikram (2016) found that majority of nutmeg trees exhibited mid season flowering (late July to early August), however late flowering and early flowering were also observed in trees with a frequency of 38 per cent and 12 per cent respectively. According to his observation female trees possessed axillary raceme inflorescence and monoecious trees had umbellate cyme inflorescence.

Krishnamoorthy *et al.* (1996) observed that the fruit weight of nutmeg ranged from 60.5 to 80.0 g, the mace weight from 2.5 to 3.7 g and the seed weight from 8.4 to 12.2 g under Kerala conditions. They reported that mace dry weight was negatively correlated with number of fruits per tree and seed weight was positively correlated with mace weight. In a conservation study of nutmeg germplasm at IISR, Krishnamoorthy (1997) observed 452 nutmeg accessions and found wide variation in number of fruits per tree after evaluating 28 trees. According to him, among the 452 nutmeg accessions, ten accessions had high fruit set and yield. Also a rare accession which had 1- 4 seeds per fruit was also identified. Vikram (2016) observed wide variation in number of fruits per cluster after evaluating 50 nutmeg accessions. He found that one to four fruit per cluster was least observed, while majority of the trees had two fruits per cluster followed by three fruits per cluster and then single fruit per cluster. The pattern of fruit bearing density was observed in nutmeg: fifty per cent of evaluated accessions had intermediate bearing followed by sparse (30.43%) and profuse (19.55%) bearing density. Different fruit shapes were noticed among the

accessions *viz.,* round, oval, ovoid and pyriform. Of these, round fruit shape was observed among majority of the accessions followed by ovoid, oval and pyriform shapes. Number of seeds per fruit in accessions varied from one to two with majority of accessions having fruit splitting into two halves and rarely into three or four splits in case of monoecious types.

In nutmeg, artificial pollination would be beneficial over open pollination, which helps in increasing fruit set and yield but fruit drop was later observed. It takes eight months for fruit development and maximum fruit drop was observed after three months of fruit set (Shigvan and Kore, 2003). Significant variation with respect to duration of anthesis and fruit set was reported in nutmeg by Haldankar *et al.* (2004a). They observed two cyclic growth peaks in January - February followed by May. The duration of anthesis was short in female flowers when compared to that of male flowers. Higher phenotypic and genotypic coefficient of variation indicated higher amount of variability. Both phenotypic as well as genotypic coefficients of variation for fruit set and duration of anthesis were higher. Haldankar *et al.* (2004b) reported higher genetic advance for fruit characters like fresh mace and nut weight. Magnitude of heritability was also high for fruit characters like fresh fruit length, breadth and weight along with fresh mace and nut weight. They concluded that the environmental coefficient of variation was low for all traits in nutmeg. In Maharashtra, thirty nutmeg genotypes were grouped into early (225 days), mid (250-275 days) and late (300 days) duration types (Haldankar *et al.*, 2009).

According to Vikram (2016) flushing pattern in nutmeg varied significantly with mid season flushing pattern in majority of the trees followed by early and late flushing patterns. The colour of flushes observed a frequency distribution of 50 per cent (yellowish green colour) followed by 44 per cent (greenish yellow colour) and 6 per cent (light green colour).

Shinde *et al.* (2006) reported occurrence of solitary flower bud in female nutmeg trees and occurrence of flower bud cluster on monoecious trees. Fruit setting percentage ranged between 18 and 40 per cent. Fruit characters showed significant variability at genotypic as well as phenotypic levels with least influence of environmental factors. They concluded that mean fresh fruit weight was 49.82g with mean fresh and dry nut weight of 7.28 g and4.59 g each respectively. Similarly the mean fresh and dry mace weight was 1.87g and 0.78g respectively. The recorded kernel weight was 3.06g and shell weight 1.53g. Thus they concluded that there existed good scope for exploiting the variation through selection on performance basis of individual trees. The selection in nutmeg should be made in such a way that trees selected should possess optimum fruit number and moderately good seed weight (Parthasarathy, 2010).

A variability study on nutmeg conducted by Vikram (2016) reported that, 92 per cent trees evaluated were dioecious and only 8 per cent trees were having monoecious nature. He also observed that most of the nutmeg trees were having broadly pyramidal canopy architecture and spreading branching pattern.

Yield in nutmeg is a complex character as reported by Vikram (2016) and it is necessary to assess association of various yield contributing components with yield and within themselves. This was made possible by determining the correlation coefficients between the combining traits and yield. Krishnamoorthy *et al*. (1991) observed high variation for number of fruits per tree and fruit weight. Fruit number per tree showed a negative correlation with mace weight and seed weight had a very high positive association with mace yield.

Haldankar *et al*. (2007) summarized in a genetic divergence study, thirty four genotypes of nutmeg into 12 clusters. Among the clusters, one of the clusters had genotypes superior for total weight of fruits, pericarp weight, fruit length, nut length and shell weight. Another cluster had genotypes with characters of large fruits and

nuts and high kernel butter yield. Significantly high amount of variability was reported in growth rate, size and shape of the leaf, flower size and shape, productivity, fruit set and size of the fruit and seed in nutmeg (Krishnamoorthy *et al.*, 1996; Haldankar *et al*., 2004a; Haldankar *et al.,* 2006; Sasikumar, 2009).

In his study, Vikram (2016) reported different mace shapes in nutmeg *viz.,* oval, round and oblong along with different fresh mace colour *viz.*, red, deep red, orange-red and yellow. Majority of the evaluated accessions had oval shaped mace with red colour, which on further drying turned to scarlet red. He also reported an accession with a distinct feature of beaked mace. Likewise, nut characters also showed wide variation and concluded that fresh nuts had mostly oval shape with shining black colour, which after drying turned into dark brown with grey coloured kernels with shallow grooves. Twenty year old nutmeg trees grown in coconut plantations showed variation in yield for continuous six years under Konkan region of Maharashtra in terms of number of fruits and nut weight (Haldankar *et al*., 2003).

Seed anatomy studies on nutmeg revealed that it had anatropus ovule with outer and inner integuments; the inner integument surrounds the micropyle region. Sastri (1953) worked on the seed anatomy in nutmeg and revealed that testa and tegmen are thick. Nucellus enlarges by cell growth without division, then it is absorbed and crushed. Endosperm is oily and starchy. Ruminations formed by outgrowth of the inner brown vascular layer of the tegmen, push the nucellar tissue into the endosperm except at the pointed micropylar end. Sastri (1955) repoted that aril arises from the outer integuments. Nair and Pillai (1959) contradicted this and reported that aril arises from funicular region. Later it was proposed that mace originated from both exostomal and funicular region.

Krishnamoorthy (2008) suggested that *Myristica malabarica* can be used as drought tolerant hardy rootstock to combat water stress condition. Sangadji *et al.* (2015) reported that in Moluccas Islands, the nutmeg flowering season started during

month of March or May and continued up to December. Fruit harvesting starts in March or April and continued up to December or January for eight to ten months. The mean fruit set percentage of nutmeg ranged from 22.63 to 47.53. A study on genetic diversity of nutmeg species by Das *et al*. (2012) in North Moluccas recorded significant variation for mature fruit shape and colour, shape as well as mace weight. Based on the phenotypic markers, accessions were formed into four clusters at 70 per cent similarity level. The variation between nutmegs from individual trees is frequently greater than the mean variations between regions, indicating an almost unlimited reservoir of material for selection or breeding purposes (Weiss, 2002).

Twenty three nutmeg accessions were evaluated for fruit and yield characters under high altitude region of Kodagu in Karnataka and results showed high variability for fresh seed yield per tree and medium to high variability for mace characters. Least variability was recorded for the leaf and seed characters as reported by Senthilkumar *et al*. (2010).

Purseglove *et al.* (1981), reported that in an individual nutmeg fruit, the aril weighs 1 to 4g and seed weighs 5 to 10 g with 20:3 as ratio of nut to mace. RAPD DNA profiling of clonal and seedling progenies of high yielding nutmeg selections was done by Sheeja *et al. (*2006) for detecting the variations among the populations and uniqueness. The Jaccard's Similarity Index values ranged from 0.72 to 1.00 which indicated the close relatedness among the progenies and mother. The similarity index within the clones and seedlings ranged from 96-100 per cent and 76-100 per cent respectively.

2.3 Pest and diseases in nutmeg

Wilson and Sathiarajan (1974) first reported die back disease of nutmeg in Trivandrum district of Kerala state, caused by *Diplodia* sp. *Marasmius pulcherima* and *Marasmius equicrinus* fungi cause two types of thread blight in nutmeg ie white thread blight and another is horse hair blight. Same authors also reported that in white

thread blight, the fungal hypha is white which aggregate underneath the leaves and also traverse along the stem in irregular manner and finally result in blight appearance. But in case of horse hair blight, the fungal hypha is black silky thread which form loose irregular network on stem and leaves. Thread blight disease incidence was more predominant in nutmeg plantations under heavy shade as reported by Nair *et al.* (1977). Skaria *et al.* (2000) observed horse hair blight disease in nutmeg in Rayamangalam panchayat of Ernakulam district in Kerala, India. They concluded the typical symptom for horse hair blight as dried leaves and branches hanging in black mycelia network giving a bird's nest like appearance. Haldankar and Rangwala (2009) reported that phytosanitation and shade regulation followed by 1% Bordeaux mixture spray was effective against thread blight infection.

Shot hole was another minor leaf disease noticed in nutmeg which is characterized by necrotic spot surrounded by a chlorotic halo which become brittle and fall off resulting in holes. Several workers reported that a prophylactic spray with 1% Bordeaux mixture was found effective against shot hole disease in nutmeg caused by *Colletotrichum gloeosporioides* (Menon and Remadevi, 1965; Nair *et al.,* 1977; Sankaran *et al*., 1980; Radhakrishnan,1986).

Seedling wilt is another major disease in nutmeg nurseries resulting in 5- 40 per cent seedling mortality. The actual causative organism is not known, but several workers implicated that causative organism for seedling blight can be *Cylindrocladium sp, Fusarium sp., Phytophthora sp., Colletorichum sp.* and *Rhizoctonia bataticola* (Philip *et al.,* 1973; Rahman *et al.,* 1981; Raju and Leelavathy, 1987). Lawate *et al.* (1986) reported that leaf spot in nutmeg was caused by *Cladiosporium oxysporum*

Vikram (2016) noticed higher incidence of *Phytophthora* leaf fall disease and mild incidence of fruit rot and die back disease among the accessions evaluated. Thread blight infestation was high in two accessions, rest of the trees were free from

this disease. *Colletotrichum* leaf spot infestation was also observed in some trees. He also reported that all nutmeg accessions evaluated were free from pest infestation.

Mathew and Beena (2012) reported occurrence of leaf fall disease during south west monsoon period in nutmeg growing tracts like Thrissur, Ernakulam, Kottayam and Idukki. The study confirmed the causative organism as *Phytophthora* sp. causing the leaf fall and shoot rot of nutmeg. The infection mainly affects the stem and leaf, which causes extensive defoliation. Mathew and Miniraj (2013) reported severe outbreak of leaf fall disease in Kerala. Sumbula and Mathew (2015) concluded that spraying of 1% Bordeaux mixture + soil drenching of copper hydroxide and spraying of 1% Bordeaux mixture + soil application of *Trichoderma viride* showed maximum effectiveness against leaf fall diseases.

2.2 Variability in Biochemical characters

Nutmeg, unlike other tree spices, is a fat rich spice with attractive split opened yellow coloured fruits showing the red aril and nut. Fully matured fruits are harvested mainly for the mace and kernel for culinary and other industrial purposes.80-85% fruit weight was mainly contributed by the rind. Due to the strong aroma and astringent taste, the fruit rind was considered as a farm waste till last few years (Teena, 2015). But now the situation changed to certain extent of having diversified products from nutmeg rind like nutmeg rind syrup, rind candy and wine. Nutmeg, mace and the essential oils extracted are generally used for preparation of confectionery items like cakes, cookies, doughnuts, fruit pies and puddings to give them a delicate smooth flavour.

Apart from nutmeg and mace, other value added products derived from *M. fragrans* are volatile oil, oleoresin and fixed oil. These high value products find their utility in the food, medicine and perfumery industries. Nutmeg volatile oil is colourless or light creamy liquid having the characteristic odour and taste of fresh nutmeg. The oil is soluble in alcohol, but insoluble in water. Volatile oil is sensitive

to light, it should be kept in tightly closed containers and protected from light. Oleoresin is sold on a neutral base such as salt, dextrose, flour or husk. The fixed oil is an orange coloured aromatic semi-solid, also known as concrete or nutmeg butter. The crude fixed oil contains essential oil in the range of 10-12 per cent. The major component of fixed oil is trimyristin. Fixed oil was composed of mainly saturated fats (90%) with 10 per cent unsaturated fats (Leela, 2008). Shree *et al.* (2008) conducted a comparative study on the chemical constituents present in *M. fragrans* and *M. malabarica*. According to her study, the essential oil constituents in *M fragrans* were eugenol,isoeugenol, methyl eugenol, myristicin, trimyristicin, safrole, elemicin, dehydroisoeugenol, β- pinene and γ- terpinene whereas the essential oil constituents in *M. malabarica* were malabaricanol, malabaricones A-D, licarin-B, myristic acid, palmitic acid and oleic acid .The major hallucinogenic constituents in both mace and kernel were myristicin and elemicin, with significantly higher myristicin (5.92%) and elemicine (3.14%) in mace oil than nutmeg oil (Teena, 2015)

The oil is used in canned stews and soups and has an important application in neutralizing the unpleasant smell of cooked cabbage (Lewis, 1984). USA import bulk quantities of nutmeg and mace oils for various culinary, industrial and pharmaceutical applications. Myristicin or methoxy safrole is the major aromatic constituent of nutmeg and mace oils. Yen *et al.* (1996) revealed that in nutmeg quality and chemical composition vary significantly according to geographic, climatic and maturity conditions.

2.2.1 Variability in rind composition of nutmeg

Proximate composition analysis of any fruit helps us to estimate the nutritional content in any food material and which can be utilized to meet the nutritional requirements of our body. In a study, the proximate composition analysis of fully ripened nutmeg fruit parts like rind, kernel and mace showed significant variation in chemical and physical characteristics (Gopalakrishnan, 1992). Parameters

like moisture content, acidity, total reducing sugars, total sugars, total ash, crude fibre and pectin recorded highest values in the rind. At the same time, kernel recorded highest concentration of volatile and non volatile oil substances. Mace was rich in starch and protein content.

Gopalakrishnan (1992) reported a moisture content of 88 per cent in nutmeg rind. Acidity content of pericarp was 2.5 per cent on fresh weight basis and 20.83 per cent on dry weight basis (Gopalakrishnan, 1992). Nutmeg rind is thick, yellow and fleshy with an average moisture content of 89.17 per cent as reported by Teena (2015). Vikram (2016) reported the range of titrable acidity content of nutmeg rind as 1.28 to 1.92 per cent. Abdullah (2009) reported that in nutmeg the moisture content was highest in the leaves followed by pericarp, mace and nutmeg except for kernel.

According to Teena (2015), nutmeg rind possessed 1.43 per cent acidity, 35.20 mg/ 100g phenol and 33.80 mg/100 g tannin content. She also reported low vitamin C content (11.45 mg/100g) in fresh nutmeg rind. Proximate analysis of nutmeg rind also revealed presence of starch (0.95g/100g), protein (1.25g/100g) and crude fibre (2.55 %).

Pectin can be extracted by using hydrochloric acid and citric acid. Pruthi and Krishnankutty (1985) found that HCl was better than citric acid in pectin extraction from nutmeg pericarp. In the same study, they observed 27 per cent crude fibre and 14 per cent pectin in nutmeg rind. Starch content was about 0.42g/100g on dry weight basis of pericarp (Gopalakrishnan, 1992).Tannin content in nutmeg rind as reported by Gopalakrishnan (1992) was 2.42 g/100g. Vikram (2016) obtained starch content within a range of 0.30 to 1.23 (g/100g). A study conducted by Burubai *et al.* (2009) on proximate analysis of African nutmeg seeds revealed that it contained 29.1% volatile oil and 8.3% acid content. Fruits are rich source of anti oxidants, ascorbic acid was the most abundant one among the plant antioxidants (Smirnoff, 2000).

2.2.2 Variability in Mace and Kernel volatiles

The hallucinogenic principles in nutmeg oil are myristicin, elemicin and safrole as reported by Purseglove *et al.* (1981). Nutmeg volatile oil comprised of a mixture of alkeneylbenzene derivatives and terpenes. Among the alkeneylbenzene derivatives, myristcin , elemicin and safrole contribute 80% of oil composition. A study was conducted by Yen *et al.* (1996) on the yield and chemical composition of essential oils from Grenadian nutmegs (*Myristica fragrans*). Oil yields decreased with increased drying temperatures, and this was associated with corresponding decreases in monoterpene hydrocarbon content. Results indicated that at a temperature of 21-23°C essential oil yield from nutmeg on dry weight basis was maximum. Oil yield was negatively correlated with drying temperature, and it was due to corresponding decreases in monoterpene hydrocarbon content. Grenadian nutmeg oils contained more sabinene but less myristicin and safrole than oils from other geographic regions. Various clinical and toxicological studies indicated that 6 to 7 mg of myristicin per kg of body weight was enough to cause psychopharmacologicaI effects. Woolf (1999) also reported the specific toxicity of nutmeg essential oils. On an average India exports 11 to 12 metric tonnes of nutmeg oil every year (Peter and Zachariah, 2000). Amaladhas *et al.* (2002) observed that hot water blanching of mace at 75° C for two minutes conserved the mace qualities during drying.

Evans (2003) conducted an experiment to study chemical composition of nutmeg volatile oil from different parts of India and observed that chemical composition of nutmeg oil was significantly influenced by location. Kumar (2012) conducted comparative analysis of volatile oil of nutmeg samples collected from North East India and Kerala. He concluded that North East Indian sample (8.50%) had higher volatile oil content than Kerala sample (7.25%).

Naveen (2013) conducted a study on drying and storage of nutmeg. He estimated the oil content in harvested and fallen nutmeg separately and concluded that in harvested nutmeg, the kernel oil content was 8.06 per cent and mace oil content was 8.41 per cent while, in fallen nutmeg the kernel oil content was 8.13 per cent and mace oil content was 8.21 per cent. Storage studies concluded that oil content decreased drastically in both cases and maximum retention of oil content was in aluminium laminated pouches.

Maya *et al*. (2004) have reported a volatile oil content of south Indian nutmeg as 3.9 to 16.5 per cent and in mace as6.0 to 26.1 per cent. Maya *et al.* (2006) in another study evaluated *Myristica* species in terms of biochemical parameters in leaf, nut and mace. Myristic acid content was high in nuts of *M. fragrans, M. beddomeii* and *M. prainii* and high content of palmitic acid was recorded in mace of *M. fragrans*. High concentrartion of phenyl alanine was recorded in *Myristica fragrans*. *K. andamanica* leaf was rich in amino acids like threonine and alanine. The average volatile oil content in kernel and mace collected from Kannur district of Kerala were 8.7 and 15.8 per cent respectively (Abdurrasheed and Janardanan, 2009). Aroma compounds of nutmeg are mainly monoterpenes (87.5%), monoterpene alcohols (5.5%) and other aromatics (7.0%). The principal constituents of mace oil are sabinene, α-pinene, myrcene, limonene, 1, 8-cineole, terpinene-4-ol, myristicin, γ-terpinene and safrole (Pooja *et al.,* 2012). Marzuki *et al.* (2014) observed that Moluccas nutmeg contained 9.99- 11.92% essential oil with highest myristicin content of 13.76%.

Rong and Tao (2011) conducted a study on GC MS analysis of volatile oil of nutmeg and reported 48 components which included myristicin (36.53%), terpinen-4-ol (12.47%), myristic acid (8.27%), elemicin (4.96%), safrole (4.75%), γ-terpinene (4.56%), and methyleugenol (4.38%). Though West Indian nutmeg is low in myristicin, some nutmeg accessions available in India also have low myristicin in oil. The predominance of myristicin in some locations of cultivation may be a reflection

of agroclimatic factors. Myristicin being an antioxidant possessed the property of scavenging cancer-causing free radicals as reported by Maya et *al.* (2004)

Another study conducted by Mallavarapu and Ramesh (1998) revealed the composition of essential oils of nutmeg and mace as follows: nutmeg oil composed of alpha pinene (13.6%), beta-pinene (12.9%), sabinene (32.19%), limonene (4.0%), gamma- terpinene (3.9%) and terpinen-4-ol (7.2%); mace oil composed of alpha terpinene (3.9%), gamma- terpinene (6.6%), terpinolene (3.3%),terpinene-4-ol (23.6%), sabinene (23.5%), eugenol(3.7%), limonene (3.9%) and elemicin (10.5%). They also concluded that Indian nutmeg oil when compared to East Indian and West Indian nutmeg oils, had moderate quality. Valente *et al.* (2011) reported radial growth inihibition in bacteria and fungi when treated with 0.1% nutmeg essential oil and growth inhibition rate reached 100% on treatment with 0.3% nutmeg oil.

In a comparative study of the volatile oil constituents of nutmeg collected from Kerala and North East India, chemoprofiles of North East Indian nutmeg oil constituted majorly, alpha-limonene (1.51 %), m-cymene (4.03 %), terpineol (23.39 %), safrole (13.23 %), isohomogenol (5.14 %) and myristicin (1.33 %), while in Kerala types volatile oil was rich in alpha-pinene (15.21 %), sabinene (38.79%), limonene (4.51 %), terpineol (4.15 %), safrole (6.34 %) and myristicin (6.15 %) (Soni , 2012).

Gas chromatography and mass spectrometry analysis of nutmeg essential oil showed the presence of 38 components representing about 99.60 per cent of the total weight. Sabinene (29.40%) was found to be a major component along with beta-pinene (10.60%), alpha pinene (10.10%) and terpene-4-ol (9.60%) as reported by Kapoor *et al.* (2013).

The supercritical fluid extraction was done on nutmeg seeds and the volatile oil obtained composed of myristicin (32.8%), sabinene (16.1%), α-pinene (9.8%), β-pinene (9.4%), β-phellandrene (4.9%), safrole (4.1%) and terpinen-4-ol (3.6%) (Piras *et al.,* 2012). Rema *et al.* (2015) identified a nutmeg germplasm A9/71(IC-537220);

INGR 10142 with high sabinene content. They reported that accessions A9/71 and A9/95 had high sabinene content and low myristicin, elemicin and safrole contents. Maya (2005) concluded that accessions with high myristicin and elemicin content in both mace oil and nutmeg oil were suitable for pharmaceutical industry whereas low myristicin and elemicin levels in both nutmeg and mace oil coupled with high sabienene content was best suited for confectionary preparations.

In his study Vikram (2016) reported that the total volatile constituents of nutmeg oil ranged between 68.96 and 90.49 per cent. The range of percentage composition of kernel oil was myristicin (2.48 - 12.48%) , elemicin (4.31 - 22.43%), safrole (2.44 - 4.64%), sabinene (1.06% - 11.75%), alpha pinene (2.55 - 6.0%) and L-4- terpineol (10.12 - 25.60%). The range of percentage composition of mace oil was 1.57 - 18.87% for myristicin, 1.39 - 27.86% for elemicin, 2.96 - 4.89% for safrole, 16.15% - 16.32% for sabinene, 4.49 - 9.50% for alpha pinene and 6.74 - 17.34% for L-4- terpineol.

2.2.2.1 Nutmeg leaf oil

Maya (2005) reported that nutmeg leaf oil also contained the same constituents as that of nutmeg and mace but in varied concentrations. Wider variation was found in constituents like sabinine, safrole, myristicin and elemicin in nutmeg leaf oil. Leaf oil recovery does not have any correlation with total yield of nuts. Weiss (2002) reported that nutmeg leaf oil obtained through steam distillation was of inferior quality when compared to oil obtained from nutmeg and mace.

Hydrodistillation of nutmeg leaves yielded 0.41 to 0.60 per cent volatile oil with light brown colour and pleasing spicy aroma. However the essential oil recovery from leaves was higher 1.58 per cent in steam distillation. There was no much visual variation among oil of mace and oil of nutmeg. On storage, mace oil became more viscous than fresh ones (Anon, 2008).

Biochemical profiling of leaf volatile oil of three *Myristica spp*. conducted by Zachariah *et al*. (2008) revealed that *M. fragrans* dominated in monoterpenes (91%) followed by *M. beddomeii* which contained mono(48%) and sesquiterpenes (35%), whereas *M. malabarica* was dominated by sesquiterpenes (73%). *M. beddomeii* contained α-pinene (19.59%), t-caryophyllene (14.63%) and β-pinene (12.46%). *M. fragrans* contained sabinene (19.07%), α-pinene (18.04%), 4-terpineol (11.83%), limonene (8.32%) and β-pinene (7.92%) as major compounds, while t-caryophyllene (20.15%), α-humulene (10.17%), nerolidol (9.25%) and δ-cadinene (6.72%) were predominant in *M. malabarica*.

Zheng *et al*. (1992) found a potent anticarcinogenic sesquiterpene, *β*-caryophyllene in *M. beddomeii* leaf essential oil. These natural anticarcinogenic substances can induce detoxifying enzymes and their activity was comparable with that of chemical carcinogens. *Myristica fragrans* leaf oil contains only 2.6% β-caryophyllene , which is significantly lower than *Myristica beddomei* (40% *β*-caryophyllene). Verghese (2001) revealed that nutmeg leaf oil from Indonesia had 80% alpha pinene and 10% myristicin.

2.2.2.2 Nutmeg rind oil

Pericarp oil on comparison with nutmeg and mace oils had higher concentration of terpinen-4-ol and α- terpineol, but lower concentration of sabienene, myristicin and safrole (Choo *et al.,* 1999). Maya (2005) found that nutmeg rind on distillation yielded 0.123% essential oil and GC MS profiling of rind oil showed the presence of myristicin (9.82%) and elemicin (18.07%). Teena (2015) identified the presence of myristicin, elemicin, terpene-4-ol, alpha-terpinol, methyl (z)-N-hydroxy benzene carboximidate, 1,2, dimethoxy-4[(z)-l-methoxprop-l-enyl), benzene and methyl laurate in GC –MS profiling of nutmeg rind oil.

2.2.3 Variability in mace and kernel oleoresin

Oleoresin may be defined as the total extract of spice that may be used to replace the spice for its aroma, taste and flavour. Mace oleoresin is inclusive of the fixed oil and nutmeg oleoresin is exclusive of the fat or fat that it possess, this can be the reason for lesser oleoresin from nutmeg kernel (Maya, 2005). Sofyana *et al.* (2013) showed that the Soxhlet method of oleoresin extraction required a longer time and higher temperature to produce oleoresin when compared to maceration and ultrasonic waves assisted oleoresin extraction methods. The ultrasonic extraction resulted in highest oleoresin yield of 6.2% at the condition of 50°C and 3 hours of extraction temperature and extraction time, respectively. Teena (2015) reported that mace yielded 27 to 32 per cent oleoresin and nutmeg kernel yielded 18 to 26 per cent oleoresin.

Oleoresin content in both harvested and fallen nutmeg was estimated by Naveen (2013) and reported 22.40 per cent mace oleoresin and 29.09 per cent kernel oleoresin in harvested nutmeg. Likewise, 22.06 per cent mace oleoresin and 29.63 per cent kernel oleoresin was found in fallen nutmeg. Oleoresin content in kernel decreased less rapidly than mace on long term storage.

In the study by Vikram (2016) kernel oleoresin ranged between 18.59 and 36.20 per cent while mace oleoresin ranged from11.38 to 31.66 per cent. He concluded that kernel oleoresin was higher than mace oleoresin in all the accessions.

2.2.4 Variability in fixed kernel oil in nutmeg

Nutmeg butter is a semisolid yellowish red coloured substrate which contained trymyristicin up to 75% (Weiss, 2002) .The main components of nutmeg fixed oil were myristic acid, trimyristicin, glycerides, stearic acid, lauric acid, linoleic acid and palmitic acid (Duarte *et al.,*2011).

Purseglove (1981) reported that nutmeg kernel contained 30-40% fat. Mace had comparatively lesser fat content than nutmeg kernel. Nutmeg kernel attacked by storage pests had low fat content. He concluded that there was no direct correlation with yield of nut in a plant and variation in fat content. Palmitic and oleic acids were predominant fatty acids in mace according to Prakashchandra and Chandrasekharappa (1984). Verghese (2001) reported that 90.6% of total fatty acids in nutmeg were saturated and only 8.7% were unsaturated. Fixed oil content in nutmeg ranged from 17.79 to 44.80 per cent (Vikram, 2016)

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present investigation “Collection and characterization of unique genotypes of nutmeg (*Myristica fragrans* Houtt.) ” was carried out at the Department of Plantation Crops and Spices, College of Horticulture, Kerala Agricultural University, Thrissur during 2014-2016. Details of the materials used and methods followed in the study are described in this chapter.

3.1 Materials

Twenty one unique accessions were identified and collected through an extensive survey throughout the nutmeg growing tracts of Kerala. The unique accessions collected from diverse locations of Kerala along with the ‘two’ improved accessions identified in an earlier study conducted by Vikram (2016) formed the material for the study. The details and their unique features are listed in the Table 3.1.

3.2 Characterization of accessions

3.2.1 Qualitative characters

All the accessions were characterized as per the nutmeg descriptor developed by Vikram (2016).

3.2.1.1 Canopy shape

The canopy shape of the mature tree was observed and grouped into narrowly pyramidal, pyramidal, oblong or globular.

3.2.1.2 Branching pattern

The branching pattern of the tree was recorded and was grouped into erect, spreading or drooping.

Table 3.1 Unique accessions of nutmeg identified and their locations

Sl no.	Accession ID	Features	Location
1	YL-1	Yellow maced type	Chalakudy
2	YL-2	Yellow maced type	Thavalakuzhy
3	SL-1	Seedless type	Muniyara
4	DS-1	Double seeded type	Pattikaad
5	DS-2	Double seeded type	Chalakudy
6	MN-1	Monoecious type	Chalakudy
7	MN-2	Monoecious type	Pattikaad
8	CF1	Cluster fruited type	Vaikkom
9	CF-2	Cluster fruited type	Chalampadam
10	LA1	Low astringent type	Chalakudy
11	LA2	Low astringent type	Vazhikadavu
12	PT1	Pyramidal type	Chalakudy
13	PT2	Pyramidal type	Kallurkadu
14	GT1	Grape type	Pattikaad
15	GT2	Grape type	Chalampadam
16	WT1	Wild type	Pattikaad
17	WT2	Wild type	Mullaringaad
18	IA1	Improved accession	Manalai
19	IA2	Improved accession	Manalai
20	OB1	Oblong type	Chalakudy
21	OB2	Oblong type	Chalakudy
22	TM1	Triangular mace type	Koottala
23	SM1	Small leaf type	Kanjirappally

3.2.1.3 Mature leaf shape

The mature leaf shape was recorded from the 5th leaf of the first primary laterals towards four directions. Five groups namely elliptic, oblong, ovate, obovate and lanceolate were recognised.

3.2.1.4 Mature leaf colour

The leaf colour of mature leaf was recorded and grouped into light green, green or dark green.

3.2.1.5 Colour of flushes

The emergence of new flushes was observed and the colour grouped as light green, greenish yellow, yellowish green and purple green.

3.2.1.6 Season of flowering

Peak flowering period was taken as the season of flowering. Season of flowering was classified as Early (July), Mid (Late July - Early August) or late (August).

3.2.1.7 Inflorescence type

The two types of inflorescence, namely axillary raceme and umbellate cyme were recorded during the peak flowering time.

3.2.1.8 Colour of perianth

The colour of perianth was recorded and grouped into creamy, creamy white, creamy yellow or greenish creamy.

3.2.1.9 Colour of filament

The colour of filament was recorded. It was creamy white, white or light yellow cream.

3.2.1.10 Colour of anther

The colour of anther was recorded and was classified into pale yellow and yellow.

3.2.1.11 Fruit colour (fresh)

Fruit colour was recorded from fresh fully mature ripe stage fruits and classified into three groups namely light yellow, yellow and yellow light green.

3.2.1.12 Mace colour (fresh)

Fresh mace colour was recorded through repeated observation of mature ripe fruits and was classified into four groups namely deep red, red, orange red and yellow.

3.2.1.13 Mace colour (dry)

The resultant changes in colour on drying the fresh mace was noted as red, scarlet red, orange red or yellow.

3.2.1.14 Nut colour (fresh)

Fresh nut colour was recorded through repeated visual observation of mature split open fruits and was classified into brown, dark brown, greyish brown or black coloured group.

3.2.1.15 Nut colour (dry)

Nut colour after drying was recorded through repeated visual observation and was classified into light brown, brown, dark brown and black.

3.2.1.16 Kernel colour (dry)

The colour of kernel after shelling the dried nuts was recorded and was classified into grey, dark grey and light brown.

3.2.1.17 Shape of mace

The shape of mace was recorded and was classified into round, oval and oblong accordingly.

3.2.1.18 Nature of fruit dehiscence

Fruit dehiscence was classified into three as given below

1) Nutmeg and mace along with pericarp drop

2) Nutmeg and mace dispersed with pericarp persistent on the tree

3) Nutmeg and mace along with pericarp persistent on the tree

Each group was then again subdivided into two halved fruit, three halved fruit and four halved fruit according to the number of halves of pericarp of the split open fruit.

3.2.2 Quantitative characters

3.2.2.1 Plant height

The fully mature tree height was measured from the ground level to the tip portion of the canopy using tape and expressed in metres.

3.2.2.2 Girth at 140 cm height

The girth of the tree was measured as the circumference of tree trunk at a height of 140cm from the ground level and expressed in centimetres.

3.2.2.3 Canopy spread (N-S) and (E-W)

Tree canopy spread was taken in both North - South and East - West directions, recorded according to the maximum branching spread in the related directions.

3.2.2.4 Leaf length

Leaf length was measured from the base to the tip of lamina. The average length of 5^{th} leaf from tip collected from all the four sides with a representative sample size of five each was measured and expressed in centimetres.

3.2.2.5 Leaf breadth

Leaf breadth was measured on the same leaves mentioned above from the widest portion of leaf blade.

3.2.2.6 Leaf area

Leaf area was recorded with leaf area meter as the area of the fifth whole leaf. Leaf area was measured on the same leaves, which were used for taking length and breadth observations and expressed in cm^2.

3.2.2.7 Number of orthotrops

Number of orthotrops were counted and recorded.

3.2.2.8 Number of female flowers/ 10 cm^2

Recorded by counting number of female flowers in 10 cm^2 area on all four sides of tree.

3.2.2.9 Number of male flowers / 10 cm^2

Recorded by counting number of male flowers in 10 cm^2 area on all four sides of tree.

3.2.2.10 Number of hermaphrodite flowers/ 10 cm^2

Recorded by counting number of hermaphrodite flowers in 10 cm^2 area on all four sides of tree.

3.2.2.11 Length of flower (cm)

The length of ten fully opened flowers from the base to the tip of flower was taken and recorded.

3.2.2.12 Breadth of flower (cm)

The breadth of ten fully opened flowers at the widest portion was measured and recorded.

3.2.2.13 Length of perianth (mm)

The length of perianth was taken from ten samples and recorded.

3.2.2.14 Breadth of perianth (mm)

The breadth of perianth at the widest portion was taken from ten samples and recorded.

3.2.2.15 Length of filament (mm)

The length of filaments was recorded from ten different flowers.

3.2.2.16 Length of anther lobe (mm)

The length of anther lobes was recorded from ten different flowers.

3.2.2.17 Number of stamens

Number of stamens was recorded by counting the number of anthers which were adnate to the central column.

3.2.2.18 Fruit weight (fresh)

Recorded the fresh weight of ten representative fruit samples per accession and worked out the average.

3.2.2.19 Thickness of pericarp (mm)

Thickness of pericarp was measured by using vernier caliper from fully ripe fruits and expressed in mm.

3.2.2.20 Mace weight (fresh)

Recorded the fresh mace weight from ten representative samples per accession and worked out the average.

3.2.2.21 Mace weight (dry)

Recorded the mace weight from ten representative samples per accession after drying at 50°C for 3 - 4 hours and worked out the average.

3.2.2.22 Nut weight (fresh)

Recorded the fresh nut weight from ten representative samples per accession and worked out the average.

3.2.2.23 Nut weight (dry)

Recorded the nut weight from ten representative samples per accession after drying at 50-55° C for 14 - 16 hours and worked out the average.

3.2.2.24 Shell thickness (dry)

Recorded the shell thickness of ten representative samples per accession after shelling using a vernier caliper and worked out the average.

3.2.2.25 Kernel weight (dry)

Recorded the kernel weight of ten representative samples per accession after shelling the dried nut and worked out the average.

3.2.2.26 Number of fruits per tree

Recorded by counting fruit number regularly during peak harvesting period.

3.2.2.27 Ratio of nut to mace

Proportion of nut and mace weight was recorded and calculated as ratio of nut to mace.

3.2.2.28 Shelling percentage

Recorded as ratio of kernel to dried nut weight and expressed as percentage.

3.2.2.29 Seed weight

Recorded the fresh nut weight of ten representative samples per accession and worked out the average.

3.2.2.30 Seed volume (cm^3)

Recorded by using water displacement method from ten representative samples per accession and expressed in terms of cm^3.

3.2.2.31 Mace volume (cm^3)

Recorded by using water displacement method from ten representative samples per accession and expressed in terms of cm^3.

3.2.3 Incidence of diseases

Incidence of diseases like leaf fall (*Phytophthora* and *Colletotrichum*), fruit rot, thread blight and die back noticed in the field was recorded.

3.2.4 Biochemical parameters of pericarp

3.2.4.1 Moisture content

The moisture content of pericarp was calculated by using the following formula and was expressed in percentage.

$$\text{Moisture content} = \frac{(\text{Fresh pericarp weight} - \text{Oven dried pericarp weight}) \times 100}{\text{Fresh pericarp weight}}$$

3.2.4.2 Acidity

Acidity of pericarp was estimated by titration method given by Ranganna (1997). Dried powdered pericarp (1 g) was dissolved in distilled water and boiled for

10minutes. The water lost by evaporation is replaced and the contents transferred to a 250 ml volumetric flask and made up to the volume. An aliquot of the sample prepared as above was diluted with recently boiled distilled water. After adding a few drops of 1% phenolphthalein, titration was done with 0.1 N sodium hydroxide. The titre value was noted and expressed as percentage citric acid (%).

$$\text{Total acid (\%)} = \text{Titre value} \times \text{normality of alkali} \times \text{volume made up} \times \frac{\text{equivalent weight of acid} \times 100}{\text{Volume of sample taken for estimation} \times \text{weight of sample taken}}$$

3.2.4.3 Starch

Starch was estimated by anthrone reagent method given by Hedge and Hofreiter (1962).The dried and powdered pericarp sample (0.1 g) was homogenized in 80% hot ethanol to remove sugars, the sample along with ethanol was centrifuged and the residue was retained. Residue was washed repeatedly with hot 80% ethanol and then dried well over a water bath. To the residue, 5.0 ml of water and 6.5 ml of 52% perchloric acid were added. The modified residue was centrifuged for 20 min at 0°C and the supernatants pooled. The extraction was repeated using fresh perchloric acid and made up to 100 ml. The supernatant measuring 0.1 or 0.2 ml was pippetted out and made up the volume to one millilitre with water. The standards were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard and made up the volume to one millilitre with water. To each of the test tube, 4 ml of anthrone reagent was added. All the test tubes were heated for eight minutes in a boiling water bath. Then the test tubes were taken out and cooled . The intensity of green to dark green colour was read at 630 nm.

3.2.4.4 Tannin

Tannin was estimated by folin denis method given by Schandrel (1970). Dried and powdered material weighing 0.5 g was transferred to a 250 ml conical flask and

75 ml of water was added. The flask was heated gently for 30 min and centrifuged at 2000 rpm for 20 min. Resultant supernatant was collected in 100 ml volumetric flask and made up to the volume. The sample extract measuring one millilitre was transferred to a container and 100 ml water was added. To this, 5 ml of Folin-denis reagent and 10 ml of sodium carbonate solution were added and diluted to 100ml with water. After shaking thoroughly the absorbance was read at 700 nm after 30 min.

3.2.4.5 Pectin

Pectin was estimated by gravimetric method given by Carre and Haynes (1922). The blended sample weighing 50 g was taken into a 1000 ml beaker. Pectin was extracted with 400 ml of 0.05 N HCl for 2 hours at a temperature ranging 80°C - 90°C. The water lost by evaporation was replaced and contents cooled. After cooling, the contents were transferred to a 500 ml volumetric flask and made up to the mark with water. Afterwards it was taken and filtered through Whatmann no.4 filter paper into a 500 ml conical flask. Two aliquots each measuring in the range of 100-200 ml was transferred to 1000 ml beaker and 250 ml water was added. The acid was neutralized with 1N sodium hydroxide using phenolphthalein as indicator. After titration, 10 ml of 1N sodium hydroxide in excess was added with constant stirring. The content was allowed to stand overnight. Fifty millilitre of 1N acetic acid was added and after 5 minutes, 25 ml of 1N Calcium chloride solution was added with stirring. After allowing the contents to stand for 1 hour, it was boiled for 1 or 2 min and contents filtered through a previously prepared filter paper. Filter paper was prepared by wetting a fresh filter paper in hot water and dried in oven at 102°C for 2 hour s and cooled in a desiccator and the same weighed in a covered dish. The precipitate was washed with almost boiling water, until free from chloride tested using silver nitrate. The filter paper containing the calcium pectate was dried overnight at 100°c and cooled in a desiccator and weighed. The pectin content was expressed as percentage calcium pectate.

$$\text{Percentage calcium pectate(\%)} = \frac{\text{Weight of calcium pectate x 500 x 100}}{\text{Volume of filterate taken x Weight of sample for estimation}}$$

3.2.5 Biochemical characterization of mace and kernel

The mace and kernel of twenty three unique accessions including 'two' improved accessions were analysed for both volatile oil and oleoresin content. Fat content was estimated by extracting the fixed oil from the kernel.

3.2.5.1 Volatile oil

The volatile oil present in both kernel and mace was extracted by using Clevenger's apparatus through hydro distillation method (Clevengers, 1982). Fifteen grammes of dried powdered sample (mace or kernel) was mixed with 150 ml distilled water and then fed into a round bottom flask attached to the Clevenger's apparatus with condenser. Then the flask was heated gently up to a temperature of 70°C - 80°C and continued for three hours. The oil was collected in the receiver end of the Clevenger apparatus, cooled to room temperature. A pinch of anhydrous sodium sulphite was added to oil extracted, in order to remove any excess moisture. The volume of oil collected was expressed as per cent volume per unit mass of the sample

$$\text{Volatile oil (\%)} = \frac{\text{Volume of oil collected (ml) x 100}}{\text{Total weight of the sample (g)}}$$

3.2.5.2 Oleoresin

The oleoresin was extracted from mace and kernel by using Soxhlet apparatus through solvent extraction method with petroleum benzene. Five grammes of dried powdered sample was packed in thimble and then placed in extraction tube. Seventy five millilitres of petroleum benzene was then added to the extraction tube, and then

kept in water bath along with a condenser. The extraction continued for three to four hours till no colour was observed for the solvent in extraction tube. At the final stage of extraction, removed the thimble and distilled further to remove all the solvents. Results were expressed as percentage (ASTA,1968).

$$\text{Oleoresin (\%)} = \frac{\text{Weight of extracted oleoresin} \times 100}{\text{Initial weight of the sample (g)}}$$

3.2.5.3 Fat

Cold percolation method using acetone was employed in determining the fixed oil content from nutmeg kernel. Fifteen grammes of dried powdered sample was taken in glass column and filled with 60 ml acetone and kept undisturbed for one day. The top of the glass column should be plugged with cotton to prevent the evaporative loss of acetone. Drained out the solvent in the very next day and repeated the procedure for up to three washings with acetone. The solvent drained from column was collected in a beaker and kept for solvent evaporation. Finally the collected butter expressed in per cent (AOAC, 1975).

$$\text{Butter (\%)} = \frac{\text{Weight of extracted butter} \times 100}{\text{Initial weight of the sample (g)}}$$

3.2.5.4 GC-MS analysis of volatile oil

Volatile oil after extraction was subjected to GC MS analysis. GC MS of the oil samples was recorded in GC MS – Make – Agilent Model – 7890 A Model – 5975 C. 50 µl of the sample diluted to 1.0 ml with Dichloromethane and injected in to GC MS equipped with capillary column DB -5MS of 30 m x 0.25 mm x 0.25 µm dimension with injector temperature of 250 ^{0} C, interface temperature of 280 ^{0} C (10 min hold) and detector MS source with 230 ^{0} C. Helium was used as carrier gas with

split ratio of 50:1.Major compound peaks were analysed by comparing its mass fragment pattern with the standard spectra available in the data base.

3.3 Statistical analysis

The qualitative data were assessed through UPGMA (Unweighted pair group method with arithematic mean) method (Rohlf, 1992) and summarized using dendrogram (NTSYS package 2.2). Analysis of variance of all quantitative data was done using General Linear Model procedure suggested by McCulloh and Searle (2001).

RESULTS

4. RESULTS

The results of the study "Collection and characterization of unique genotypes of nutmeg (*Myristica fragrans* Houtt.)" carried out at the Department of Plantation Crops and Spices , College of Horticulture, in the nutmeg growing tracts of Kerala are presented in this chapter:

The extensive survey was conducted with the specific objective of identifying unique genotypes of nutmeg; "unique" as regards any of the qualitative / quantitative characteristics that are descriptors of the tree 'Nutmeg'. The survey could sort out 21 accessions that were unique as regards a characteristic / a group of characteristics. Though seemingly alike at times, with respect to a characteristic, a separating line could be easily drawn with respect to the featured characteristic of any of the pair of identified trees. A formal listing of the 21 accessions according to its specific feature is given below. This type of study will be quite inconclusive, if a comparative evaluation based on a commonly occurring nutmeg tree is not done. As such two "improved" accessions were co-opted into the study and designated accordingly. The list of the unique accessions and their respective featured characteristic is given in Table 4.1.The uniqueness was noticed as regards: shape of canopy, leaf size, wildness in general, sex expression, shape of fruit, size of fruit, clusterness in fruits, number of seeds, seedlessness, mace colour, shape of mace and astringency of the rind.

4.1 Unique nutmeg accessions

4.1.1 Yellow maced nutmeg

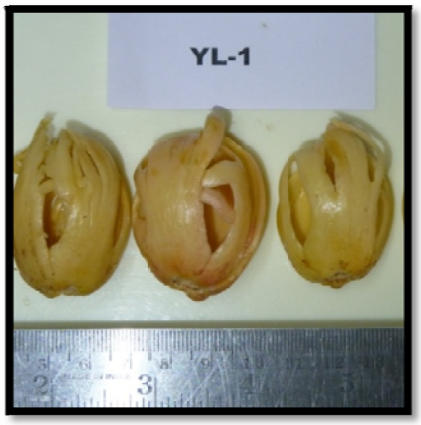
Yellow colour of mace is a preferred characteristic that is rarely spotted in the nutmeg family (Plate 4.1a). Normal mace colour in nutmeg is deep red in fresh form and scarlet red when dry. In the present study, two collections were spotted with yellow mace, YL-1 from Chalakudy (Plate 4.1b and 4.1c) and YL-2 from Kottayam. Both the accessions were budded trees of age 17 years and 13 years respectively. Data presented in Table 4.1 shows that the tree height ranged from

Table 4.1 Unique accessions of nutmeg and their featured characteristic

Accession ID	Featured characteristic
YL-1	Yellow maced type 1
YL-2	Yellow maced type 2
SL-1	Seedless type 1
DS-1	Double seeded type 1
DS-2	Double seeded type 2
MN-1	Monoecious type 1
MN-2	Monoecious type 2
CF-1	Cluster fruited type 1
CF-2	Cluster fruited type 2
LA-1	Low astringent type 1
LA-2	Low astringent type 2
PT-1	Pyramidal tree type 1
PT-2	Pyramidal tree type 2
GT-1	Grape type 1
GT-2	Grape type2
WT-1	Wild type 1
WT-2	Wild type 2
IA-1	Improved accession 1
IA-2	Improved accession 2
OB-1	Oblong type 1
OB-2	Oblong type 2
TM-1	Triangular mace type 1
SM-1	Small leaf type 1

a. Yellow mace (fresh)

b. Fruit



c. Yellow mace (dry)

Plate 4.1 Features of yellow maced nutmeg

6.48 -7.9 metres with a canopy spread of 5- 6.2 metres in North-South and 5.2 meters in East -West direction. Both the trees were having broadly pyramidal canopy shape with spreading branching pattern in YL-1 and erect branching pattern in YL-2. Mature leaf shape was elliptic in YL-1 as against the ovate leaf in YL-2. YL-1 showed late flowering habit where as YL-2 exhibited mid season flowering nature.

4.1.2 Seedless nutmeg

Seeding is extremely essential as regards any tree crop is concerned. However, a unique germplasm with rudimentary sterile seed / seedless nature was noticed in Muniyara, Idukki district and is included in the present study as SL-1 (Plate 4.2 a).The tree of seedling origin was of eighteen years age and had a height of 11 metres with 9.11 metres and 8.35 metres canopy spread in North - South and East- West direction.

Eighty per cent of fruits had rudimentary sterile seed inside the mace and rest twenty per cent fruits were totally seedless (Plate 4.2 d and4.2 e). Longitudinal section of the rudimentary seed showed hollow nature as depicted in (Plate 4.2 f and 4.2 g). The mace was thick, compactly arranged with several inner folding (Plate 4.2 b). A longitudinal section of mace shows the peculiar mace structure which resembles the folding of cerebrum in human brain. Fruits of this accession had rough netted skin (Plate 4.2 c).

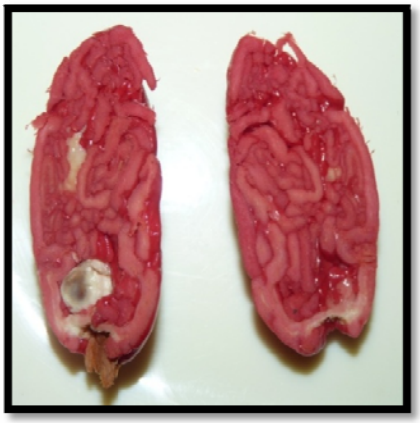
4.1.3 Double seeded nutmeg

Two double seeded nutmeg accessions were spotted at Pattikkad (DS-1) and Chalakudy (DS-2) regions respectively. Accession DS-1 was budded tree and DS-2 was seedling tree of age 14 years and 18 years respectively. These nutmeg types with two seeds may be regarded as accessions having aberration from the normal features, a characteristic worth to be pulsed in, towards the question of incorporation in the future studies (Plate 4.3 a). Both the accessions were monoecious in nature. Few abnormal flowers were also noticed in double seeded

a.Seedless nutmeg

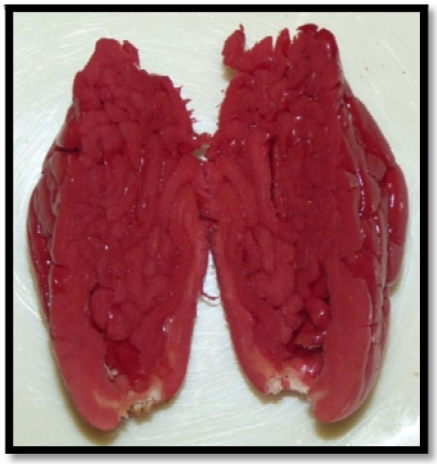
b. Seedless nutmeg fruit

c. Netted appearance on rind



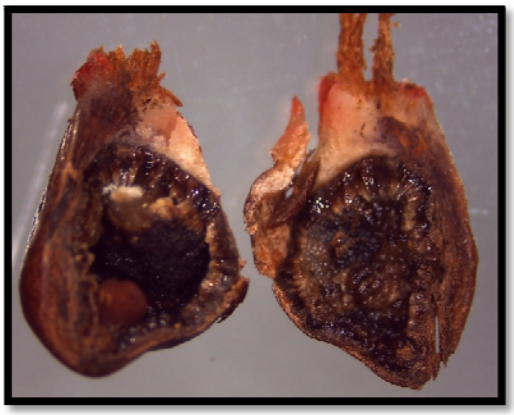
d. L .S. of mace with rudimentary sterile seed

Plate 4.2 Features of seedless nutmeg



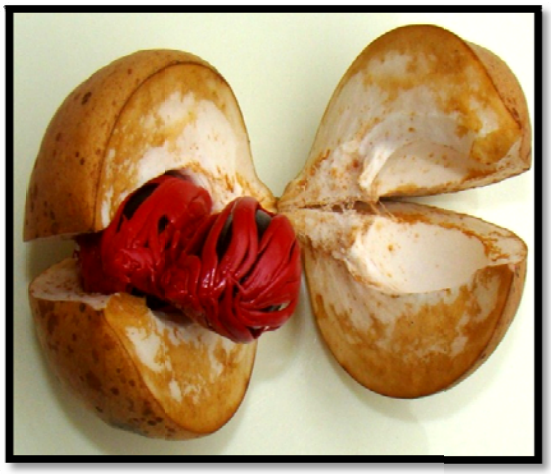
e. L.S. of mace without rudimentary sterile seed

f. Rudimentary seed in seedless nutmeg



g. L.S. of rudimentary seed

Plate 4.2 Features of seedless nutmeg



a. Double seeded nutmeg fruit

b. Four halved fruit splitting

c. Abnormal flower

d. Abnormal flower

Plate 4.3 Features of double seeded nutmeg

accessions (Plate 4.3 c and 4.3 d). Typical four halved splitting nature was noticed in double seeded nutmeg types as depicted in Plate 4.3 b.

4.1.4 Monoecious nutmeg

Monoecy is a very much desirable characteristic as nutmeg is usually classified as dioecious in nature. Towards this end, the spotting of two accessions namely MN-1 and MN-2 is definitely an asset as regards a researcher is concerned. The two monoecious nutmeg genotypes were collected from Chalakudy and Pattikkad regions. Accession MN-1 was 18 year old seedling tree and MN-2 was 28 year old budded tree. Both the accessions had umbellate cyme inflorescence (Plate 4.4b and 4.4 c). Few abnormal flowers were observed in these accessions (Plate 4.4 e). Stereomicroscopic view of male flowers in monoecious nutmeg is depicted in Plate 4.4 d.

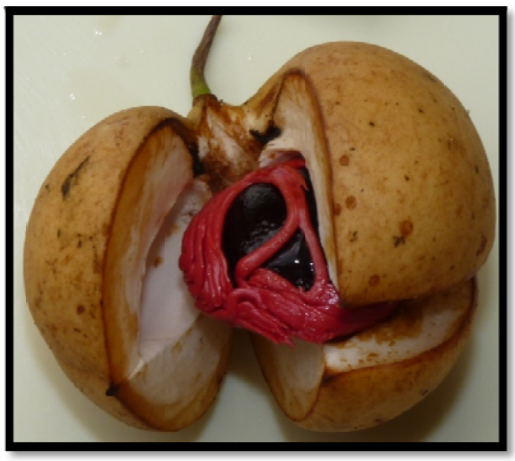
Monoecious nutmeg had yellow coloured fruits which split open into three halves (Plate 4.4 a). Mace and nut along with the pericarp persistent on the tree even after fruit splitting was another peculiar feature observed in MN-1 and MN-2. Higher incidence of rotting of mace due to fungal attack was noticed, which also affected the quality of mace during rainy season.

4.1.5 Cluster fruited nutmeg

Clustering of fruits is not a phenomenon at all in nutmeg, since two accessions CF-1 and CF-2 were spotted as cluster bearing, this divergent nature of parent may be co-opted with reservation on the quality of fruit. The two unique accessions namely CF-1 and CF-2 were collected from Vaikkom and Chalampadam areas (Plate 4.5 a). In CF-1, eighty per cent of the fruits were in clusters of three, fifteen per cent clusters in two and remaining 4-8 fruits per cluster (Plate 4.5 b). In CF-2, the clusters were of 2-3 fruits per bunch.

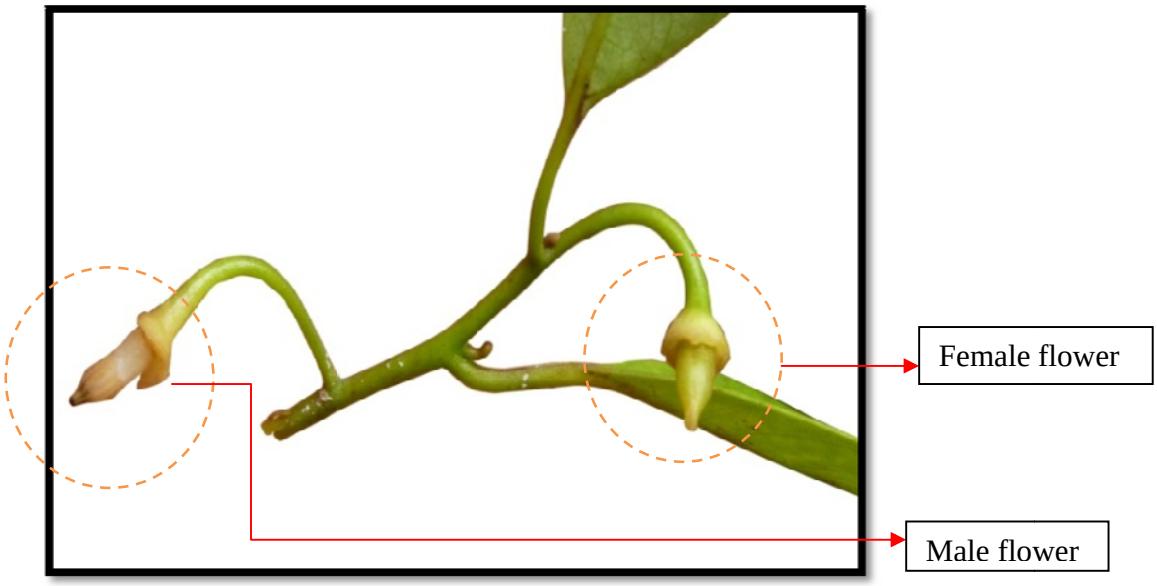
4.1.6 Low astringent nutmeg

The astringency of rind is usually a reckoned characteristic, deviation from this towards a low level is very much desirable and the spotted accessions LA-1



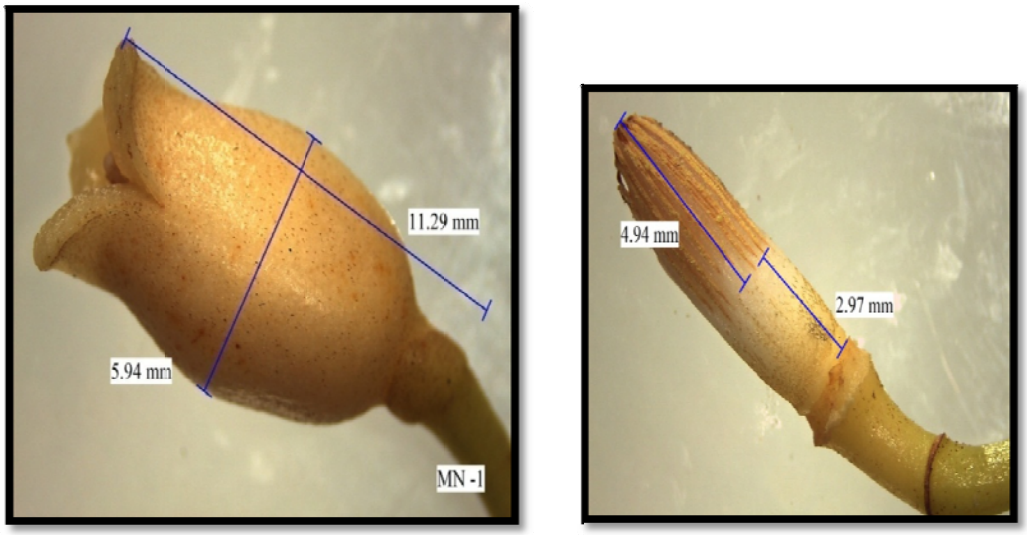
a. Monoecious nutmeg

b. Umbellate cyme



c. Male and female flower

Plate 4.4 Features of monoecious nutmeg



e. Steriomicroscopic view of male flowers in monoecious nutmeg

f. Abnormal flowers in monoecious nutmeg

Plate 4.4 Features of Monoecious nutmeg

a. Cluster of 3 fruits

b. Cluster of 4 fruits

c. Cluster of 5 fruits

d. Cluster of 8 fruits

Plate 4.5 Features of cluster fruited nutmeg

a. Fruit **b. Rind**

Plate 4.6 Features of low astringent nutmeg

a. Tree **b. Erect branching pattern**

Plate 4.7 Features of narrowly pyramidal nutmeg

and LA-2 adds robustness towards further research in nutmeg towards low astringency for use in the value addition of nutmeg rind. The low astringent nutmeg accessions namely LA-1 and LA-2 were spotted from Chalakudy and Vazhikadavu regions (Plate 4.6). Accession LA-1 was 15 years old budded tree and LA-2 was 23 year old seedling tree. Accession LA-1 was devoid of orthotropic shoots and LA-2 had 15 orthotropic shoots. Mace and nut along with pericarp was persistent on the tree even after fruit splitting. Accession LA-2 exhibited thread blight and fruit rot incidence at moderate level.

4.1.7 Narrowly Pyramidal (conical) nutmeg

Shape of tree navigates the spacing between the trees, and also acts as a cover against sunlight in most of the cases. The farmers of Kerala are having only limited land holding in most of the cases. To accommodate more trees and also to accommodate intercrops it is most desirable that natural light infiltration percolates the interspacing between any two trees. Utilization of tree shape is possible if a narrowly pyramidal (conical) shape is spotted like the unique accessions labeled PT-1 and PT-2. Accession PT-1 and PT-2 were collected from Chalakudy and Kallurkadu (Plate 4.7). Both the accessions were budded female nutmeg trees of age 15years and 25 years old respectively. The tree height ranged from 9.5-13.78 metres with a canopy spread of 4.3-5.9 metres in North -South direction and 4.8 -5.7 metres in East- West direction. Erect branching pattern was observed in these accessions with dark green coloured leaves with elliptic shape in PT-1 and obovate shape in PT-2. Least canopy spread was the distinct feature observed in these accessions.

4.1.8 Grape nutmeg

Certain spice crops can also be exploited to act as an ornamental, at the same time giving an economical point. The name grape nutmeg denotes trees bearing numerous small grape sized fruits. The grape types can definitely be exploited as ornamental types suitable for landscape horticulture. The grape nutmeg namely GT-1 and GT-2 were collected from Pattikkad and Chalampadam

a. Bearing tree

b. abnormal flowers

Plate 4.8 Features of grape nutmeg

areas (Plate 4.8). Both the accessions are budded female nutmeg trees with 20 years and 15 years age respectively. The tree height ranged from 5 -7.5 metres with a canopy spread of 6.2 -7.4 metres in North-South and 5.8-6.5 metres in East -West direction. Few abnormal flowers were observed in grape nutmeg. GT-1 and GT -2 showed two halved fruit splitting with nut and mace drop while pericarp remains attached to the tree after ripening.

4.1.9 Wild nutmeg

Wildness is a characteristic that may of course be desirable in many respects towards development of an all round healthy tree. The two wild nutmeg accessions collected from Pattikkad (WT-1) and Mullaringaad (WT-2) regions were identified as *M. malabarica*, both the accessions were seedling trees of 18 years and 15 years respectively. The tree height ranged between 16.1 to 19.3m with a canopy spread in the range of 11.13-13.1 metres in North -South direction and 11.78-14.7 metres in East -West direction.

The trees were having broadly pyramidal canopy shape and large green elliptic mature leaf. Both the accessions were having small greenish creamy female flowers born in axillary raceme and exhibited late flowering nature. Orthotropic shoots were absent in WT-1 and WT-2. Wild nutmeg had brown coloured fruits with velvety texture, which on splitting exposed the attractive yellowish orange coloured oblong mace tightly covering the brown coloured nut (Plate 4.9). On drying, the mace colour turns to orange red and nut colour changes to brown and light brown colour in WT-1 and WT-2 respectively. Kernel colour varied from light brown colour in WT-2 to dark grey colour in WT-1. Fruits split into two halves on ripening, followed by mace and nut drop with persistent pericarp on the tree.

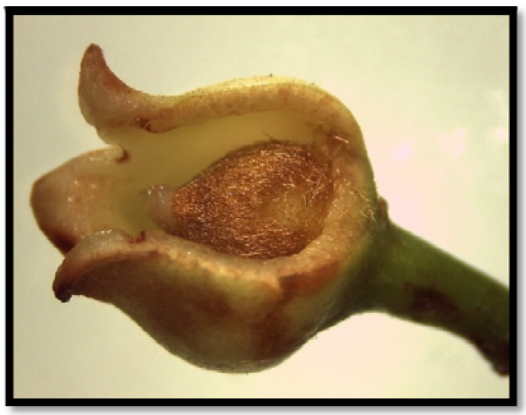
4.1.10 Oblong nutmeg

Oblong shaped nutmeg was another interesting distinct feature due to the deviation in fruit shape from the normal nutmeg (Plate 4.10). Two oblong

a. Fruits

b. Velvety rind in wild nutmeg

Plate 4.9 Features of wild nutmeg



Female flower **Male flower**

Female flower **Male flower**

Plate 4.9 c. Steriomicroscopic view of flowers in wild nutmeg

Plate 4.10 Features of oblong nutmeg

accessions namely OB-1 and OB-2 were spotted from Chalakudy and Pattikkad regions. Accession OB-1 and OB-2 had pyramidal canopy shape and green lanceolate shaped mature leaves with spreading branching pattern. Yellowish green flushes were observed in both the oblong nutmeg accessions. Both the accessions had late season flowering habit with creamy yellow coloured female flowers born in axillary raceme inflorescence. OB-1 had dark brown coloured nut while OB-2 had black coloured nut. The mace in both accessions was oblong shaped and thin. After drying, mace colour changed to red in OB-1 and scarlet red in OB-2.

4.1.11 Triangular mace nutmeg

In nutmeg, normally the mace shape varied among round, oval and oblong shapes, any deviation from this shape may be a distinct feature that needs attention. Triangular shape of mace was much distinct when mace was attached to the nut. Triangular mace nutmeg labeled TM-1 was spotted from Koottala area (Plate 4.11). This seedling tree was 13 years old and had 9 m height with a canopy spread of 6.6 meters in North-South and 7.6 meters in East-West direction. The number of orthotropic shoots in TM-1 was found to be 10, which is a preferred characteristic in nutmeg. Accession TM-1 had yellow coloured fruits with black coloured fresh nut and red coloured fresh mace, which after drying faded to scarlet red coloured triangular shaped mace and black coloured nut.

4.1.12 Small leaved nutmeg

Small leaf type nutmeg labelled SM-1 was spotted from Kanjirappally area. Accession SM-1 had pyramidal tree canopy with spreading branching pattern. SM-1 possessed dark green coloured elliptic leaves (Plate 4.12). Leaf dimensions in this accession makes it distinct from the normal nutmeg types with lowest leaf length and breadth along with a minimum leaf area of 15.05 cm^2.

Plate 4.11 Features of triangular mace nutmeg

Plate 4.12 Feature of small leaved nutmeg

Plate 4.13 Features of improved nutmeg accession

4.1.13 Improved nutmeg

Improved accessions from an earlier study namely IA-1 and IA-2 respectively were collected from Chalakudy region. Tree height ranged from 7.21-7.5 metres with a canopy spread of 10.3-10.6 metres in North - South and 9.95-10.2 metres in East - West direction. Accession IA-1 and IA-2 had spreading branching pattern and globular canopy shape. Both the accession had late season flowering nature and flowers were born in axillary raceme inflorescence. Data presented in table 4.5 shows that improved accessions had yellow coloured fruits with scarlet red coloured round mace, shiny black coloured fresh nut and grey coloured kernel (Plate 4.13).

4.2 Evaluation of unique nutmeg accessions

Data with respect to the evaluation of the unique accessions are presented in Tables 4.2, 4.3 and 4.4.

4.2.1 Tree characters

The tree characteristics of all the unique accessions observed were recorded and a firsthand measure of similarity among the tree characteristics revealed the divergence of such type of characteristics. They may be regarded as singletons in statistical point of view. The prominent tree characteristics measured were plant height, girth at 140 cm height, canopy spread in North-South and East-West directions, leaf length, leaf breadth, leaf area and number of orthotrops are listed in Table 4.2 accession wise.

A bird's eye view of the said characters asserts the peaked variability among the genotypes as highlighted by the observed range of characters; 5m-19 m for plant height, 18.8cm -149.2cm for girth, 4.3m-13.1m for canopy spread in N-S direction and 4.9m -14.7m for canopy spread in E-W directions. Similarly, the leaf length ranged from 8.6cm-14.28cm, leaf breadth ranged from 2.52cm-5.68 cm and leaf area ranged from 15.05cm^2-49.09cm^2. The number of orthotrops ranged from zero to forty five.

4.2.2 Flower characters

The prominent flower characters measured were length of flower, breadth of flower, length of perianth, breadth of perianth, length of filament, length of anther lobe, number of stamens, number of male, female and hermaphrodite flowers per $0.1m^2$ respectively and are given below in Table 4.3.

The range of various floral characters observed were as: length of flower 6.35 mm - 14.1 mm; breadth of flower 4.20 mm -7.27 mm; length of perianth 5.25 mm -10.51 mm; breadth of perianth 1.18 - 6.08 mm; length of filament 1.79 mm - 5.45 mm; length of anther lobe 1.02 mm -3.69 mm; number of stamens 16-22; number of female flowers /10 cm^2 (2-10); number of male flowers / 10 cm^2 (1-4).

4.2.3 Fruit characters

The fruit characteristics being of ultimate importance and an ultimate economic end point as regards a farmer, a comprehensive assessment of quantitative characters is being done after collecting the representative samples of fruits from all the trees. All the fruit characteristics were measured from the samples collected and the respective characteristics were analyzed for identification of sub groups among the 23 accessions using Univariate General Linear Model. The results of the analysis for all the characteristics are discussed in chronology of its economic importance and depicted in Table 4.4 (a), 4.4 (b) and 4.4 (c).

4.2.3.1 Fruit weight (fresh)

The fruit weight was the highest in DS-1 (126.05g) followed by Yl-1(108.96 g). The fruit weights of CF-1 (99.56g), IA-2 (98.43 g), IA-1 (98.33g), TM-1 (95.17g) and PT-1 (92.82g) were on par. Thus in comparisons with improved accessions, the accessions DS-1, YL-1and CF-1 were superior. The least fruit weight was recorded by GT-2 (38.87g) followed by GT-1 (47.14g).

Table 4.2 Tree characters of unique accessions of nutmeg

Sl. no.	Accession	Plant height (m)	Girth at 140 cm height (cm)	Canopy spread (m)		Leaf length (cm)	Leaf breadth (cm)	Leaf area (cm^2)	No.of orthotrops
				(N-S)	(E-W)				
1	YL-1	7.90	43.0	6.20	5.30	13.54	5.68	49.09	12
2	YL-2	6.48	44.0	5.00	5.20	12.90	5.40	43.30	27
3	SL-1	11.0	72.0	9.11	8.35	12.56	5.32	43.20	10
4	DS-1	7.10	66.0	7.34	9.75	12.38	5.10	33.75	1
5	DS-2	10.9	58.4	8.50	7.91	12.62	5.30	38.20	1
6	MN-1	5.70	128	7.40	7.20	11.30	4.96	29.68	3
7	MN-2	8.90	75.0	9.50	8.60	10.80	5.50	27.85	1
8	CF-1	13.1	73.0	8.36	7.63	14.12	4.72	42.64	9
9	CF-2	8.32	90.0	9.10	8.90	10.62	4.24	32.2	10
10	LA-1	6.40	23.63	6.20	6.10	12.06	4.10	27.85	Nil
11	LA-2	9.0	78.0	8.20	7.30	13.14	4.20	35.76	15
12	PT-1	9.50	18.8	4.30	4.80	10.36	4.28	25.55	7
13	PT-2	13.78	81.0	5.90	5.70	12.76	4.90	33.75	1
14	GT-1	7.50	52.0	7.40	6.50	10.20	4.50	30.25	5
15	GT-2	5.00	41.0	6.20	5.80	9.80	3.80	27.16	6
16	WT-1	19.3	149.2	11.13	11.78	14.28	5.22	44.61	Nil
17	WT-2	16.1	175	13.10	14.70	16.90	4.60	47.74	Nil
18	IA-1	7.21	65.0	10.60	10.20	11.55	3.65	30.84	12
19	IA-2	7.50	61.0	10.30	9.95	11.35	4.70	40.56	10
20	OB-1	6.58	24.2	4.95	4.90	11.54	4.86	37.81	Nil
21	OB-2	7.70	41.0	5.05	5.30	11.02	4.50	29.57	Nil
22	TM-1	9.00	40.0	6.60	7.60	11.10	4.50	36.51	10
23	SM-1	12.1	63.0	8.50	8.10	8.60	2.52	15.05	45

4.2.3.2 Thickness of pericarp (fresh)

The highest value recorded for thickness of fresh pericarp was in YL-2 (16.98 mm). The thickness of fresh pericarp of DS-1 (15.86mm), SL-1(15.66 mm), SM-1 (15.53 mm), IA-1 (15.36mm) and PT-1(15.29mm) were on par with the highest recorded thickness. With respect to this characteristic, superiority of accession YL-2 over IA was noticed. The lowest thickness was observed in WT-1 and WT-2 (6.21 mm) followed by CF-2 (10.49 mm).

4.2.3.3 Mace weight (fresh)

Accession SL-1 recorded highest value for fresh mace weight (9.74 g) followed by WT-1 (8.70 g) and WT-2 (8.33 g), which were on par with the aforesaid accession. Accessions GT-2 and GT-1 recorded least values for fresh mace weight as 0.99g and 1.07 g each respectively. As regards this characteristic, superiority of accessions SL-1, WT-1 and WT-2 over both the improved accessions were noticed.

4.23.4 Mace weight (dry)

The highest mace dry weight was observed in WT-1 (6.75g) and WT-2(5.28 g) followed by SL-1 (3.82g), which showed on par dry weight. Least mace dry weight was observed in GT-1 (0.35 g) and GT-2 (0.39 g). As regards mace dry weight, unique accessions WT-1, WT-2 and SL-1 showed superior nature over improved accessions.

4.2.3.5 Nut weight (fresh)

The highest nut fresh weight was recorded in IA-1(17.02g), followed by DS-1 (16.18 g), IA-2 (14.97 g) and WT-2 (14.96 g) with their relative significant differences also in order. Accessions DS-2 (5.55g), WT-1 (6.21g) and GT-1(6.33 g) recorded lowest fresh nut weight.

Table 4.3 Floral characters of unique accessions of nutmeg

Sl. no.	Accession	Length of flower (mm)	Breadth of flower (mm)	Length of perianth (mm)	Breadth of perianth (mm)	Length of filament (mm)	Length of anther lobe (mm)	No. of stamens	No.of female flowers/ 10 cm^2	No. of male flowers/ 10 cm^2	No.of hermaphrodite flowers/ 10 cm^2
1	YL-1	8.46	4.61	6.48	3.55	NA	NA	NA	4	NA	NA
2	YL-2	8.36	5.51	6.86	4.45	NA	NA	NA	4	NA	NA
3	SL-1	12.10	5.30	8.21	4.27	NA	NA	NA	3	NA	NA
4	DS-1	9.3	6.38	8.07	4.93	3.9	2.45	22	4	1	NA
5	DS-2	9.58	7.27	8.34	6.08	4.37	2.83	18	4	2	NA
6	MN-1	14.1	5.77	10.51	4.8	5.45	3.94	20	3	3	2
7	MN-2	8.79	4.94	8.21	3.54	1.79	3.02	18	4	3	2
8	CF-1	9.17	6.25	7.45	5.12	NA	NA	NA	10	NA	NA
9	CF-2	7.40	4.20	6.11	3.53	2.05	1.02	16	4	1	NA
10	LA-1	10.01	5.68	9.02	4.1	NA	NA	NA	5	NA	NA
11	LA-2	11.38	5.46	8.59	4.37	NA	NA	NA	6	NA	NA
12	PT-1	9.16	5.3	8.07	3.95	NA	NA	NA	5	NA	NA
13	PT-2	8.39	5.78	7.64	3.72	NA	NA	NA	4	NA	NA
14	GT-1	9.38	6.18	6	4.52	4.16	1.6	18	2	4	NA
15	GT-2	7.35	5.82	7.17	4.99	NA	NA	NA	3	NA	NA
16	WT-1	9.13	4.62	7.43	1.18	3.6	3.69	16	4	1	NA
17	WT-2	5.47	5.32	5.25	3.97	NA	NA	NA	4	NA	NA
18	IA-1	9.32	5.91	8.2	5.8	NA	NA	NA	5	NA	NA
19	IA-2	9.9	5.86	7.34	5.39	NA	NA	NA	4	NA	NA
20	OB-1	8.54	4.95	5.86	3.93	NA	NA	NA	3	NA	NA
21	OB-2	8.40	5.14	6.53	4.21	NA	NA	NA	3	NA	NA
22	TM-1	6.35	5.68	5.84	4.36	NA	NA	NA	4	NA	NA
23	SM-1	9.11	6.52	7.79	5.67	NA	NA	NA	5	NA	NA

Table 4.4 (a) Fruit characters of unique accessions of nutmeg

Accessions	Fruit wt (g)	Thickness of rind (mm)	Mace weight fresh (g)	Mace weight dry (g)	Nut weight fresh (g)	Nut weight dry (g)	Shell thickness dry (g)	Kernel weight dry (g)
YL-1	108.9 ^{b}	13.02^{hi}	4.524^{d}	1.45^{h}	17.02^{a}	8.35^{fg}	0.72^{k}	5.53^{cde}
YL-2	90.34^{def}	16.98^{a}	3.55^{fg}	1.266^{hi}	12.16^{c}	5.97^{i}	0.80^{hi}	4.41^{fgh}
SL-1	86.35^{efg}	15.66^{bc}	9.744^{a}	3.82^{c}	NA	NA	NA	NA
DS-1	126.05 ^{a}	15.86^{b}	5.70^{c}	3.31^{d}	16.18^{ab}	13.03^{a}	1.02^{b}	9.55^{a}
DS-2	50.33^{l}	10.93^{lm}	1.79^{j}	0.90^{j}	5.55^{i}	4.23^{kl}	077^{ij}	2.63^{i}
MN-1	63.52^{jk}	12.28^{ij}	2.57^{hi}	1.41^{h}	13.11^{c}	8.13^{fg}	0.93^{d}	4.92^{efg}
MN-2	71.65^{hij}	14.06^{fg}	2.17^{hij}	1.28^{h}	8.42^{fg}	5.90^{i}	0.66^{l}	4.14^{gh}
CF-1	99.56 ^{c}	14.75^{def}	4.37^{de}	2.35^{ef}	12.82^{c}	10.75^{bc}	0.94^{d}	7.70^{b}
CF-2	61.23^{k}	10.49^{m}	1.99^{ij}	0.86^{j}	7.65^{gh}	4.98^{ijk}	0.93^{d}	3.55^{h}
LA-1	72.85^{hi}	13.58^{gh}	2.16^{hij}	0.94^{j}	13.11^{c}	8.13^{fg}	0.74^{jk}	5.60^{cde}
LA-2	61.65^{k}	11.95^{jk}	2.37^{hij}	1.28^{hi}	12.28^{c}	9.54^{de}	0.97^{d}	5.24^{cdef}
PT-1	92.82^{cdef}	15.29^{bcd}	2.87^{gh}	1.342^{hi}	12.14^{c}	5.65^{ij}	0.61^{m}	4.19^{gh}
PT-2	85.90^{fg}	14.22^{efg}	1.89^{ij}	1.20^{h}	12.69^{c}	8.75^{ef}	1.05^{b}	5.93^{cd}
GT-1	47.14^{l}	11.19^{klm}	1.07^{k}	0.35^{k}	6.33^{i}	3.54^{lm}	0.83^{gh}	2.10^{i}
GT-2	38.87^{m}	11.59^{jkl}	0.99^{k}	0.39^{k}	6.57^{hi}	3.06^{m}	0.63^{lm}	1.84^{i}
WT-1	85.08^{fg}	6.22^{n}	8.70^{b}	6.75^{a}	6.21^{i}	4.74^{jk}	0.85^{efg}	1.89^{i}
WT-2	88.13^{efg}	6.22^{n}	8.33^{b}	5.28^{b}	14.96^{b}	11.44^{b}	0.93^{d}	8.05^{b}
IA-1	98.33^{cd}	15.36^{bcd}	3.73^{ef}	2.58^{e}	17.02^{a}	10.04^{cd}	0.88^{e}	6.00^{c}
IA-2	98.44^{cd}	13.96^{fg}	2.90^{gh}	2.36^{ef}	14.98^{b}	8.68^{ef}	0.86^{ef}	5.69^{cde}
OB-1	64.69^{ijk}	12.29^{ij}	1.96^{ij}	1.16^{hi}	10.86^{d}	7.48^{gh}	0.81^{gh}	5.34^{cde}
OB-2	73.15^{hi}	11.56^{jkl}	4.39^{de}	2.43^{ef}	10.41^{de}	7.34^{gh}	1.01^{c}	4.99^{defg}
TM-1	95.18^{cde}	14.95^{cde}	5.28^{c}	2.60^{e}	12.66^{c}	8.03^{fgh}	1.22^{a}	5.44^{cde}
SM-1	79.74^{gh}	15.53^{bcd}	3.88^{def}	1.98^{g}	10.25^{de}	7.02^{h}	1.06^{b}	5.40^{cde}

Superscripts a,b,c denote the rank scores

4.2.3.6 Nut weight (dry)

The highest value for nut weight dry was in DS-1 (13.03 g) followed by WT-2 (11.44g), CF-1 (10.75g) and IA-1 (10.04g). Lowest value recorded was in GT-2 (3.06 g) and GT-1 (3.54 g). The accessions DS-1, WT-2 and CF-1 were superior over improved accessions with respect to dry nut weight.

4.2.3.7 Shell thickness (dry)

Highest value for shell thickness was recorded in TM-1 (1.22 mm), followed by SM-1 (1.06 mm), PT-2 (1.05 mm) and DS-1 (1.02 mm). Lowest value for shell thickness was recorded in PT-1(0.61 mm) and GT-2(0.63mm). The accessions TM-1, SM-1, PT-2 and DS-1 showed superiority in shell thickness over IA-1 and IA-2.

4.2.3.8 Kernel weight (dry)

The highest value for dry kernel weight was observed in DS-1(9.55g), being followed by WT-2 (8.05g) and CF-1(7.70g). Accessions GT-2(1.84g) and WT-1 (1.89 g) recorded least value for dry kernel weight. The accessions DS-1, WT-2 and CF-1 showed superiority over both the improved accessions.

4.2.3.9 Ratio of nut to mace

The highest ratio of nut to mace was observed in PT-2 (6.75), followed by LA-1 (6.40) and GT-1 (6.16). Lowest value for ratio of nut to mace was observed in WT-1(0.71) and WT-2(1.81). Accessions PT-2, LA-1 and GT-1 showed superiority over both the improved accessions.

4.2.3.10 Shelling percentage

The highest shelling percentage was observed in SM-1(76.16%). The shelling percentage of other accessions; YL-2(74.23%), PT-1(73.83%), CF-1 (71.62%), OB-1 (70.98%), CF-2 (70.75%), WT-2 (70.33%) and MN-2 (70.03%) were on par with that of SM-1. Lowest value for shelling percentage was

Table 4.4 (b) Fruit characters of unique accessions of nutmeg

Accessions	Number of fruits per tree	Ratio of nut to mace	Shelling percentage (%)	Seed weight (g)	Seed volume (cm^3)	Mace volume (cm^3)
YL-1	$980^{(14)}$	2.68^{hi}	66.12^{cde}	17.02^{a}	10.80^{cde}	4.2^{d}
YL-2	$1000^{(13)}$	2.61^{hi}	74.23^{ab}	12.16^{c}	8.20^{gh}	3.5^{de}
SL-1	$800^{(15.5)}$	NA	NA	NA	NA	10.5^{a}
DS-1	$700^{(17)}$	2.90^{gh}	58.84^{fg}	16.18^{ab}	13.30^{b}	5.00^{c}
DS-2	$280^{(19)}$	3.63^{fg}	61.88^{def}	5.55^{i}	5.20^{jk}	1.60^{j}
MN-1	$250^{(21)}$	4.45^{de}	58.19^{fg}	13.11^{c}	11.60^{cd}	2.00^{ghij}
MN-2	$275^{(20)}$	3.98^{ef}	70.03^{abc}	8.42^{fg}	7.50^{hi}	2.60^{fgh}
CF-1	$2250^{(3)}$	2.92^{gh}	71.62^{abc}	12.82^{c}	11.20^{cde}	3.60^{de}
CF-2	$1800^{(7)}$	3.95^{ef}	70.74^{abc}	7.65^{gh}	7.40^{hi}	2.00^{ghij}
LA-1	$800^{(15.5)}$	6.40^{a}	68.24^{bcd}	13.11^{c}	11.6^{cd}	2.30^{ghij}
LA-2	$1750^{(8)}$	5.22^{cd}	54.59^{g}	12.28^{c}	10.2^{def}	2.60^{fgh}
PT-1	$850^{(14)}$	4.27^{ef}	73.83^{ab}	12.14^{c}	11.00^{cde}	2.70^{fg}
PT-2	$1150^{(12)}$	6.75^{a}	67.75^{bcd}	12.69^{c}	11.90^{c}	2.50^{fghi}
GT-1	$2000^{(5)}$	6.16^{ab}	57.10^{fgh}	6.33^{i}	6.60^{i}	1.80^{hij}
GT-2	$1900^{(6)}$	4.79^{de}	60.03^{efg}	6.57^{hi}	4.80^{k}	1.70^{ij}
WT-1	$3500^{(2)}$	0.71^{j}	38.56^{h}	6.21^{i}	6.40^{ij}	9.00^{b}
WT-2	$4000^{(1)}$	1.81^{i}	70.03^{abc}	14.96^{b}	13.90^{ab}	9.50^{b}
IA-1	$1450^{(10)}$	4.72^{de}	59.30^{fg}	17.02^{a}	14.70^{a}	3.30^{ef}
IA-2	$1350^{(11)}$	4.72^{d}	65.50^{cde}	14.98^{b}	13.40^{b}	4.00^{de}
OB-1	$2200^{(4)}$	5.65^{bc}	70.98^{abc}	10.86^{d}	9.30^{fg}	2.00^{ghij}
OB-2	$1500^{(9)}$	2.45^{hi}	67.58^{bcd}	10.41^{de}	10.00^{ef}	3.80^{de}
TM-1	$680^{(18)}$	2.41^{hi}	67.71^{bcd}	12.66^{c}	10.2^{def}	4.00^{de}
SM-1	$1580^{(8)}$	2.65^{hi}	76.16^{a}	10.25^{de}	9.90^{ef}	3.90^{de}

Values in the brackets and superscripts denote the rank scores

observed inWT-1 (38.56%).Thus in comparison with the improved accessions; the accessions SM-1, YL-2, PT-1, CF-1, OB-1, CF-2, WT-2 and MN-2 were superior with respect to the shelling percentage.

4.2.3.11 Seed weight

The highest fresh seed weight was recorded in IA-1(17.02g), followed by DS-1 (16.18g) and IA-2(14.97g). Accessions DS-2 (5.55g), WT-1 (6.21g) and GT-1 (6.33g) recorded lowest values for fresh seed weight. However, none of the accessions was superior to the improved accessions.

4.2.3.12 Seed volume

The highest seed volume were observed in IA-1(14.7cm^3), WT-2 (13.9 cm^3) and WT-1(13.9 cm^3). Lowest seed volume was recorded in GT-2 (4.8 cm^3) and DS-2 (5.2 cm^3). However, no accessions showed superiority over the improved accessions.

4.2.3.13 Mace volume

The highest mace volume was observed in SL-1 (10.5 cm^3), followed by WT-2 (9.5 cm^3) and WT-1(9 cm^3). Lowest mace volume were observed in DS-2 (1.6 cm^3) and GT-2 (1.7 cm3). The accessions SL-1, WT-2 and WT-1 showed superiority over both the improved accessions.

4.2.3.14 Number of fruits per tree

Data pertaining to the per tree yield of nutmeg and mace are presented in Table 4.5 (c).The highest number of fruits per tree was observed in WT-2 (4000), followed by WT-1 (3500) and CF-1 (2250). Lowest number of fruits were observed in MN-1 (250), followed by MN-2 (275) and DS-2 (280). The accessions WT-2, WT-1 and CF-1 showed superiority over both the improved accessions.

Table 4.4 (c) Yield of nutmeg and mace in unique nutmeg accessions

Accessions	Mace weight dry (g)	Nut weight dry (g)	Number of fruits per tree	Mace yield per tree (kg)	Nut yield per tree (kg)
YL-1	1.45	8.35	980	1.42	8.18
YL-2	1.27	5.97	1000	1.27	5.97
SL-1	3.82	NA	800	3.06	NA
DS-1	3.31	13.03	700	2.32	9.12
DS-2	0.90	4.23	280	0.25	1.18
MN-1	1.41	8.13	250	0.35	2.03
MN-2	1.28	5.90	275	0.35	1.62
CF-1	2.35	10.75	2250	5.29	24.19
CF-2	0.86	4.98	1800	1.55	8.96
LA-1	0.94	8.13	800	0.75	6.50
LA-2	1.28	9.54	1750	2.24	16.70
PT-1	1.34	5.65	850	1.14	4.80
PT-2	1.20	8.75	1150	1.38	10.06
GT-1	0.35	3.54	2000	0.70	7.08
GT-2	0.39	3.06	1900	0.74	5.81
WT-1	6.75	4.74	3500	23.63	16.59
WT-2	5.28	11.44	4000	21.12	45.76
IA-1	2.58	10.04	1450	3.74	14.56
IA-2	2.36	8.68	1350	3.19	11.72
OB-1	1.16	7.48	2200	2.55	16.46
OB-2	2.43	7.34	1500	3.65	11.01
TM-1	2.60	8.03	680	1.77	5.46
SM-1	1.98	7.02	1580	3.13	11.09

4.2.3.15 Mace yield per tree

The highest mace yield per tree was observed in WT-1 (23.63 kg/tree), followed by WT-2 (21.12 kg/tree) and CF-1 (5.29 kg /tree). Lowest mace yield per tree was observed in DS-2 (0.25 kg/ tree) followed by MN-1 and MN-2 (0.35 kg/tree). The accessions WT-2, WT-1 and CF-1 showed superiority over both the improved accessions.

4.2.3.16 Nut yield per tree

The highest nut yield per tree was observed in WT-2 (45.76 kg/tree), followed by CF-1 (24.19 kg/tree) and WT-1 (16.59 kg/tree) and CF-1. Lowest nut yield per tree was observed in DS-2 (1.18 kg/ tree) followed by MN-2 (1.62 kg/tree) and MN-1 (2.03 kg/tree). The accessions WT-2, CF-1 and WT-1 showed superiority over both the improved accessions.

4.3 Biochemical parameters

Biochemical analysis of the rind, mace and kernel were done and the results of the analysis are furnished below in the Tables 4.5 and 4.6.

4.3.1 Biochemical analysis of rind

4.3.1.1 Moisture content

The moisture content in the rind ranged from 81.40% (WT-2) to 91.09% (LA-1).The accessions IA-2 (90.69%), IA-1 (90.08%), CF-1 (90.06%) and CF-2 (89.67%) were on par with LA-1.

4.3.1.2 Acidity

Significant difference was recorded among the unique accessions for acidity content in the rind. The highest acidity content was recorded by IA-2(1.73%) followed by IA-1 (1.62%) and MN-2(1.60%). The lowest recorded acidity content was in YL-2 (0.53%) and SL-1(0.59%).

Table 4.5 Biochemical parameters of rind in the unique nutmeg accessions

Sl no.	Accession ID	Moisture content (%)	Acidity (%)	Starch (mg /100g)	Tannins (g/100g)	Pectin (%)
1	YL-1	88.25^{bc}	0.93^{J}	16.65^{g}	1.55^{def}	2.17^{i}
2	YL-2	86.79^{cde}	0.53^{n}	38.62^{a}	2.77^{b}	2.48^{h}
3	SL-1	88.29^{bc}	0.59^{n}	26.32^{c}	0.50^{J}	0.92^{k}
4	DS-1	89.57^{ab}	1.44^{cd}	16.01^{gh}	0.32^{J}	4.10^{e}
5	DS-2	85.89^{ef}	1.20^{gh}	14.60^{hi}	1.36^{fg}	1.88^{j}
6	MN-1	87.26^{cde}	1.25^{fg}	30.25^{b}	1.60^{def}	4.12^{e}
7	MN-2	84.92^{f}	1.60^{c}	27.08^{c}	1.25^{gh}	2.91^{g}
8	CF-1	90.07^{ab}	1.33^{ef}	15.93^{ghi}	1.45^{efg}	0.15^{n}
9	CF-2	89.67^{ab}	1.25^{fg}	15.74^{ghi}	1.60^{def}	0.59^{l}
10	LA-1	91.09^{a}	1.39^{de}	19.70^{f}	1.59^{def}	0.18^{mn}
11	LA-2	87.29^{cde}	0.82^{kl}	10.84^{J}	1.04^{hi}	0.48^{l}
12	PT-1	88.41^{bc}	1.49^{d}	8.86^{k}	1.77^{d}	11.65^{a}
13	PT-2	86.43^{cde}	1.07^{i}	31.02^{b}	1.66^{de}	5.41c
14	GT-1	86.05^{def}	1.14^{hi}	22.58^{de}	1.25^{gh}	5.14^{d}
15	GT-2	85.23^{ef}	1.25^{fg}	22.70^{d}	0.86^{i}	5.11^{d}
16	WT-1	86.68^{cde}	0.91^{Jk}	20.06^{f}	3.82^{a}	0.23^{mn}
17	WT-2	81.40^{g}	0.75^{lm}	9.30^{Jk}	2.86^{b}	0.31^{m}
18	IA-1	90.08^{ab}	1.62^{b}	22.37^{de}	2.18^{c}	8.02^{b}
19	IA-2	90.69^{a}	1.73^{a}	20.90^{ef}	2.38^{c}	3.30^{f}
20	OB-1	88.07^{bcd}	1.28^{fg}	10.92^{J}	1.69^{de}	2.07^{i}
21	OB-2	84.97^{f}	0.72^{m}	20.86^{ef}	0.84^{i}	5.25^{d}
22	TM-1	87.12^{cde}	0.77^{lm}	14.20^{I}	2.10^{c}	0.15^{n}
23	SM-1	84.73^{f}	1.39^{de}	21.18^{def}	0.49^{J}	2.51^{h}

Superscripts a, b, c denote the rank scores

4.3.1.3 Starch

The starch content differed significantly among the unique accessions. The highest starch content was recorded in YL-2 (38.62 mg/100g) and the lowest starch content was recorded in WT-2 (9.30mg/100g). The accessions MN-1 (30.25mg/100g) and PT-2 (31.02mg/100g) were on par.

4.3.1.4 Tannins

Tannin content in the rind showed significant differences among the accessions. Accession WT-1 (3.82 g/100g) and WT-2(2.86 g/100g) recorded highest tannin content in rind. Lowest tannin content was recorded in DS-1 (0.32 g/100g), SM-1 (0.49 g/100g) and SL-1 (0.50 g/100g).

4.3.1.5 Pectin

Pectin content in rind varied from 0.18% to 11.65%. Accession PT-1 recorded the highest pectin content of 11.65%. Accessions LA-1 and WT-1 recorded lowest pectin content of 0.18% and 0.23% pectin each respectively.

4.3.2 Biochemical characterization of mace

4.3.2.1 Volatile oil

The highest volatile oil content of mace was recorded in SL-1 (16.72%) where as the lowest volatile oil content was recorded in CF-2 (4.29%).

4.3.2.2 Oleoresin

The oleoresin content in mace showed significant differences among the accessions. Accession WT-2 (37.40%) recorded highest mace oleoresin content and accession GT-1 (6.2%) recorded lowest mace oleoresin. As regards mace oleoresin, WT-1 (35%) and SL-1 (31.30%) were on par.

Table 4.6 Volatile oil and oleoresin content of unique accessions of nutmeg

Sl no.	Accession ID	Mace oil (%)	Kernel oil (%)	Mace oleoresin (%)	Kernel oleoresin(%)	Butter (%)
1	YL-1	8.33^{fgh}	5.49^{bcdef}	19.60^{c}	30.50^{de}	21.01^{g}
2	YL-2	11.99^{bc}	5.33^{cdef}	20.20^{c}	26.10^{fg}	19.61^{h}
3	SL-1	16.72^{a}	NA	31.30^{b}	NA	NA
4	DS-1	12.75^{b}	5.15^{defg}	22.20^{c}	26.20^{fg}	22.55^{f}
5	DS-2	6.91^{hij}	5.96^{bcde}	21.80^{c}	38.80^{bc}	20.15^{gh}
6	MN-1	10.05^{de}	5.00^{defg}	15.80^{d}	20.35^{hi}	42.06^{b}
7	MN-2	8.58^{fg}	4.50^{fghi}	21.20^{c}	45.60^{a}	19.94^{h}
8	CF-1	6.16^{jkl}	3.78^{hi}	12.60^{d}	28.90^{ef}	43.95^{a}
9	CF-2	4.29^{m}	4.62^{fgh}	20.50^{c}	21.80^{h}	11.25^{l}
10	LA-1	5.20^{lm}	4.15^{ghi}	13.50^{d}	33.55^{d}	22.30^{f}
11	LA-2	5.06^{lm}	3.15^{i}	14.90^{d}	33.45^{d}	38.69^{c}
12	PT-1	5.40^{klm}	6.05^{bcd}	15.65^{d}	31.80^{de}	29.15^{d}
13	PT-2	6.70^{ijk}	5.49^{bcdef}	13.00^{d}	32.90^{d}	26.50^{e}
14	GT-1	4.52^{m}	8.66^{a}	6.20^{e}	16.30^{jk}	12.60^{k}
15	GT-2	5.58^{jklm}	6.30^{bc}	7.05^{e}	13.24^{kl}	22.37^{f}
16	WT-1	NA	NA	35.00^{ab}	9.75^{l}	NA
17	WT-2	NA	NA	37.40^{a}	17.05^{ij}	12.65^{k}
18	IA-1	10.83^{cd}	6.55^{b}	15.70^{d}	26.00^{fg}	26.47^{e}
19	IA-2	10.75^{cd}	5.06^{defg}	14.30^{d}	23.80^{gh}	26.26^{e}
20	OB-1	9.50^{def}	4.63^{fgh}	32.50^{b}	37.45^{c}	22.40^{f}
21	OB-2	8.40^{fg}	4.41^{fgh}	15.00^{d}	28.75^{ef}	16.72^{j}
22	TM-1	7.73^{ghi}	4.90^{efg}	14.40^{d}	22.40^{h}	25.70^{e}
23	SM-1	8.88^{efg}	5.83^{bcde}	22.60^{c}	41.80^{b}	18.20^{i}

Superscripts a,b,c denote the rank scores

4.3.3 Biochemical characterization of kernel

4.3.3.1 Volatile oil

The highest volatile oil was recorded in the kernel of GT-1(8.33) and lowest volatile oil content was recorded in LA-2 (3.15%).

4.3.3.2 Oleoresin

Significantly higher kernel oleoresin content was recorded in MN-2 (45.60%). The lowest oleoresin content was recorded in GT-2 (13.24%).

4.3.3.3 Fat

Fixed oil present in the kernel is the fat. The fat content ranged from 11.25% (CF-2) to 43.95% (CF-1). Accession MN-1 (42.06%) closely followed CF-1, as regards the fat content.

4.3.4. GC MS profiling of mace and kernel volatile oils

Significant difference was observed among the GC MS profile of mace and kernel volatile oils of unique nutmeg accessions. Chemo profiling data of volatile oils of the unique nutmeg accessions are presented in Table 4.7 and 4.8 and the chromatogram in Appendix I and II.

4.3.4.1 Chemo profiling of mace volatile oil

About thirty eight constituents were identified from the mace volatile oils of the unique nutmeg accessions. The major constituents of mace volatile oil were myristicin, elemicin, sabinene and safrole.

Significantly highest myristicin content was recorded in the accession GT-1(17.17%), followed by TM-1 (15.68%) and LA-1 (14.04%).The lowest recorded myristicin content was in SM-1 (0.70%) and YL-1(0.73%).

Table 4.7 (a) GC MS profile of mace oil of unique nutmeg accessions

Constituents	YL-1 (%)	YL-2 (%)	DS-1 (%)	DS-2 (%)	MN-1 (%)	MN-2 (%)	CF-1 (%)	CF-2 (%)	LA-1 (%)	LA-2 (%)
R-alpha pinene	*	15.45	7.21	16.78	*	9.62	*	10.94	7.09	9.99
Sabinene	29.88	21.66	26.61	22.83	17.05	30.25	23.85	0.44	12.55	15.23
Beta - Pinene	11.54	8.16	26.61	8.08	*	4.01	11.31	11.71	6.80	6.56
Pinene	3.74	2.61	2.48	2.65	1.75	2.67	3.42	*	1.52	1.87
I-Phelandren	*	2.43	1.75	5.95	1.08	1.36	0.10	*	0.62	0.75
3-carene	*	2.43	1.75	0.21	2.94	2.32	6.94	2.70	1.16	1.29
Alpha terpine	3.05	2.68	4.14	2.28	1.29	2.34	3.79	*	3.30	3.59
o- cymol	2.47	1.02	1.54	1.67	1.13	0.99	3.89	*	1.80	1.57
Cinen	11.25	0.02	7.94	8.33	5.79	7.27	10.56	*	5.25	5.48
alpha terpine	*	2.68	4.14	2.28	1.29	2.34	3.79	1.12	3.30	3.59
Moslene	4.76	3.89	6.09	2.76	1.95	3.50	5.41	*	4.94	5.18
Terpinolen	5.14	3.96	3.31	3.04	2.32	2.38	3.80	1.29	1.97	2.07
Styrene	0.17	0.11	0.16	0.24	0.20	0.11	0.67	*	0.14	0.16
Linalol	0.60	0.35	0.45	1.02	0.33	0.71	0.64	0.36	0.30	0.25
L-4-terpineol	8.14	5.78	11.05	3.67	3.22	6.70	8.25	8.14	10.66	9.89
Terpenol	1.39	1.20	1.19	0.78	0.60	0.86	1.46	1.12	1.64	1.47
trans piperitol	0.18	0.09	0.22	0.08	0.09	0.17	0.26	*	0.39	0.35
Borneol	0.36	0.28	0.23	0.30	0.14	0.24	0.37	0.25	0.29	0.77
Safrole	0.99	0.91	0.74	3.22	3.61	0.95	8.03	2.37	11.23	10.18
Anisylacetone	*	0.16	0.13	0.08	0.12	0.08	0.11	*	0.17	0.88
delta elemene	0.40	0.38	0.15	0.64	0.12	0.36	0.72	*	0.58	0.41
p- eugenol	0.06	0.06	0.49	0.05	0.05	0.58	0.08	0.36	0.22	0.15
p-eugenol	0.06	0.06	0.49	0.05	0.05	0.58	0.08	0.36	0.22	0.15
p- eugenol	0.06	0.04	0.21	0.04	0.17	0.38	0.10	0.36	0.24	0.15
copaen	0.23	0.17	0.06	0.09	0.09	0.20	0.38	0.59	0.33	0.24
Geraniol acetate	0.11	0.09	0.17	0.06	0.02	0.17	0.09	*	0.15	0.09
Methyleugenol	1.89	3.53	0.27	2.39	5.97	0.36	0.07	*	1.50	1.03
Isoeugenol	*	*	1.28	0.03	0.01	0.24	1.19	2.19	1.27	0.57
Beta Farnesene	*	*	0.15	0.03	0.01	0.07	*	*	0.11	0.07
beta cubebene	0.24	0.16	0.06	0.04	0.10	0.28	0.13	*	0.16	0.10
Methylisoeugenol	0.28	0.72	0.16	1.82	0.44	0.25	0.17	*	1.80	1.06
cadinine	*	*	*	0.04	*	0.07	*	*	0.06	0.03
cadinene	*	*	9.29	*	0.02	0.07	*	*	0.14	0.08
Myristicin	0.73	1.50	9.29	0.95	15.06	12.86	1.71	*	14.04	8.85
Elemicin	2.46	7.20	0.86	3.99	26.89	1.05	0.17	0.64	2.54	1.31
Methoxyeugenol	*	*	0.17	*	0.03	0.03	0.06	*	0.09	*
Myristic acid	*	*	0.10	*	*	*	*	*	*	*
Manoyl oxide	*	*	0.07	0.07	0.05	0.06	0.11	*	0.03	*

* denotes not detected

Table 4.7 (b) GC MS profile of mace oil of unique nutmeg accessions

Constituents	PT-1 (%)	PT-2 (%)	IA-1 (%)	IA-2 (%)	OB-1 (%)	OB-2 (%)	GT-1 (%)	GT-2 (%)	TM-1 (%)	SM-1 (%)	SL-1 (%)
R-alpha pinene	11.96	11.13	13.33	*	10.07	*	14.13	3.03	7.47	*	18.67
Sabinene	14.87	10.29	18.62	*	16.50	32.94	13.78	29.07	26.18	32.41	21.25
Beta – Pinene	6.83	7.62	7.21	6.48	6.28	6.10	10.28	29.07	2.74	1.87	8.72
Pinene	1.71	1.62	2.40	*	2.62	3.51	2.31	2.03	2.33	*	2.32
I-Phelandren	0.04	0.93	5.45	*	0.96	10.80	0.96	0.97	*	1.90	1.16
3-carene	3.09	2.11	5.45	1.00	1.19	0.10	2.60	2.07	4.39	1.90	3.31
Alpha terpine	1.14	2.56	1.59	2.03	4.30	2.47	2.45	3.91	3.10	1.39	0.78
o- cymol	1.05	1.08	2.37	*	0.81	2.76	1.89	1.44	1.27	1.12	1.06
Cinen	5.26	4.97	7.38	*	5.83	12.51	6.34	7.11	7.30	8.08	7.04
alpha terpine	1.14	2.56	1.59	2.03	4.30	2.47	2.45	3.91	3.10	1.12	0.78
Moslene	1.74	3.80	2.54	*	6.24	3.46	3.62	5.89	4.52	2.09	1.20
Terpinolen	1.51	2.08	3.35	2.03	2.51	4.63	2.23	2.41	2.77	3.03	2.02
Styrene	0.18	0.14	0.15	*	0.07	4.24	0.24	0.17	0.16	0.15	0.23
Linalol	0.36	0.26	0.45	*	0.22	0.82	0.45	0.52	0.42	0.41	0.32
L-4-terpineol	3.36	5.47	5.69	2.45	12.84	6.00	4.78	10.83	8.05	3.93	2.02
Terpenol	0.84	1.16	1.46	*	3.01	0.71	0.98	0.91	1.05	0.63	0.43
trans piperitol	0.08	0.15	0.16	0.43	0.90	0.17	0.17	0.30	0.25	0.10	0.04
Borneol	0.19	0.16	0.27	*	0.19	0.44	0.24	0.24	0.21	0.20	0.22
Safrole	3.03	0.97	0.07	1.37	0.21	0.19	1.59	0.24	2.04	0.27	2.23
Anisylacetone	0.08	0.19	0.11	0.76	0.09	0.09	0.21	0.10	0.14	0.06	0.03
delta elemene	0.29	0.18	0.21	*	0.49	0.42	0.42	0.55	0.18	0.28	1.16
p- eugenol	0.09	0.07	0.10	*	0.05	*	0.29	0.13	0.02	0.05	0.04
p-eugenol	0.09	0.07	0.10	*	0.05	*	0.29	0.13	0.02	0.05	0.04
p- eugenol	0.27	0.09	0.13	*	0.04	*	0.24	0.05	0.48	0.05	0.28
Copaen	0.57	0.14	0.15	0.49	0.32	0.38	0.12	1.22	0.16	0.34	2.09
Geraniol acetate	0.05	0.02	0.04	*	0.13	0.05	0.05	0.11	0.03	0.35	0.19
Methyleugenol	4.50	7.10	5.01	*	1.17	*	0.53	0.73	0.64	14.33	0.22
Isoeugenol	0.21	0.09	*	*	0.07	0.14	2.66	0.16	2.14	0.17	0.42
Beta Farnesene	0.03	0.07	0.21	*	0.07	*	0.03	0.04	*	0.06	0.05
beta cubebene	0.10	0.06	0.24	*	0.19	0.22	0.07	0.71	0.12	0.17	0.23
Methylisoeugenol	0.42	3.68	1.43	*	0.86	0.16	0.20	1.08	0.13	15.61	0.06
Cadinine	0.03	0.06	0.04	*	0.18	0.16	0.04	0.07	*	15.61	0.04
Cadinene	0.18	0.05	*	*	0.40	0.10	0.04	0.42	15.68	0.09	0.50
Myristicin	11.92	7.03	1.35	*	1.82	6.50	17.17	1.25	15.68	0.70	17.13
Elemicin	20.20	20.40	11.73	13.81	8.31	0.41	2.53	9.01	1.10	3.00	0.70
Methoxyeugenol	0.05	*	0.05	*	0.03	0.05	0.43	0.09	0.36	0.04	0.08
Myristic acid	*	*	*	*	0.03	*	0.12	*	*	*	*
Manoyl oxide	0.19	*	0.23	*	0.09	*	0.29	0.05	0.03	*	*

* denotes not detected

The highest recorded elemicin content was in MN-1 (26.89%), followed by PT-2 (20.40%) and PT-1 (20.20%). The accessions CF-1 (0.17%) and OB-2 (0.41%) recorded the lowest elemicin content.

Significantly wide variation was observed in safrole content, ranged from 0.07% to 11.23%. Safrole content was highest in the accession LA-1 (11.23%), followed by LA-2 (10.18%).

Sabinene content was found more prominent among the other constituents present in volatile oil. The maximum sabinene content was recorded in OB-2 (32.94%), followed by SM-1 (32.41%) and MN-2 (30.25%).The accessions CF-2 (0.44%) recorded the least sabinene content.

R-alpha pinene content in mace oil ranged from 3.03% to 16.78%. The accession DS-2 recorded highest value of 16.78% and accession GT-2 recorded lowest value of 3.03%. The accession SM-1 (1.87%) and GT-2 (29.07%) recorded the minimum and maximum content, as regards the constituent β pinene .

L-4 terpineol content was highest in the accession OB-1 (12.84%) and lowest in the accession MN-1 (3.22%). Manoyl oxide was found to be the least prominent constituent, which ranged from 0.11% in CF-1 to 0.29% in GT-1.

4.3.4.2 Chemo profiling of kernel volatile oil

GC MS profiling of the kernel volatile oil detected thirty eight constituents, of which the major share were for myristicin, elemicin, sabinene and safrole.

Highest myristicin content was recorded in the accession LA-2 (15.85%) followed by MN-1(15.50%) and lowest myristicin content was recorded in DS-2 (0.33%) followed by GT-2 (0.57%).

Significantly higher elemicin content was recorded in MN-1 (31.18%) and PT-2 (21.45%). The accessions MN-2 (0.40%), LA-1 (0.60%), GT-1(0.61%) and OB-2 (0.67%) showed significantly lower elemicin content in kernel volatile oil.

Table 4.8 (a) GC MS profile of kernel oil of unique nutmeg accessions

Constituents	YL-1 (%)	YL-2 (%)	DS-1 (%)	DS-2 (%)	MN-1 (%)	MN-2 (%)	CF-1 (%)	CF-2 (%)	LA-1 (%)	LA-2 (%)
R-alpha pinene	*	7.80	8.37	13.71	4.71	11.48	*	*	11.60	2.17
Sabinene	31.61	21.73	33.04	26.02	15.91	36.19	21.17	11.53	28.59	16.14
Beta – Pinene	5.14	4.23	33.04	26.02	3.25	3.96	6.06	12.42	28.59	1.63
Pinene	2.83	2.09	2.60	2.52	0.99	3.96	2.75	12.81	2.64	1.45
I-Phelandren	0.09	1.55	1.81	1.13	0.44	1.47	0.76	0.47	0.73	0.70
3-carene	4.64	2.19	1.81	1.84	1.12	2.58	1.11	0.83	0.98	1.06
Alpha terpine	1.41	2.13	1.69	2.09	0.56	1.77	3.52	1.58	3.19	1.72
o- cymol	2.44	0.73	0.89	0.91	0.66	1.71	1.97	*	1.97	0.85
Cinen	8.67	6.66	7.93	7.19	3.22	8.66	7.58	*	7.66	4.96
Alpha terpine	1.41	2.13	1.69	2.09	0.56	1.77	3.52	1.58	3.19	1.72
Moslene	2.27	3.22	2.74	3.21	0.96	2.60	5.39	*	4.73	2.89
Terpinolen	2.85	2.85	3.25	2.08	0.89	2.41	2.19	*	1.73	1.8
Styrene	0.11	0.07	0.07	0.09	0.06	0.19	0.16	*	0.13	0.09
Linalol	0.73	0.42	0.94	0.68	0.56	1.08	0.62	0.43	0.66	0.37
L-4-terpineol	4.79	5.90	7.36	4.89	3.35	4.81	7.68	3.54	7.01	6.36
Terpenol	0.92	1.06	1.49	0.75	0.64	0.71	1.40	0.58	0.98	1
trans piperitol	0.16	0.20	0.32	0.17	0.12	0.14	0.31	*	0.22	2.46
Borneol	0.37	0.34	0.44	0.28	0.17	0.25	0.43	*	0.25	0.71
Safrole	1.43	1.20	0.99	1.39	3.21	0.54	9.28	1.76	3.84	8.69
Anisylacetone	0.09	0.12	0.03	0.11	0.08	0	0.55	*	0.7	1.15
delta elemene	0.46	0.39	0.29	0.53	0.13	0.38	0.89	*	0.49	0.48
p- eugenol	0.60	0.54	2.43	0.22	0.30	1.26	0.45	*	0.85	0.78
p-eugenol	0.60	0.54	2.43	0.22	0.30	1.26	0.45	*	0.85	0.78
p- eugenol	0.17	0.21	0.05	0.22	0.57	*	0.63	*	0.85	0.57
Copaen	0.27	0.24	0.33	0.11	0.22	0.58	1.41	1.38	0.42	0.56
Geraniol acetate	0.66	0.63	0.87	0.17	0.13	0.33	0.40	*	0.64	0.21
Methyleugenol	4.39	4.75	0.38	2.16	5.53	0.15	0.5	*	0.27	1.09
Isoeugenol	*	0	0.44	0.08	0.06	0.18	3.44	*	0.30	0.26
Beta Farnesene	*	0.03	0.05	0.02	0.04	0.06	0.11	*	0.09	0.07
Beta cubebene	0.36	0.47	0.21	0.08	0.29	0.75	0.63	*	0.23	1.27
Methylisoeugenol	0.93	1.49	0.33	2.95	1.12	0.25	0.99	*	0.76	0.25
Cadinine	0.05	0.08	0.04	0.09	0.07	0.21	0.10	*	0.05	0.03
Cadinene	*	0.07	0.08	0.04	*	0.18	0.51	*	0.11	0.24
Myristicin	3.07	3.20	5.40	0.33	15.50	5.51	10.40	*	4.77	15.9
Elemicin	15.52	18.79	1.16	9.88	31.18	0.40	2.29	2.61	0.6	13.72
Methoxyeugenol	*	*	0.03	*	0.03	0.37	0.31	*	0.03	0.10
Myristic acid	*	*	*	0.16	0.03	0	0.36	*	*	0.09
Manoyl oxide	0.61	*	0.04	*	*	0.03	0.3	*	0	0

* denotes not detected

Table 4.8 (b) GC MS profile of kernel oil of unique nutmeg accessions

Constituents	PT-1 (%)	PT-2 (%)	IA-1 (%)	IA-2 (%)	OB-1 (%)	OB-2 (%)	GT-1 (%)	GT-2 (%)	TM-1 (%)	SM-1 (%)
R-alpha pinene	12.15	15.85	12.11	*	9.00	*	21.27	*	6.87	*
Sabinene	20.33	16.96	27.19	0.70	29.50	31.68	22.74	*	35.25	30.50
Beta – Pinene	6.53	9.80	4.73	14.43	4.19	9.04	12.43	*	2.45	1.60
Pinene	2.04	1.98	2.66	*	2.47	3.33	2.66	3.12	3.03	2.32
I-Phelandren	0.90	0.37	4.57	0.90	0.96	1.38	1.25	0.68	1.88	1.44
3-carene	1.43	0.50	4.57	1.97	1.26	3.84	1.25	2.76	1.88	2.57
Alpha terpine	1.20	1.01	1.63	*	2.90	2.78	2.30	1.04	3.14	1.17
o- cymol	0.53	0.56	2.69	*	1.09	3.64	3.48	3.96	1.84	0.98
Cinen	5.77	5.02	7.90	*	7.15	10.50	7.95	9.32	9.14	7.40
alpha terpine	1.20	1.01	1.63	*	2.91	2.78	2.30	1.04	3.14	1.17
Moslene	1.88	1.55	2.45	*	4.32	3.67	3.48	1.95	4.81	1.86
Terpinolen	1.60	0.80	2.53	2.50	1.99	2.47	1.76	1.77	3.19	2.46
Styrene	0.04	0.03	0.21		*	0.31	0.11	0.19	0.11	0.09
Linalol	0.55	0.48	1.02	0.94	0.37	1.09	0.75	1.17	0.64	0.45
L-4-terpineol	2.48	2.32	5.99	8.90	7.50	7.00	4.98	6.84	7.73	3.48
Terpenol	0.66	0.56	1.10	1.61	0.90	0.93	1.04	0.81	0.92	0.61
trans piperitol	0.073	0.06	0.22	*	0.24	0.22	0.13	0.19	0.24	0.12
Borneol	0.19	0.17	0.28	*	0.21	0.42	0.27	0.34	0.26	0.04
Safrole	3.03	1.88	0.70	0.38	0.77	0.47	0.63	0.47	0.81	0.36
Anisylacetone	0.05	0.09	0.05	*	0.04	0.12	0.15	0.14	0.04	0.05
delta elemene	0.86	0.30	0.18	*	0.57	0.62	0.47	0.91	0.26	0.33
p- eugenol	0.52	0.46	0.27	*	0.21	0.14	1.24	1.06	0.25	0.13
p-eugenol	0.52	0.46	0.27	*	0.21	0.14	1.24	1.06	0.25	0.13
p- eugenol	0.46	0.12	0.08	*	0.08	0.08	1.24	*	0.18	0.07
Copaen	2.64	0.22	0.19	0.19	0.82	0.83	0.16	1.69	0.37	0.68
Geraniol acetate	*	0.14	0.22	*	0.48	0.37	0.20	0.19	0.23	0.19
Methyleugenol	5.34	5.53	4.40	*	2.15	0.05	0.12	1.39	0.22	20.27
Isoeugenol	0.19	0.05	*	*	0.06	0.25	0.33	*	0.78	0.17
Beta Farnesene	0.11	0.07	0.44	*	0.091	0.07	0.02	0.08	0	0.05
beta cubebene	0.647	0.12	0.38	*	0.52	0.37	0.12	0.67	0.26	0.37
Methylisoeugenol	0.76	0.93	1.17	*	1.17	0.30	0.10	1.75	0.19	15.03
Cadinine	0.13	0.10	0.02	*	0.06	0.16	0.09	0.11	0	0.03
Cadinene	0.77	0.10	0.05	*	0.26	0.19	*	0.40	0.10	0.19
Myristicin	8.40	6.22	0.79	*	2.55	9.62	4.41	0.57	5.85	0.77
Elemicin	12.42	21.45	5.94	*	10.06	0.67	0.61	6.08	0.63	2.66
Methoxyeugenol	0.02	*	*	*	0.02	0.10	0.04	0.08	0.07	*
Myristic acid	0.04	*	*	*	0.06	0.05	0	*	0.07	0.23
Manoyl oxide	0.02	*	*	*	*	*	0.03	*	0.04	*

* denotes not detected

Safrole content ranged from 0.36% - 9.28% in the unique nutmeg accessions. Accession CF-1 (9.28%) recorded the highest safrole content in mace oil, followed by accession LA-2 (8.69%). The lowest safrole content was recorded in SM-1(0.36%)

Sabinene content significantly showed wider variation in kernel oils of unique nutmeg accessions. Highest sabinene content was found in MN-2 (36.19%), followed by DS-1 (33.04%) and TM-1 (35.25%). Lowest sabinene content was found in LA-2(16.14%) and PT-2 (16.96%).

R-alpha pinene content was found to be highest in GT-1 (21.275%) and lowest in MN-1 (4.71%).The lowest β pinene content was recorded in SM-1(1.61%) and LA-2 (1.634%), similarly β pinene content recorded highest value in LA-1 (28.59%) and DS-2 (26.02%).

L-4 terpineol content recorded highest value in TM-1 (7.74%) and lowest value in PT-2 (2.32%). Manoyl oxide ranged from 0.04% (TM-1) to 0.61% (YL-1).

4.4 Incidence of diseases

Incidence of major diseases like leaf fall, fruit rot, thread blight and die back noticed during the course of investigation are recorded and presented in the Table 4.9. Trees were mainly affected with fruit rot and thread blight diseases. Accessions YL-1, CF-1, LA-2 and GT-1were affected with fruit rot. Thread blight infestation was found mainly in accessions YL-1, LA-2 and GT-2. Accession DS-2 was susceptible to leaf fall disease. Accessions MN-1 and PT-2 showed symptoms of die back disease.

Table 4. 9 Incidence of diseases in unique accessions of nutmeg

Diseases	Accessions affected
Leaf fall	DS-2
Fruit rot	YL-1,CF-1,LA-2,GT-1
Thread blight	YL-1,LA-2,GT-2
Die back	MN-1,PT-2

4.5 Clustering of unique nutmeg accessions

The qualitative parameters noticed were quantified in terms of ordinal scale, tallying with the descriptor developed by Vikram (2016).

The similarities if any noticeable as regards any of the characteristics among the different unique accessions were assessed through UPGMA (Unweighted pair group method with arithematic mean) method and a dendrogram drawn accordingly using NTSYS (Numerical Taxonomy System) package2.2.

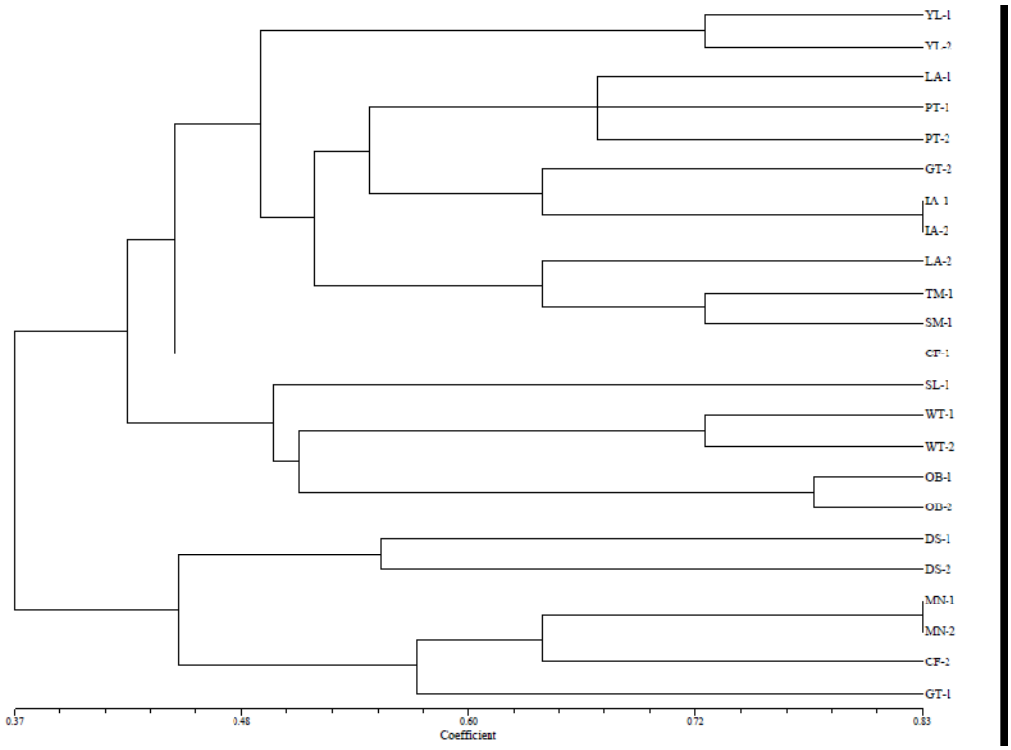


Fig 4.1 Dendrogram based on qualitative parameters of unique nutmeg accessions

The dendrogram in Fig 4.1 is itself an indicator of the dissimilarity among the genotypes present as the accessions were unique, mostly with respect to a single characteristic. If a grouping of accessions was conceived of, so as to reduce the dimensionality, it could be done only after accommodating 40% dissimilarity. Thus clusters were formed only at the default 60 % similarity. The cluster ID, the individual members, representative measure of characteristics binding them as similar, are listed in Table 4.10, 4.11, 4.12a and 4.12 b.

4.5.1 Tree characters

The canopy shape noticed was pyramidal in majority, conical, globular and oblong types were also present. The branching pattern was predominantly spreading, with erect and drooping patterns rarely noticed. The shape of leaf was oscillating among the commonly noticeable shapes – elliptic, ovate, lanceolate, oblong and obovate. The mature leaf colour has no option but to confine to its most visible green shade extending to dark green and light green at times. The colour of flushes showed predominance in greenish yellow over yellowish green, followed by light green.

4.5.2 Flower characters

Early flowering trees were less when compared to mid and late flowering trees. Majority of the trees had axillary raceme followed by umbellate cyme. The pigmentation of perianth was prominently creamy yellow /creamy, with creamy white and greenish creamy at times observed. The creamy white and white filament colours were prominent; with pale yellow coloured anthers, if the colouring of anthers was visible in male flowers.

4.5.3 Fruit characters

The ultimate resourcefulness of a tree is scaled based on the quality as also the quantity characteristics of the fruit. The fruit colour was skewed towards light yellow /yellow with a brown colour rarely spotted. In contrast, the fresh mace colour narrowed down to deep red starting from yellow with the red

Table 4.10 Cluster ID, accessions and their measured tree characters

Cluster ID	Accessions	Canopy shape	Branching pattern	Mature leaf shape	Mature leaf colour	Colour of flushes
Cluster 1	YL-1 YL-2	Pyramidal	Spreading Erect	Elliptic Ovate	Green	Light green, Greenish yellow
Cluster 2	LA-1 PT-1 PT-2	Pyramidal, Conical	Erect	Oblong Elliptic Obovate	Green, Dark green	Greenish yellow
Cluster 3	GT-2 IA-1 IA-2	Globular	Spreading	Elliptic Lanceolate Oblong	Green	Greenish yellow
Cluster 4	LA-2 TM-1 SM-1	Pyramidal	Spreading	Ovate Oblong Elliptic	Green, Dark green	Light green
Cluster 5	WT-1 WT-2	Pyramidal	Spreading	Elliptic	Green	Greenish yellow Yellowish green
Cluster 6	OB-1 OB-2	Pyramidal	Spreading	Lanceolate	Green	Yellowish green
Cluster 7	MN-1 MN-2 CF-2	Globular, pyramidal, pyramidal	Spreading	Elliptic Oblong	Green	yellowish green Greenish yellow
Cluster 8	CF-1	Pyramidal	Drooping	Ovate	Dark green	Greenish yellow
Cluster 9	SL-1	Pyramidal	Spreading	Elliptic	Green	Light green
Cluster 10	DS-1	Globular	Erect	Ovate	Light green	Light green
Cluster 11	DS-2	Oblong	Erect	Elliptic	Green	Greenish yellow
Cluster 12	GT-1	Pyramidal	Spreading	Elliptic	Green	Greenish yellow

Table 4.11 Cluster ID, accessions and their measured flower characters

Cluster ID	Accessions	Season of flowering	Inflorescence type	Colour of perianth	Colour of filament	Colour of anther
Cluster 1	YL-1 YL-2	Late Mid	Axillary raceme	Creamy	NA	NA
Cluster 2	LA-1 PT-1 PT-2	Mid Late Late	Axillary raceme	Creamy yellow Creamy Creamy white	NA	NA
Cluster 3	GT-2 IA-1 IA-2	Mid Late Late	Umbellate cyme, Axillary raceme	Creamy yellow Creamy white Creamy	NA	NA
Cluster 4	LA-2 TM-1 SM-1	Early Late Early	Axillary raceme	Creamy yellow, Creamy white, Creamy	NA	NA
Cluster 5	WT-1 WT-2	Late	Axillary raceme	Greenish creamy	Creamy white, NA	Pale yellow, NA
Cluster 6	OB-1 OB-2	Late	Axillary raceme	Creamy yellow	NA	NA
Cluster 7	MN-1 MN-2 CF-2	Mid, Mid, Late	Umbellate cyme	Creamy yellow	White	Pale yellow
Cluster 8	CF-1	Mid	Axillary raceme	Greenish creamy	NA	NA
Cluster 9	SL-1	Mid	Axillary raceme	Greenish creamy	NA	NA
Cluster 10	DS-1	Mid	Axillary raceme	Creamy yellow	Creamy white	Pale yellow
Cluster 11	DS-2	Late	Umbellate cyme	Creamy yellow	Creamy white	Pale yellow
Cluster 12	GT-1	Mid	Umbellate cyme	Creamy yellow	White	Yellow

Table 4.12 (a) Cluster ID, accessions and their fruit characters

Cluster ID	Accessions	Fruit colour (fresh)	Mace colour (fresh)	Mace colour (dry)	Nut colour (fresh)	Nut colour (dry)	Kernel colour (dry)	Shape of mace	Nature of fruit dehiscence
Cluster 1	YL-1 YL-2	Light yellow Yellow	Yellow	Yellow	Brown	Brown	Light brown	Round	Two halves
Cluster 2	LA-1 PT-1 PT-2	Light yellow Yellow Light yellow	Red	Scarlet red	Black	Brown, dark brown, brown	Grey Grey Light brown	Oval	Two halves
Cluster 3	GT-2 IA-1 IA-2	Yellow	Red Red Deep red	Scarlet red	Dark brown, Black, Black	Brown, dark brown, dark brown	Light brown, grey, grey	Round	Two halves
Cluster 4	LA-2 TM-1 SM-1	Light yellow yellow	Red	Scarlet red	Brown, Black, Black	Light brown, black, Black	Light brown	Oval Triangular Oblong	Two halves
Cluster 5	WT-1 WT-2	Brown	Yellowish orange	Orange red	Brown	Brown Light brown	Dark grey light brown	Oblong	Two halves
Cluster 6	OB-1 OB-2	Light yellow	Deep red Red	Red scarlet red	Dark brown, Black	Brown, Black	Dark grey	Oblong	Two halves

Table 4.12 (b) Cluster ID, constituent members and their fruit characters

Cluster ID	Accessions	Fruit colour (fresh)	Mace colour (fresh)	Mace colour (dry)	Nut colour (fresh)	Nut colour (dry)	Kernel colour (dry)	Shape of mace	Nature of fruit dehiscence
Cluster 7	MN-1 MN-2 CF-2	Yellow	Red	Scarlet red	Black, Black, Brown	Dark brown, Brown, Brown	Light brown Light brown Grey	Oval	Three halves Three halves Two halves
Cluster 8	CF-1	Yellow	Deep red	Scarlet red	brown	brown	Grey	Oval	Two halves
Cluster 9	SL-1	Yellow - light green	Deep red	Scarlet red	Nil	Nil	Nil	Oblong	Two halves
Cluster 10	DS-1	Light yellow	Red	Scarlet red	Black	Brown	Light brown	Oval	Four halves
Cluster 11	DS-2	Light yellow	Red	Scarlet red	Brown	Brown	Grey	Round	Four halves
Cluster 12	GT-1	Yellow	Red	Red	Black	Black	Grey	Round	Two halves

pigment increasingly getting added turning to yellowish orange and finally to red and deep red. When such maces were sun dried, the retention colour again was scarlet red in majority with a nominal distribution over yellow, orange and red colours.

The nut colour was brown with the range of brownness running from light to very deep, ultimately resulting in black colour at times. The kernel colour was mostly light brown with grey and dark grey colours getting intermixed at times. The shape of mace had a random walk over oval, round, oblong and triangular shapes. The nature of fruit dehiscence was “two haves” in majority with “three halves” and “four halves” also attainable at times. The widely audited qualitative characteristics highlight the very quantum of dissimilarity among the unique genotypes.

4.6 Summary statistics

The investigations so far has brought out the multidimensional variability with respect to all the characters. A comparative evaluation so as to assimilate at least some similarity among the genotypes with respect to the fruit characters has been achieved in a step by step manner as follows:

The ranking technique as suggested by Arunachalam and Bandyopadhyay (1984) was adopted to use in the importance of the different characteristics present in the unique accessions into a comparable scaled value in one dimension. Ranking technique method was proposed to make decisions jointly on a number of dependent character variables. A score was allotted to each entry for each character. The scores were added across characters to provide a final score for each entry. Based on the final scores, the entries were ranked on their performance over a set of characters. The result of such an analysis is given in Table 4.13.

Table 4.13 List of unique accessions of nutmeg and their pooled rank score

Accession ID	Pooled rank score
YL-1	30
YL-2	37.5
SL-1	2
DS-1	10.5
DS-2	49.5
MN-1	57
MN-2	78.5
CF-1	30.5
CF2	80.5
LA-1	66
LA-2	61.5
PT-1	40.5
PT-2	51.5
GT-1	54.5
GT-2	57
WT-1	31.5
WT-2	8.5
IA-1	27
IA2	27
OB-1	66
OB-2	36
TM-1	28
SM-1	41.5

One of the lone accessions possessed an odd characteristic namely seedless nature, a contrasting seed characteristic not being very much desirable. Since this was not measurable at all in that lone accession, the pooled ranking for this accession based on other fruit characteristics was computed and the calculation yielded a rank score of 2. It was initially separated and singled out as "extra ordinary" accession. The rest of the 22 accessions had a ranking in the wide range 8.5 - 80.5. This wide range among the accessions was resolved into smaller ranges by finding the quartiles. The quartiles are partioned values grouping the accessions based on their respective values as Q_1 (below25% and above 75%), Q_2 (below 50 and above 50) and Q_3 (below 75 % and above 25%). This served as the basis for grouping the accessions as, those having the score below Q_1; between Q_1 to Q_2, between Q_2 to Q_3 and above Q_3. Accordingly the accessions were grouped and the groups were named as **extra ordinary** (rank score below 2), **very good** (rank score below 29.5), **good** (rank score between 29.5 to 41), **moderate** (rank score between 41 to 58.125), **heteroscedastic** group (rank score above 58.125).The rule of thumb is that, a smaller rank score portraits the more desirable characteristics of the concerned accessions.

The 'extra ordinary' group consisted of SL-1. The 'very good' group comprised the accessions WT-2, DS-1, IA-1, IA-2, TM-1. The 'good' group consisted of YL-1, CF-1, WT-1, OB-2,YL-2, PT-1, the 'moderate' group consisted of SM-1, DS-2, PT-2, GT-1, GT-2, MN-1, the heteroscedastic group consisted of LA-2, OB-1, LA-1, MN-2, CF-2; with group sizes of 1,5,6,6,5 members respectively in each group.

The summary statistics namely range, mean and also coefficient of variation for the different characteristics are presented in Table 4.14 (a) and 4.14 (b) group wise. The coefficient of variation (CV) of a character is a pointer towards the extent of variability present, can be exploited for further improved measures especially by applying the principles of genetics.

Table 4.14 (a) Group wise summary statistics of tree characters of unique accessions of nutmeg

Group ID	Constituent members	Rank scores	Plant height (m)	Girth at 140 cm height (cm)	Canopy spread		Leaf length (cm)	Leaf breadth (cm)	Leaf area (cm^2)	Number of orthotrops
					N-S (m)	E-W (m)				
Extra ordinary	SL-1	2	11	72	9.11	8.35	12.56	5.32	43.2	10
Very good	WT-2, DS1, IA-1, IA-2, TM-1	8.5 , 10.5, 27,27,28	7.1 -16.1 **(42.24%)**	40-175 **(65.57%)**	6.6-13.1 **(27.45%)**	7.6 -14.7 **(24.81%)**	11.1 -16.9 (**19.13%**)	3.65-5.10 **(11.75%)**	30.84-47.74 **(17.34%)**	1 -12
Good	YL-1, CF-1, WT-1, OB-2, YL-2, PT-1	30,30.5,31.5, 36,37.5, 40.5	6.48 - 19.3 **(45.12%)**	18.8 - 149.2 **(75.27%)**	4.3 -11.13 **(38.98%)**	4.8 -11.78 (**40.39%**)	10.36 - 14.28 **(12.91%)**	4.28 -5.68 **(11.06%)**	25.55 -49.09 **(23.82%)**	0 -9
Moderate	SM-1, DS-2, PT-2 , GT-1, GT-2, MN-1	41.5,49.5,51. 5,54.5,57, 57	5 - 13.78 **(33.02%)**	41 -128 **(44.04%)**	5.9 -8.5 **(15.05%)**	5.7 – 8.1 **(15.01%)**	8.6 -12.76 **(15.07%)**	2.52 -5.3 (**23.56%**)	15.05 -38.20 **(26.99%)**	1 – 27
Heteroscedastic	LA-2, LA-1, OB-1, MN-2, CF-2	61.5 ,66 , 66, 78.5,80.5	6.4 -9 **(16.07%)**	23.63 -90 **(54.61%)**	4.95 -8.90 **(25.56%)**	10.62 - 13.14 **(23.46%)**	10.62 - 13.14 **(12.88%)**	4.1 -5.5 **(12.97%)**	27.85 -37.81 **(14%)**	1- 15

Table 4.14 (b) Group wise summary statistics of flower characters of unique accessions of nutmeg

Group ID	Constituent members	Rank scores	No. of female flowers/ 0.1m^2	No. of male flower/ 0.1 m^2	No. of hermaphrodite flowers/0.1 m^2	Length of flower (mm)	Breadth of flower (mm)	Length of perianth (mm)	Breadth of perianth (mm)	Length of filament (mm)	Length of anther lobe (mm)	Number of stamens
Extra ordinary	SL-1	2	2	0	0	12.20	5.88	8.21	4.27	nil	nil	nil
Very good	WT-2, DS-1, IA-1, IA-2, TM-1	8.5 ,10.5, 27,27, 28	4 -5	1	0	5.47-9.90 **(24.81%)**	5.32-6.38 **(6.52%)**	5.25-8.2 **(19.16%)**	3.97 – 5.8 **(15.65%)**	3.9	2.45	22
Good	YL-1, CF-1, WT-1, OB-2, YL-2, PT-1	30, 30.5, 31.5,36,37.5, 40.5	3- 10	1 – 5	0	8.36-9.17 **(4.67%)**	4.61-6.25 **(11.74%)**	6.48 - 8.07 **(8.71%)**	1.18 - 5.21 **(36.36%)**	3.6	3.69	16
Moderate	SM-1, DS-2, PT-2, GT-1, GT-2, MN-1	41.5,49.5 ,51.5,54.5, 57,57	2 – 5	2 – 4	2	7.35-14.10 **(24.04%)**	5.77-7.27 **(9.50%)**	6.00 – 10.51 **(18.86%)**	3.72 - 6.08 **(18.67%)**	4.16 - 5.45	1.6 - 3.94	18 -22
Heteroscedastic	LA-2, OB-1, LA-1, MN-2, CF-2	61.5 , 66,66, 78.5, 80.5	4 – 6	1 – 3	2	7.404 - 11.38 **(16.38%)**	4.2 – 5.68 **(11.35%)**	5.86 - 9.02 **(19.42%)**	3.53 - 7.10 **(34.04%)**	1.79 - 2.05	1.02-3.02	16 -18

The CV for all the major characters were computed group wise, so as to assess the extent of variability of a character, as regards the members comprising that group. The major variable characteristics group wise are: the CV for plant height was 42.24%, 45.12%, 33.02% and 16.07% in order the groups listed in table no. 4.14 (a) and 4.14 (b). Similarly, the CV for other parameters namely for girth 65.57%, 75.27%,44.04% and 54.61% ; for canopy spread in N-S direction 27.45%, 38.98%,15.05% and 25.56%; for canopy spread in E-W direction 24.81%, 40.39%, 15.01% and 23.46%; for leaf length was 19.13%, 12.91%, 15.07% and 12.88%; for leaf breadth 11.75%,11.06%,23.56% and 12.97%; for leaf area 17.34%, 23.82%, 26.99% and 14%; for length of flower 24.81%, 4.67%, 24.04% and 16.38%; breadth of flower 6.52%,11.74%, 9.50% and 11.35%; for length of perianth 19.16%,8.71%,18.86% and 19.42%; breadth of perianth 15.65%,36.36%,18.67% and 34.04%.

A perusal of such measured variability for each of the characteristics that were prominent among the five groups are listed as: Leaf length (19.13%) and length of flower (24.81%) in '**Very good'** group; plant height with a CV of 45.12%, Girth with a CV of 75.27%, canopy spread N-S (38.98%)and E-W (40.39%), breadth of flower with a CV of (11.74%) and breadth of perianth (36.36%) in '**Good' group**; leaf breadth (23.56%) and leaf area (26.99%)in **'Moderate'** group; length of perianth in **'Heteroscedastic'** group.

DISCUSSION

5. DISCUSSION

Spotting of accessions of any tree with rare characteristics is of academic interest to a researcher. The importance of unique characteristics spotted in the accessions, because of their specific features is worthy of discussion. In the present study, thirteen unique nutmeg types were identified *viz.* yellow mace type, seedless type, double seeded type, monoecious type, cluster fruited type, low astringent type, pyramidal type, grape type, wild type, oblong type, triangular mace type, small leaf type and Improved ones. In this chapter, discussion about unique accessions one by one is presented.

5.1 Yellow maced nutmeg

Yellow colour of mace is a preferred characteristic that is rarely found in the nutmeg family. Normal mace colour in nutmeg is deep red in fresh form and scarlet red when dry. In the present study, two collections were identified with this feature. In the trail of elite nutmeg tree expedition, Rahul *et al.* (2014) and Miniraj (2015) have reported the occurrence of a precious unique nutmeg with yellow mace in Kottayam district. Yellow mace colour is an exciting unique feature when compared with normal nutmeg types, as it fetches premium price in the market. In normal nutmeg trees, the dry mace which is red in colour gradually fades to orange and then to yellow after a period of storage for 6-12 months. Hence these genetically yellow types could be directly exploited in the nutmeg trade. In crops like cardamom the superiority of Mysore cultivars is well established for the retention of green colour after artificial drying (Prasanth and Venugopal, 2009). Saxena *et al.* (2010) reported that higher consumer preference and market price are obtained for green podded pigeon pea compared to the stripped ones. Maya *et al.* (2005) reported that the mace colour in *Myristica prainii* was due to lycopene pigment but in *M. beddomeii* , the pigment responsible for mace colour was not lycopene. Pigment responsible for yellowness in this accession is to be found out.

5.2 Seedless nutmeg

Seeding is extremely essential as regards any tree crop is concerned but here in the seedless nutmeg identified, eighty per cent of fruits had rudimentary sterile seed inside the mace and rest twenty per cent fruits were totally seedless. Seedlessness and soft seeded fruits have been reported in many fruit crops like pomegranate, grapes etc. (Abu- Zahra, 2013) and vegetables like watermelon (Pradeepkumar, 2015) by several workers. Occurrence of a seedless mango cultivar Ataulfo with high shelf life was reported by Hernandez (2015). Parthenocarpy, triploidy and several other factors contribute to seedlessness (Vardi *et al.,* 2008) Here in nutmeg, further studies are required to exactly find out the reason for seedlessness.

5.3 Double seeded nutmeg

A noticeable feature of these double seeded accessions was that they appeared to be in transition of sex expression as evidenced by the presence of few male flowers and an umbellate cyme inflorescence, typical of male trees (Vikram, 2016). Rendle (1971) reported number of stamens in male nutmeg flower as 3-18 but these double seeded accessions had higher number of stamens borne in a central column. Number of fruits per tree was less than average which nullifies the advantage of being double seeded. In both the accessions ninety per cent of the fruits were double seeded and the remaining 10 per cent slightly deformed. Double seeded nutmeg types have been identified from central parts of Kerala and it was observed that double seededness and multiple splitting of fruits were generally associated with monoecious types (Miniraj *et al.,* 2015; Vikram, 2016).

5.4 Monoecious nutmeg

Monoecy is a very much desirable characteristic as nutmeg is usually classified as dioecious in nature. If we have a monoecious tree with other desirable attributes, it will definitely be a boon to the nutmeg planters. Several workers

reported the existence of monoecious nutmeg trees in state of Kerala and Karnataka (Miniraj *et al.,* 2012; Sasikumar *et al.,* 2015; Rema *et al.,* 2015).

Occurrence of flowers in bunches with varied proportion of male and female flowers per bunch has been reported in monoecious trees (Krishnamoorthy *et al.,* 2012).In the monoecious trees located in the study, number of female flowers per 10 cm^2 was 3- 4 while number of male flowers per 10 cm^2 was three . In addition to male and female flowers, few abnormal flowers (2 per 10 cm^2) were also noticed in both the accessions. Hermaphroditic nature is rarely reported in nutmeg, but for the record of a hermaphrodite variety Konkan Sugandha from Maharashtra (Parthasarathy, 2010). However more flowers have to be examined to confirm the abnormal type of flowers noticed in these types.

5.5 Cluster fruited nutmeg

Clustering of fruits is not a common phenomenon in nutmeg, but it definitely is a desirable attribute. In the cluster fruited nutmegs, eighty per cent of the fruits were in cluster of three, fifteen per cent fruits in two and remaining 4-8 fruits per cluster. Of all the 23 accessions evaluated in the study, including the improved accessions, fruit yield per tree was significantly high in one of the cluster fruited accessions (CF-1). One-two fruits in a cluster were observed as common in nutmeg by Vikram, (2016). In crops like cardamom, extensive branching at terminal end of panicle resulted in higher yield as reported by Prasanth and Venugopal (2009). Singh and Bhatt (2012) identified a unique line with four pods per node in faba bean, which is not a commonly seen feature.

5.6 Low astringent nutmeg

The astringency of pericarp is usually a reckoned characteristic; deviation from this towards a low level is very much desirable for use in the value addition of nutmeg rind.

While considering the biochemical components of nutmeg rind, acidity and tannin contents were lower and pectin content was higher in LA-2, which signifies its use in value addition. Vikram (2016) reported the range of titrable acidity in nutmeg rind as 1.28 -1.92 per cent. In the present study, low astringent types had acidity content below the range mentioned by Vikram (2016). Even though LA-1 was initially designated as low astringent type, on final analysis it was other unique accessions which recorded comparatively lesser acidity and tannin content than these low astringent types.

5.7 Narrowly Pyramidal (conical) nutmeg

Narrowly pyramidal (conical) tree shape is the unique feature of PT-1 and PT-2, which can be utilized in high density planting system as it will occupy only less horizontal ground space coverage. High density planting in nutmeg is an upcoming area of research due to reduced land availability. Erect branching pattern was observed in these types which resulted in the narrowly pyramidal canopy shape. Ratio of nut to mace was the highest in PT-2 accession (6.75) as compared to the normal ratio of 20:3, which is a highly desirable feature (Vikram, 2016).

5.8 Grape nutmeg

Grape nutmeg can definitely be exploited as ornamental types suitable for landscape horticulture. The fruits were quite small when compared to the normal nutmeg, but number of fruits per tree was on the higher side. As the fruit itself is small, fresh mace and nut weight were also significantly low.GT -2 had lowest measures of fruit characteristics among all the unique accessions studied. Sasikumar *et al.*(2014) also reported a distinct nutmeg type with small fruits and thin shell with high yield in terms of number of fruits along with profuse bearing habit. A black pepper accession Nedumchola with small leaf and spike was reported by IISR (2015).Small fruitedness may not be a desirable character from the economic point of view. But it can definitely be utilized in landscaping. Dwarfness with short and

spreading growth habit has been reported in clove (Krishnamoorthy and Rema, 1994).

5.9 Wild nutmeg

Even though nutmeg is an introduced crop to India, there exist natural populations of other Myristicaceae members in the forest of Kerala (Miniraj, 2012). WT-1 and WT-2 can be exploited for their valuable features. Krishnamoorthy (2008) reported the significance of *Myristica malabarica* as drought hardy rootstock in water scarcity areas. The roots of wild nutmeg are quite stronger and can penetrate deep inside soil till it attains the water table. The wild nutmegs were monoecious in sex form. Another noticeable feature in wild nutmeg was the comparatively small flower size compared to *M. fragrans*. Fruits of wild nutmeg varied significantly from normal nutmeg in terms of fruit colour, fruit shape, fruit size, mace shape and nut shape.

Wide variation was observed in nut and mace characteristics in terms of its quantitative nature in WT-1 and WT-2. Kernel development was improper in WT-1 and on drying it could not be separated from the thin shell. The seeds did not germinate indicating the poorly filled endosperm. The viability of seeds of *Myristica malabarica* was only up to one week under natural condition as reported by Kumar (2002).

Fruit yield per tree was significantly high in *Mristica malabarica* when compared to *Myristica fragrans* accessions. Unlike the cultivated nutmeg, wild nutmegs are usually collected by the forest dwellers for use in the ayurvedic drug manufacturing sector. Shareif (2007) has reported the use of *Myristica praini, M. andamanica* and *Knema andamanica* in tribal medicine There are reports about the destructive harvesting of wild *Myristica sp*. which has lead to the depletion of these valuable species from the natural habitats (Miniraj, 2012).In the present study, fruits of both the wild accessions are reported to be collected by traders for use in paint industry.

5.10 Oblong nutmeg

Another interesting unique feature observed was oblong nutmeg, due to the deviation in fruit shape from the normal nutmeg. The nut and mace in both accessions was oblong shaped and had thin mace; it is very difficult to get flower grade quality mace. But the number of fruits per tree was significantly higher and was comparable with other unique nutmeg accessions evaluated in the study. Nitta (1993) reported from Moluccas Island about the occurrence of nutmeg trees with different fruit shapes *pala tidore* which has pear shaped fruits and *pala bali* which has large globoid fruit.

5.11 Triangular maced nutmeg

Triangular shape of mace was much distinct when mace was attached to the nut. Normally the mace shape in nutmeg varied among round, oval and oblong shapes. Occurrence of beaked nutmeg accessions was reported by Vikram (2016). Jerard *et al*. (2014) reported Andaman horned cocos, a unique coconut germplasm with distinct horny nuts.

5.12 Small leaved nutmeg

Small leaf type possessed dark green coloured small elliptic leaves. Leaf dimensions in this accession makes it distinct from the normal nutmeg types with lowest leaf length and breadth along with a minimum leaf area of 15.05 cm^2. Vikram (2016) reported that leaf area is one of the parameters which influence the photosynthesis and also the final biomass output of a tree including the yield.

Even though the leaves are small, the foliage density was abundant. However, the number of orthotropic shoots in SM-1 was found to be 45, which is a highly preferred characteristic in nutmeg as regards the vegetative propagation of nutmeg is concerned, a feature that is highly useful to a nutmeg grower in propagating this unique nutmeg type through budding / grafting. Studies on vegetative propagation of nutmeg have revealed that flowering commenced in the second year of planting when

straight shoots were used as scion. This solidifies the significance of orthotropic shoot production in nutmeg. Unlike the normal trees, more number of orthotropic shoot production was observed in SM-1, which may be due to more sunlight infiltration through fine textured leaves. Kakkappara-2, a narrow leaved thick mace type nutmeg tree with open canopy structure was spotted by Sasikumar *et al.* (2014). In the trail of elite nutmegs, narrow leaved thick mace type nutmeg trees were spotted by Sasikumar *et al.* (2015).

5.13 Improved nutmeg

Improved accessions from an earlier study namely IA-1 and IA-2 respectively were collected from Chalakudy region. These accessions which possessed above average economic characters were included to make a comparative evaluation with the unique germplasm collected.

5.14 Evaluation of unique accessions of nutmeg

5.14.1 Quantitative characteristics

The quantitative parameters were recorded on ten fruits randomly collected from each unique accession. The data were analyzed using Univariate General Linear Model procedure for each of the individual characteristics. The observations of improved accessions were included, to have a comparative evaluation.

The fruit weight ranged from 126.05g (DS-1) to 38.8g (GT-2) with DS-1,YL-1 and CF-1 accessions possessing a superiority in weight over improved accessions (IA-1, 98.43g and IA-2, 98.33g) (Fig 5.1) The thickness of pericarp ranged from 16.978 mm (YL-2) to 6.219 mm (WT-1 and WT-2) (Fig 5.2).

The accessions SL-1 (9.74g), WT-1(8.70g) and WT-2(8.33g) had relatively higher mace weight over all the accessions inclusive of improved accessions .The dry weight also followed the same rowster with a slight deviation in order of accession

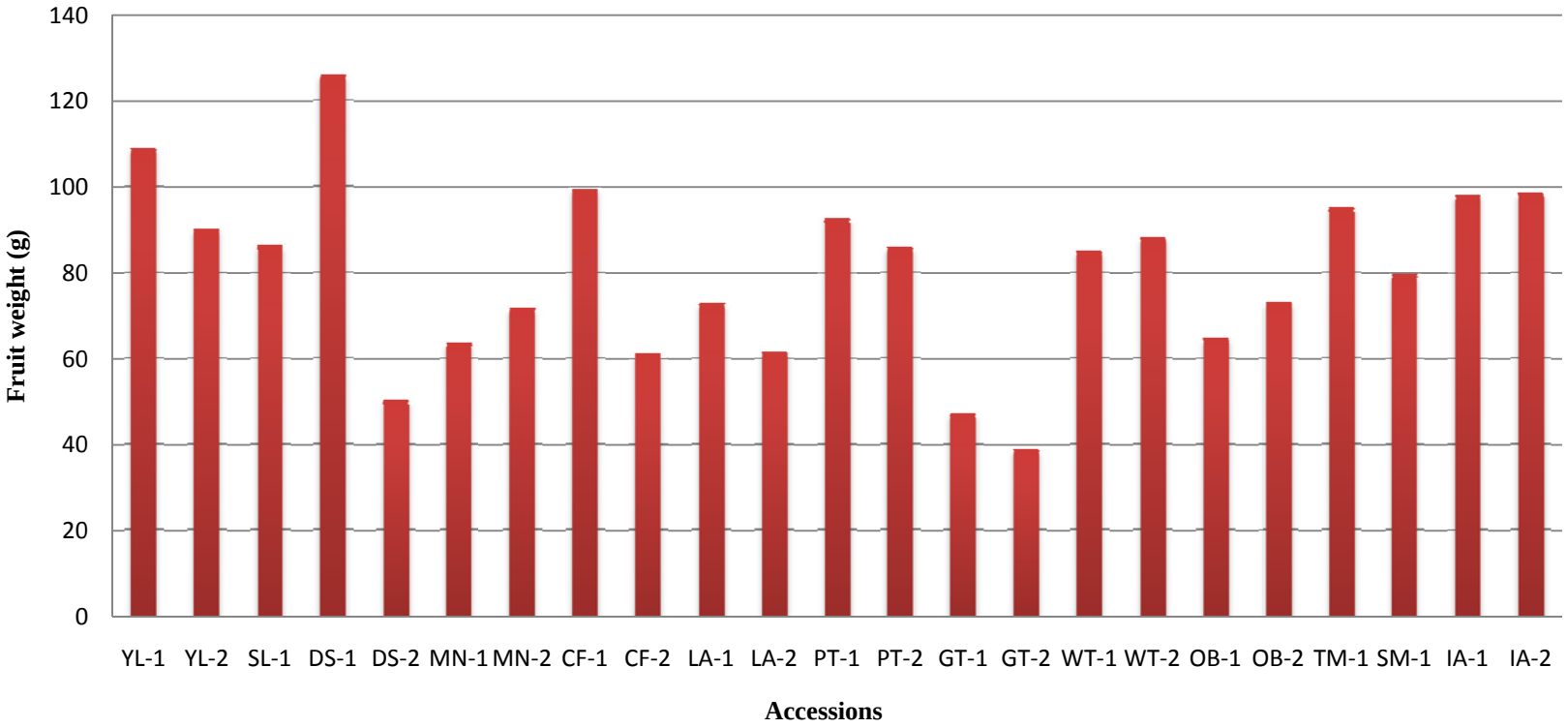


Fig 5.1 Fruit weight in unique accessions of nutmeg

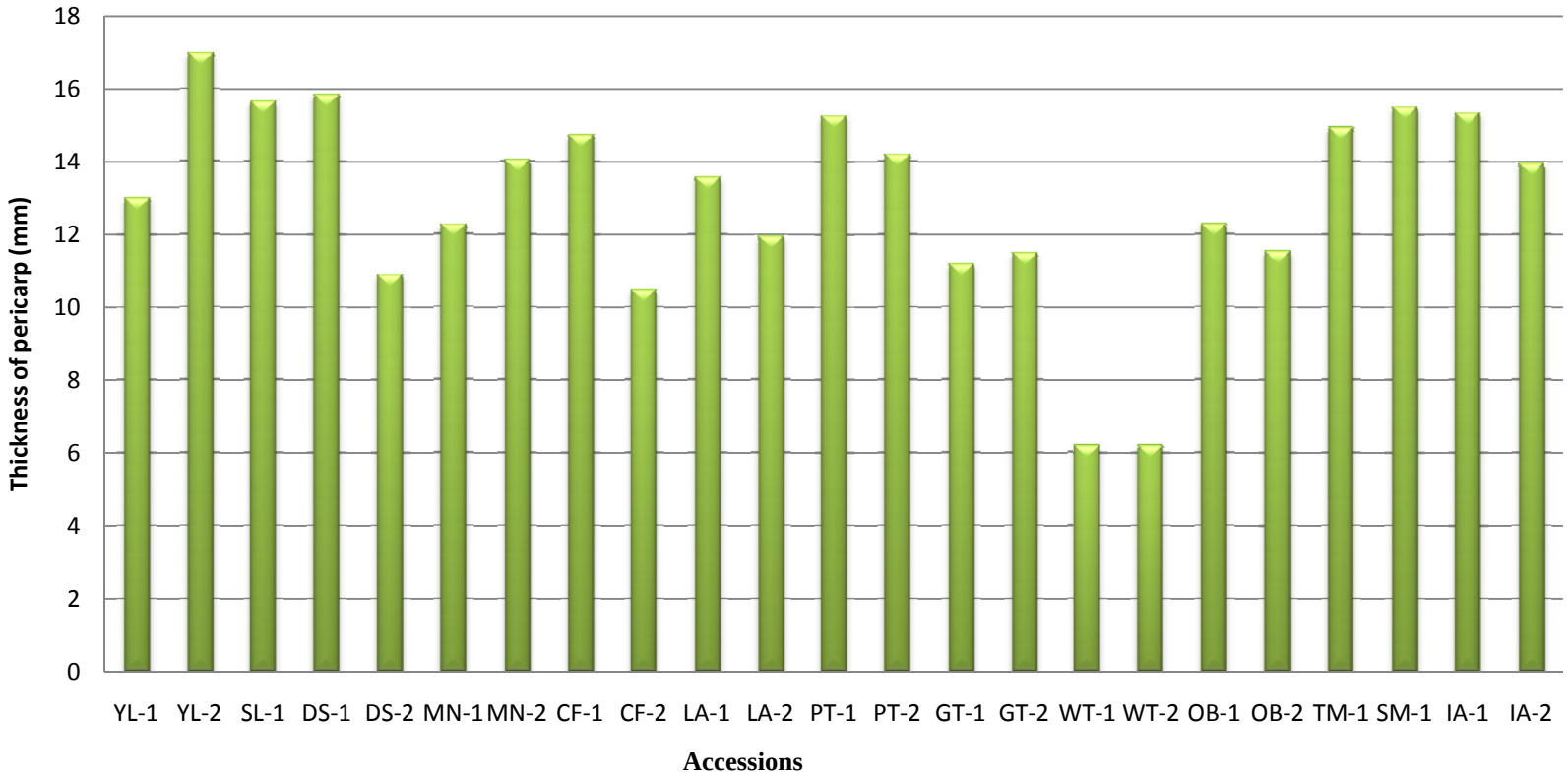


Fig 5.2 Thickness of pericarp in unique accessions of nutmeg

WT-1 (6.75g), WT-2 (5.28g) and SL-1(3.82g) (Fig 5.3). Though the nut weight of IA-1 (17.02g) was the highest, the dry nut weight had a reversal towards the unique accession DS-1(13.03g) (Fig 5.4). Krishnamoorthy *et al.* (1996) concluded that the fruit weight of nutmeg ranged from 60.5 to 80.0 g, the mace weight from 2.5 to 3.7 g and the seed weight from 8.4 to 12.2 g under Kerala conditions.

The dried shell thickness ranged from 1.22 mm (TM-1) to 0.63 mm (GT-2), with accessions TM-1, SM-1, PT-2 and DS-1 showing superiority over both the improved accessions. The highest recorded dry kernel weight was 9.55g (DS-1) followed by 8.05g (WT-2) and 7.70g (CF-1). All these three accessions were found superior over both the improved accessions (Fig 5.5).

The accessions which had a mace ratio satisfying the criteria 20:3 were PT-2 (6.75), LA-1 (6.40) and GT-1 (6.16) (Fig 5.6). The majority of the accessions namely YL-2 (74.23%), PT-1(73.83%), CF-1(71.62%), OB-1 (70.98%), CF-2 (70.75%), WT-2 (70.33%) and MN-2 (70.033%) had a relatively higher shelling percentage over the improved accessions IA-1(59.30%) and IA-2 (65.50%).

As a contrasting phenomenon, none of the accessions had a superior seed weight over the improved accession IA-1 (17.02g), as such the seed inference on the seed volume is straight. The accessions SL-1 (10.5cm^3), WT-2 (9.5 cm^3) and WT-1(9 cm^3) had a superior mace volume over that of both the improved accessions.

Number of fruits per tree was highest in accession WT-2(4000) followed by WT-1 (3500) and CF-1 (2250) and was superior over the improved accessions (Fig 5.7). Mace yield per tree was highest in accession WT-1 (23.63 kg per tree) followed by WT-2 (21.12 kg per tree) and CF-1 (5.29 kg per tree). Nut yield per tree was highest in accession WT-2 (45.76 kg per tree) followed by CF-1 (24.19 kg per tree) (Fig 5.8). Both WT-2 and CF-1 were superior over improved accessions in terms of mace and nut yield.

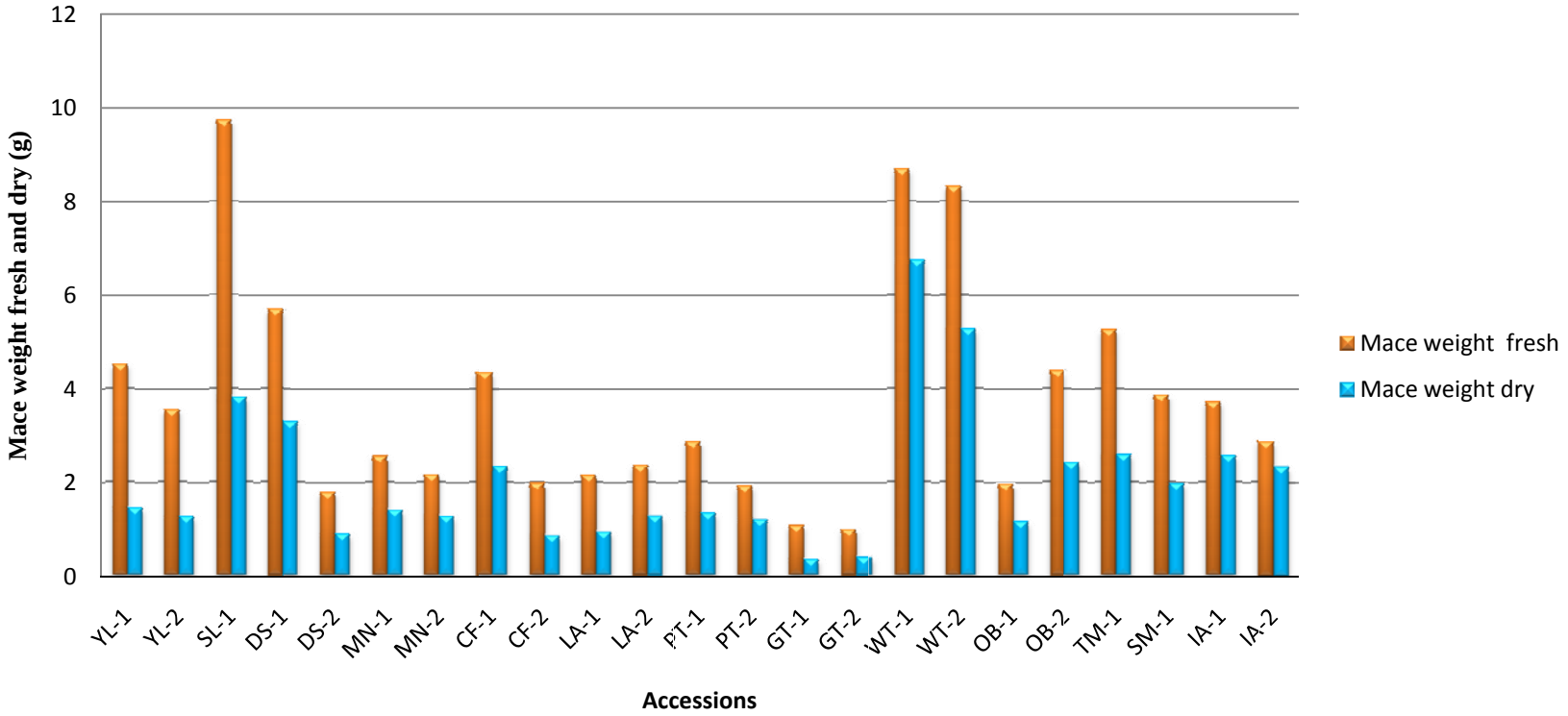


Fig 5.3 Variation in mace weight in unique accessions of nutmeg

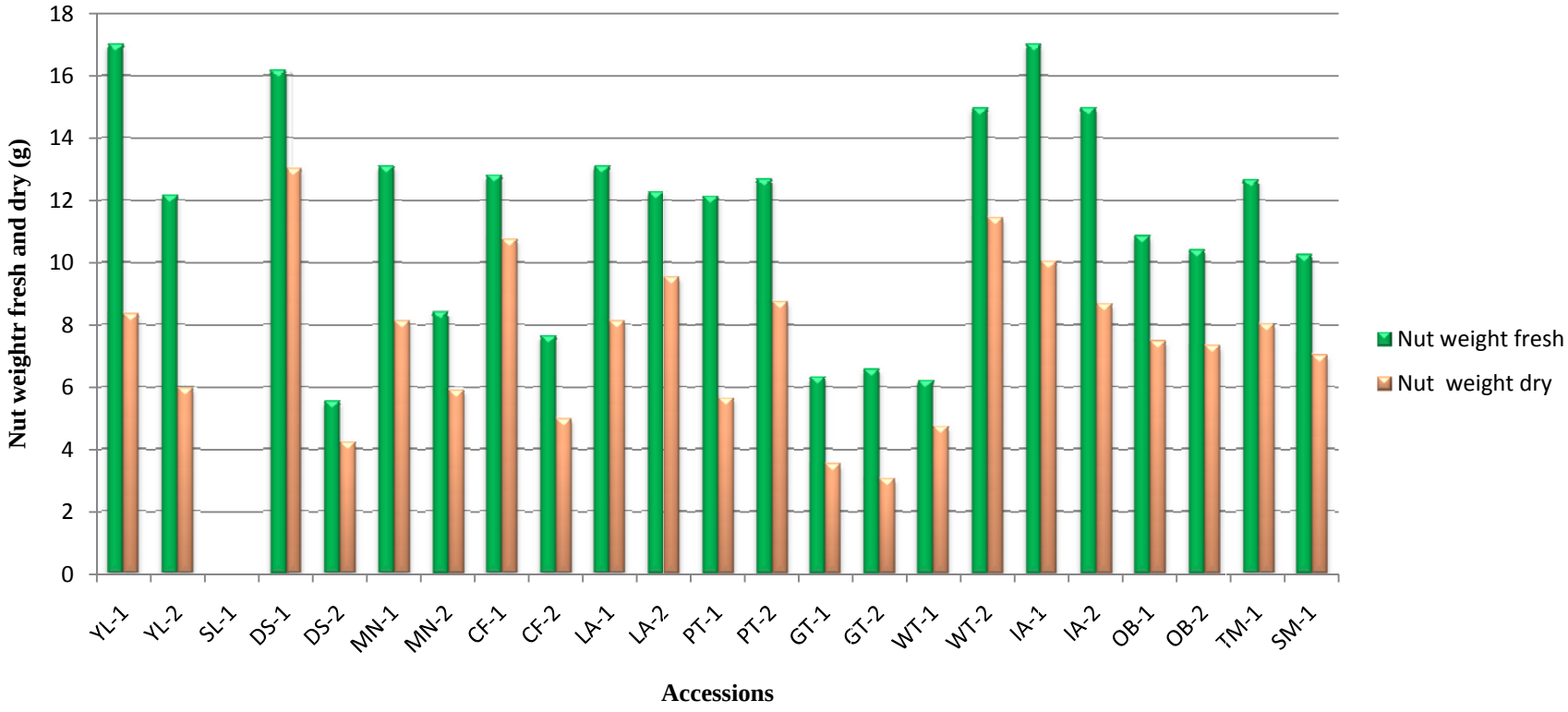


Fig 5.4 Variation in nut weight in unique accessions of nutmeg

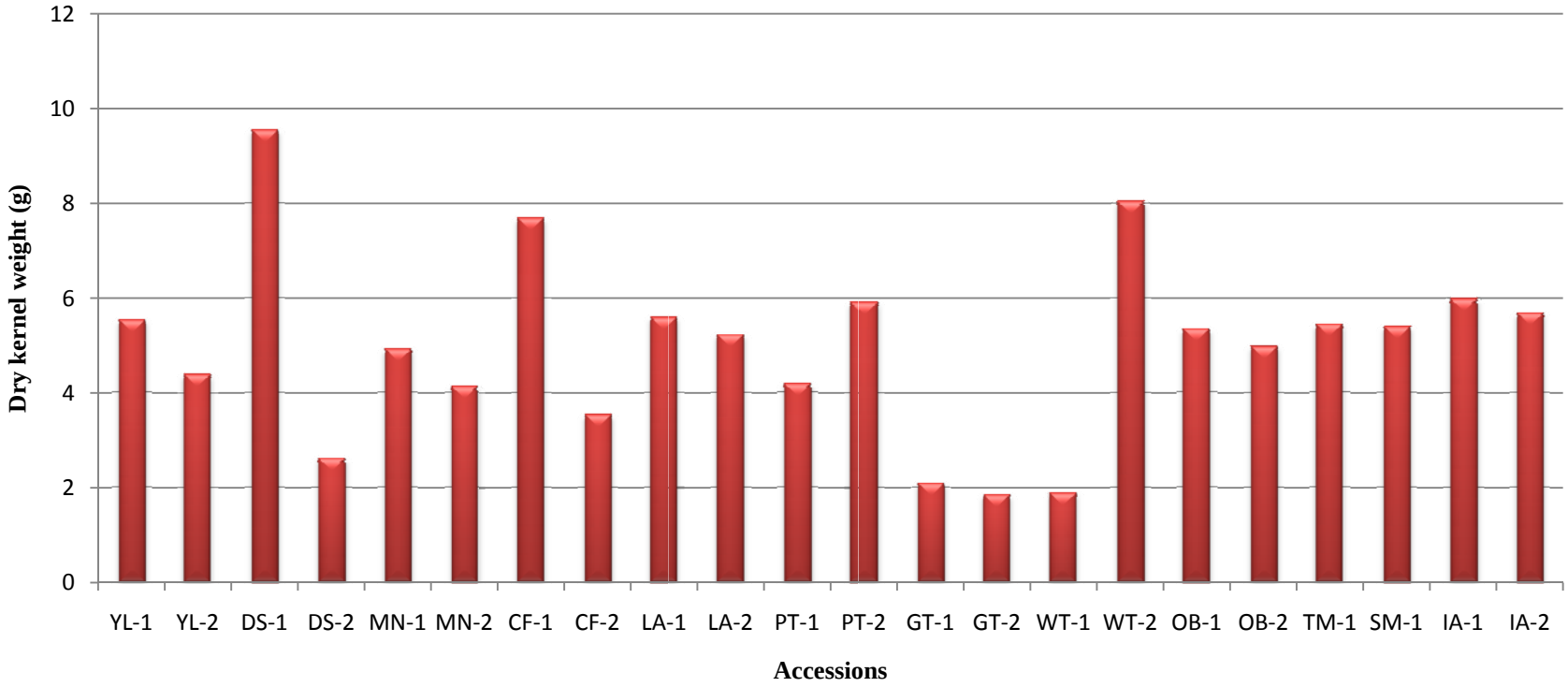


Fig 5.5 Dry kernel weight in unique accessions of nutmeg

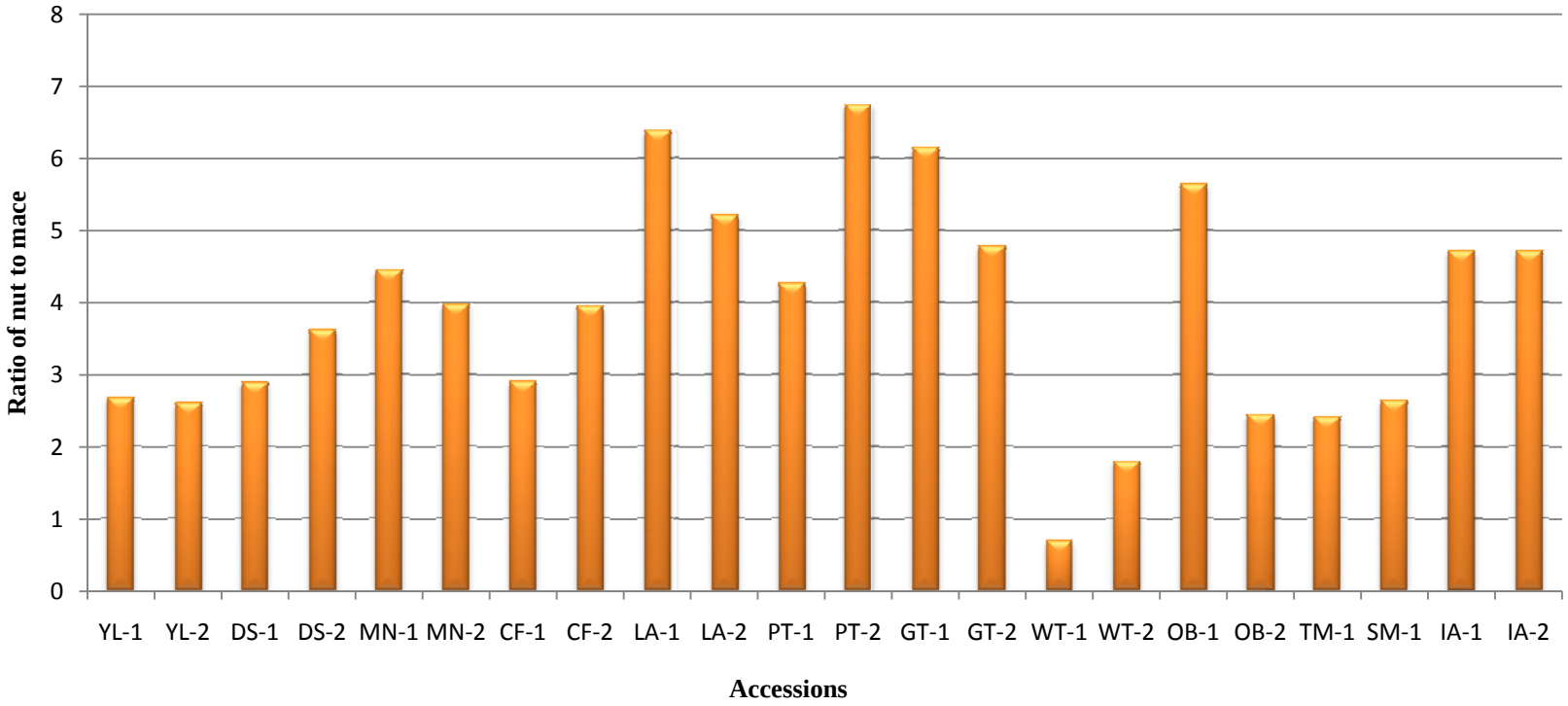


Fig 5.6 Ratio of nut to mace in unique accessions of nutmeg

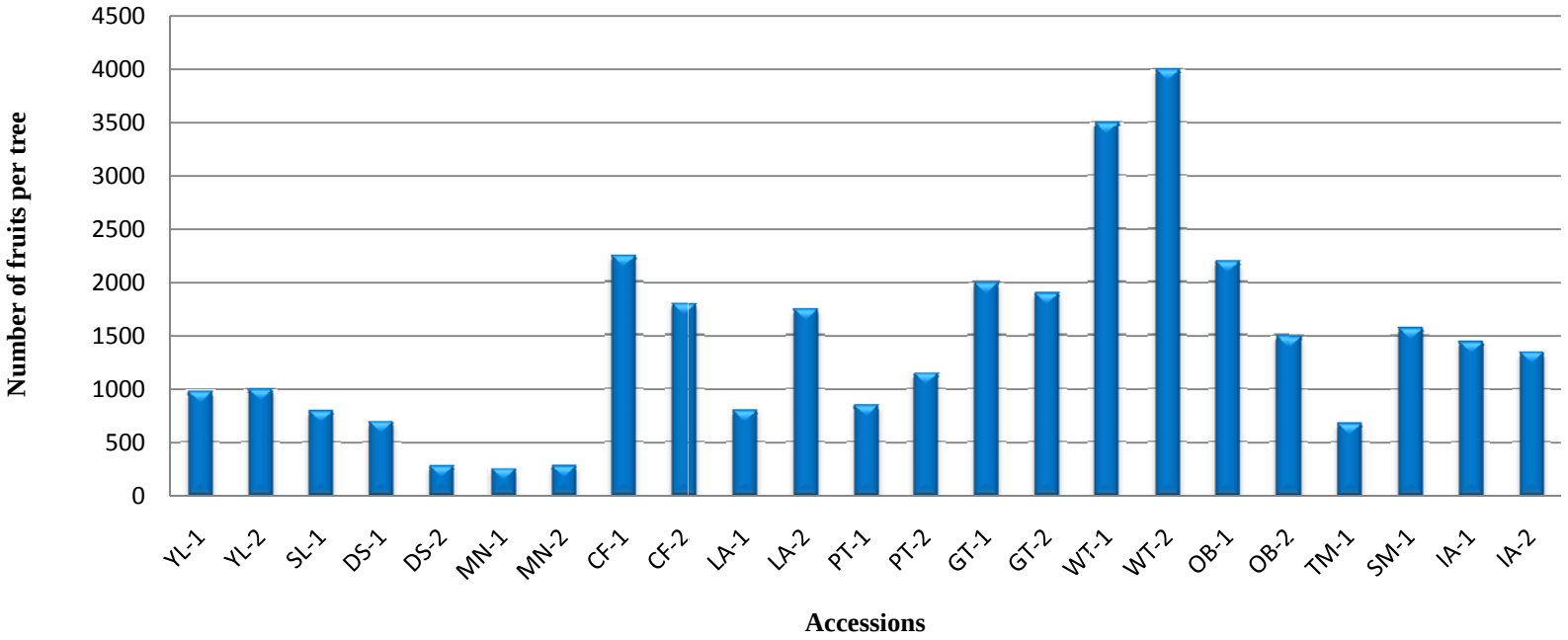


Fig 5.7 Number of fruits per tree in unique accessions of nutmeg

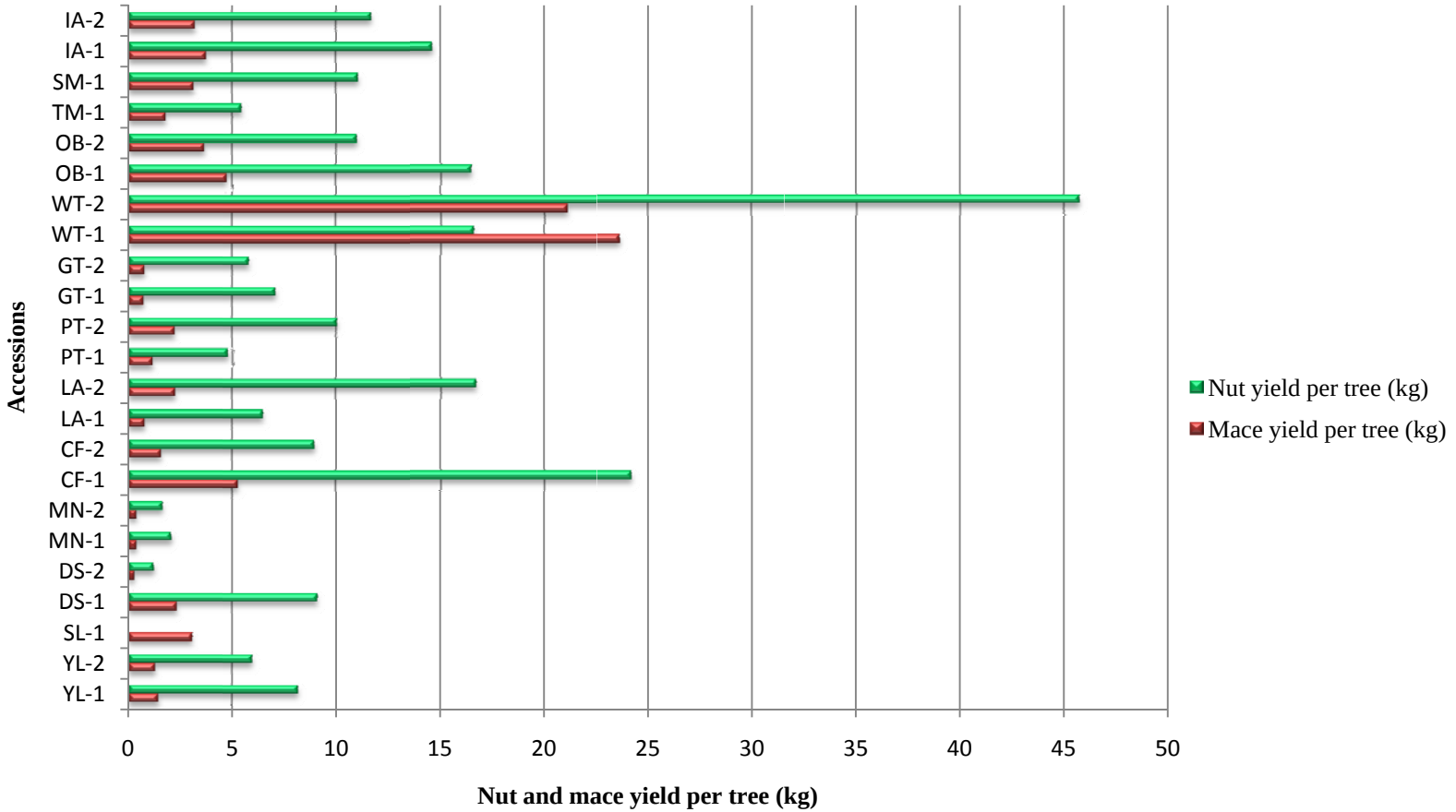


Fig 5.8 Nut and mace yield per tree in unique accessions of nutmeg

5.14.2 Biochemical parameters

5.14.2.1 Biochemical parameters of mace and kernel

The mace volatile oil recorded a maximum value of 16.73 per cent in the unique accession SL-1, whereas the kernel oil recorded a maximum value of 8.33 per cent in GT-1 (Fig 5.9). The mace oil content in SL-1 was estimated to be 16.72 per cent, which was highest among all the unique accessions. Kumar (2012) claimed that North East Indian nutmeg samples had higher volatile oil content than the Kerala nutmegs. Burubai *et al.* (2009) concluded that proximate analysis of African nutmeg seeds had 29.1 per cent volatile oil and 8.3% acid content

The mace oleoresin was highest in WT-2 (37.04%) and kernel oleoresin was highest in MN-2(45.06%). Oleoresin content in kernel varied from 18.59 to 36.20 per cent as reported by Vikram (2016). Naveen (2013) concluded that under different processing methods, the oleoresin content in nutmeg varied from 22.09 to 29.09 per cent . Also he reported that fixed oil extracted from the kernel of accessions varied from 17.79 to 44.80 per cent .The butter content recorded in the present study was highest in CF-1 (43.95%) (Fig 5.10).

Extraction of volatile oils from both kernel and mace in wild nutmeg accessions were very difficult, only a trace of non quantifiable oil layer was observed in accession WT-2. Accession WT-1 was not showing even trace amount of oil, as the fixed oil could not be extract from WT-1. The kernel was not properly filled in this accession and this could be the reason why fixed oil was not recovered from kernel. Bhat and Kaveriappa (1998) concluded that nonvolatile oil content in *Myristica fatua* var. *magnifica* (an endemic species) was about half that of nutmeg, which is in accordance with the findings of the present study.

GC MS profiling studies of mace and kernel oil revealed the percentage composition of mace and kernel oil. The four major constituents in both mace and

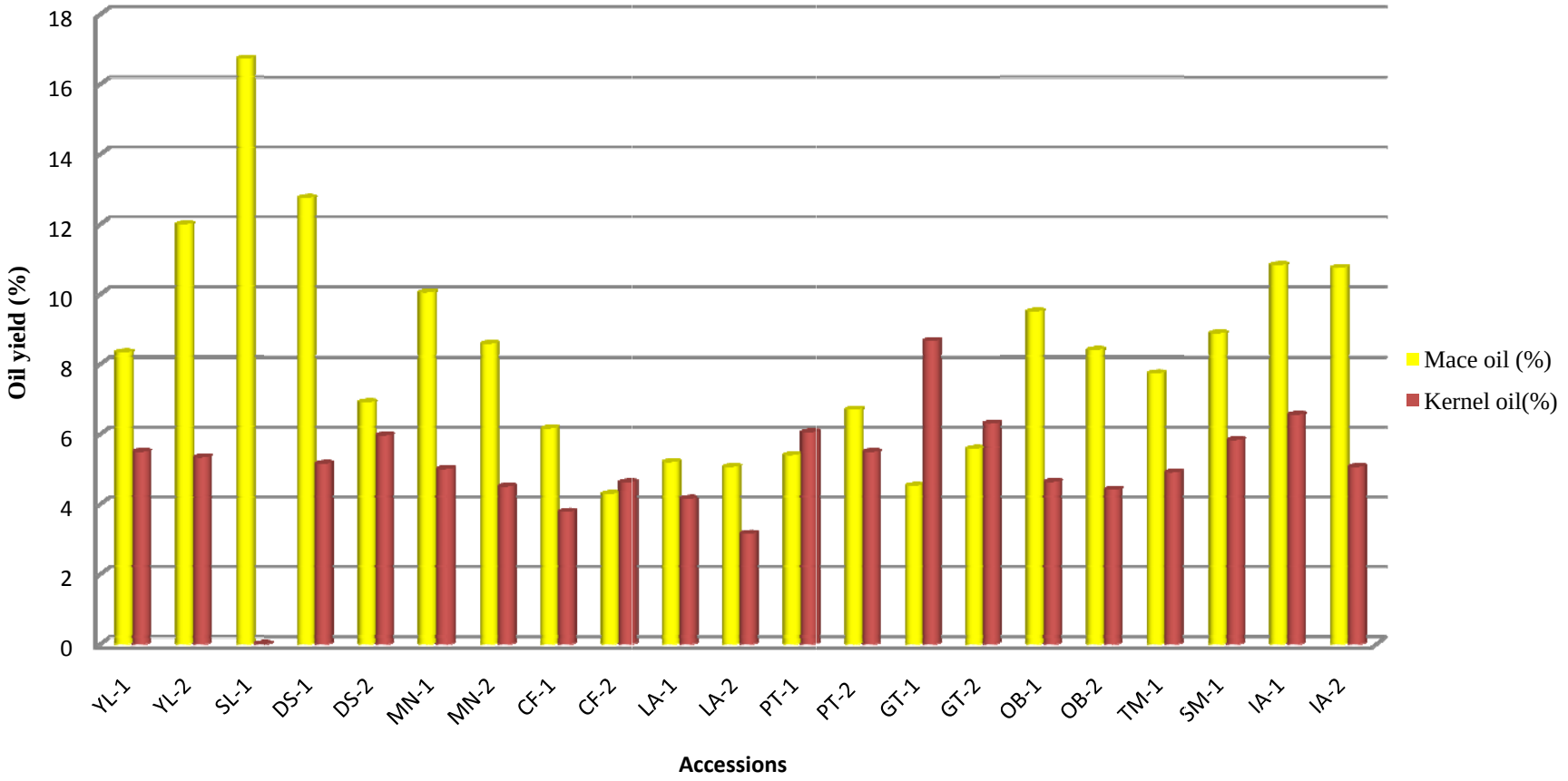


Fig 5.9 Volatile oil content in unique accessions of nutmeg

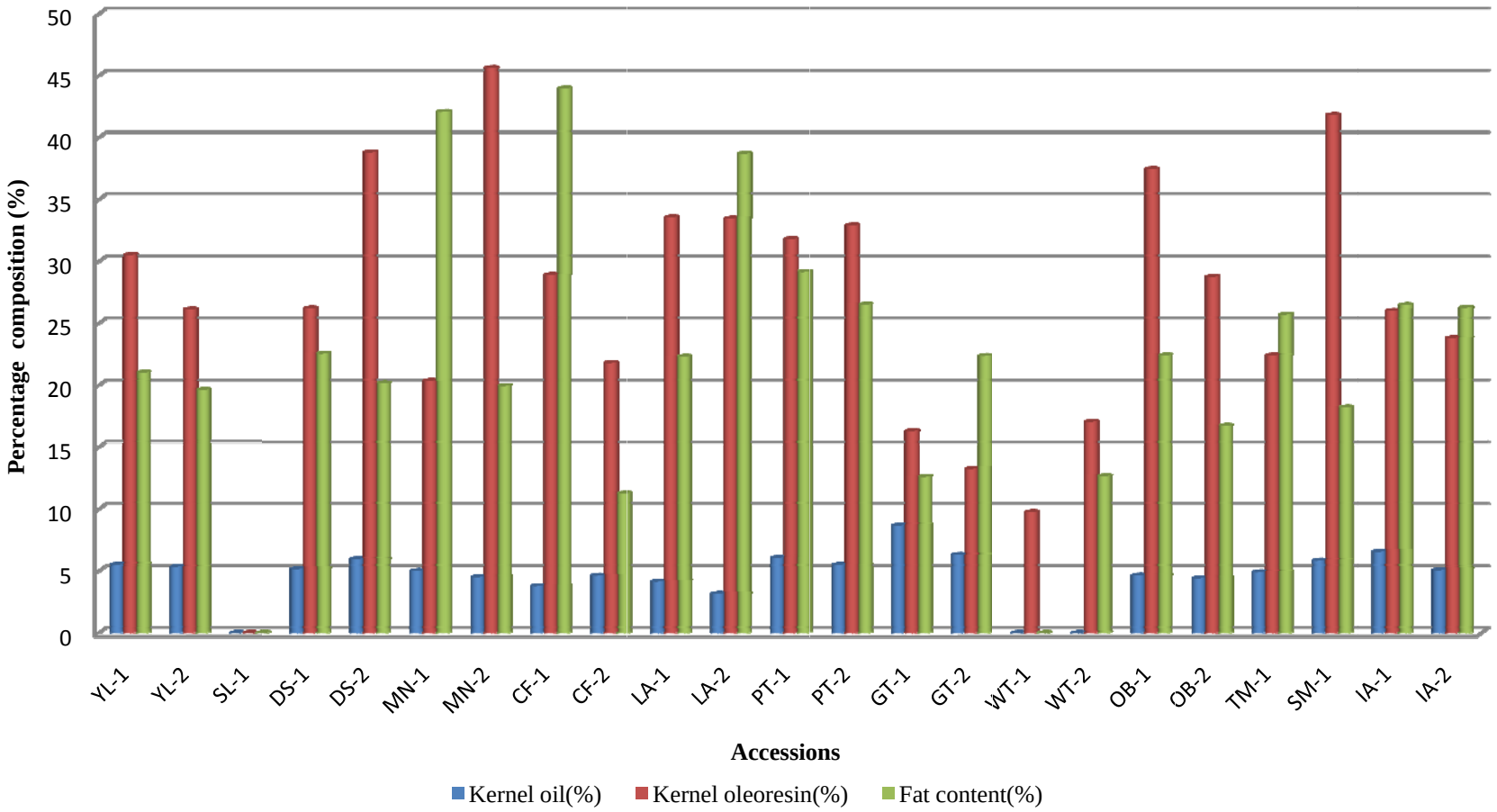


Fig 5.10 Oil, oleoresin and fat content of kernel in unique accessions of nutmeg

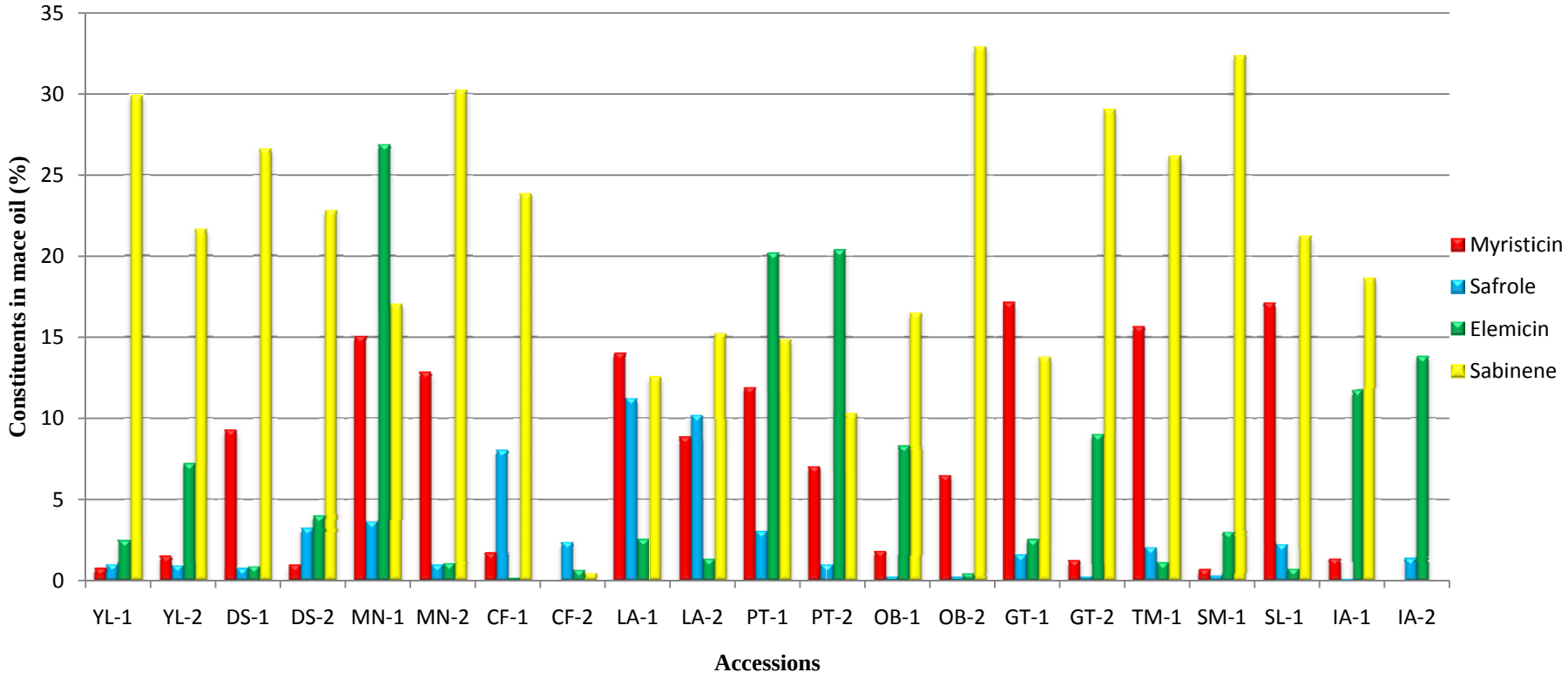


Fig 5.11 Major constituents of mace oil in unique accessions of nutmeg

kernel oil was myristicin, elemicin, sabinene and safrole. The highest percentage composition in mace oil are as follows: myristicin 17.17%(GT-1); elemicin 26.89%(MN-1); safrole 11.23% (LA-1) and sabinene 32.94%(OB-2) (Fig 5.11).

Kernel oil contain highest content of myristicin of 15.85%(LA-2); elemicin 31.18%(MN-1); safrole 9.29%(CF-1) and sabinene 36.19%(MN-2) (Fig 5.12). Rema *et al.,* (2013) identified a nutmeg germplasm A9/71(IC-537220); INGR 10142 with high sabinene content. In a study,accessions A9/71 and A9/95 had high sabinene content and low myristicin, elemicin and safrole content.

GC MS analysis of volatile oil of nutmeg identified 48 components which included myristicin (36.53%), terpinen-4-ol (12.47%), myristic acid (8.27%), elemicin (4.96%), safrole (4.75%), γ-terpinene (4.56%), and methyleugenol (4.38%) (Rong and Tao, 2011).

Another study conducted by Mallavarapu and Ramesh (1998) revealed the composition of essential oils of nutmeg oil as alpha pinene (13.6%), beta-pinene (12.9%), sabinene (32.19%), limonene (4.0%), gamma- terpinene (3.9%) and terpinen-4-ol (7.2%); mace oil was composed of alpha terpinene (3.9%), gamma-terpinene (6.6%), terpinolene (3.3%),terpinene-4-ol (23.6%), sabinene (23.5%), eugenol(3.7%), limonene (3.9%) and elemicin (10.5%). The supercritical fluid extraction was done on nutmeg seeds and the volatile oil obtained was composed of myristicin (32.8%), sabinene (16.1%), α-pinene (9.8%), β-pinene (9.4%), β-phellandrene (4.9%), safrole (4.1%) and terpinen-4-ol (3.6%) (Piras *et al.,* 2012)

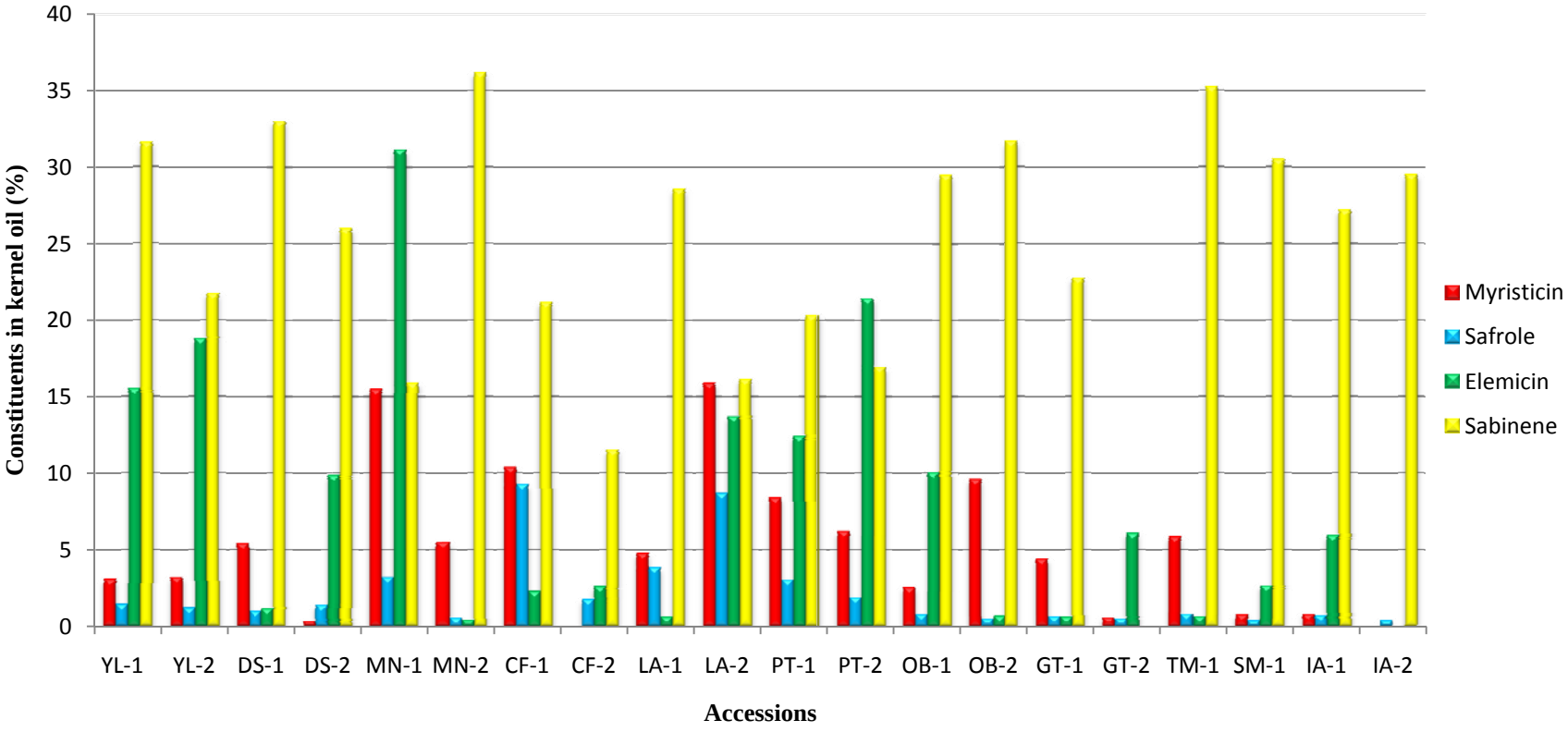


Fig 5.12 Major constituents of kernel oil in unique accessions of nutmeg

5.14.2.2 Biochemical parameters of nutmeg rind

Nutmeg rind is thick, yellow and fleshy with an average moisture content of 89.17 per cent as reported by Teena (2015). Gopalakrishnan (1992) reported a moisture content of 88 per cent in nutmeg rind. Highest moisture content recorded among the unique nutmeg accession was 91.09% (LA-1). Acidity content of pericarp was 2.5 per cent on fresh weight basis and 20.83 per cent on dry weight basis (Gopalakrishnan, 1992). Vikram (2016) reported a range of titrable acidity content of nutmeg rind as 1.28 to 1.92 per cent. The highest recorded acidity content of rind among the unique accession was 1.60% (MN-2) (Fig 5.13).

In an earlier study, Vikram obtained starch content with in a range of 0.30 to 1.23 (g/100g).Starch content was about 0.42g/100g on dry weight basis of pericarp (Gopalakrishnan,1992). The accession YL-2 recorded highest starch content of 38.62 mg/100g (Fig 5.14). Tannin content in nutmeg rind as reported by Gopalakrishnan (1992) was 2.42 g/100g. At the same time accession WT-1 showed highest tannin content of 3.82g/100g in rind (Fig 5.15).

Pectin an important parameter for determining the jelly grade can be extracted from nutmeg rind. It has been reported that pectin content in rind was 14.10 % on dry weight basis (Gopalakrishnan, 1992). In the present study pectin recorded the highest value of 11.66% in PT-1 (Fig 5.16).

5.14.2.3 Qualitative characteristics

Unique genotypes identified in this investigation can improve the species richness of nutmeg. The similarities if any noticeable as regards any of the characteristics among the different unique accessions were assessed through UPGMA method.

At the very outset, a researcher usually judges a tree based on its most visually measureable qualitative characteristics. The qualitative characteristics

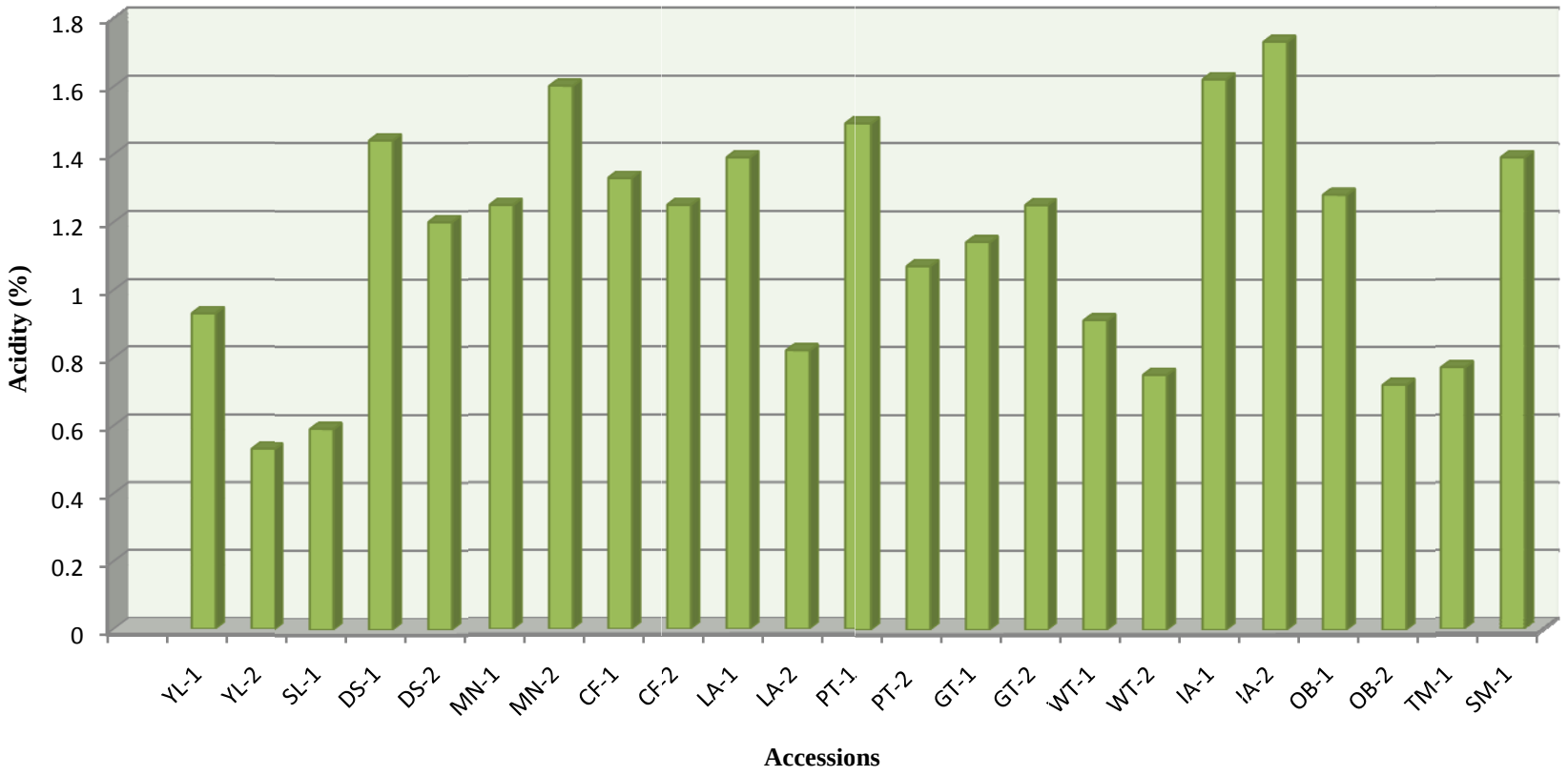


Fig 5.13 Acidity content of fruit rind in unique nutmeg accessions

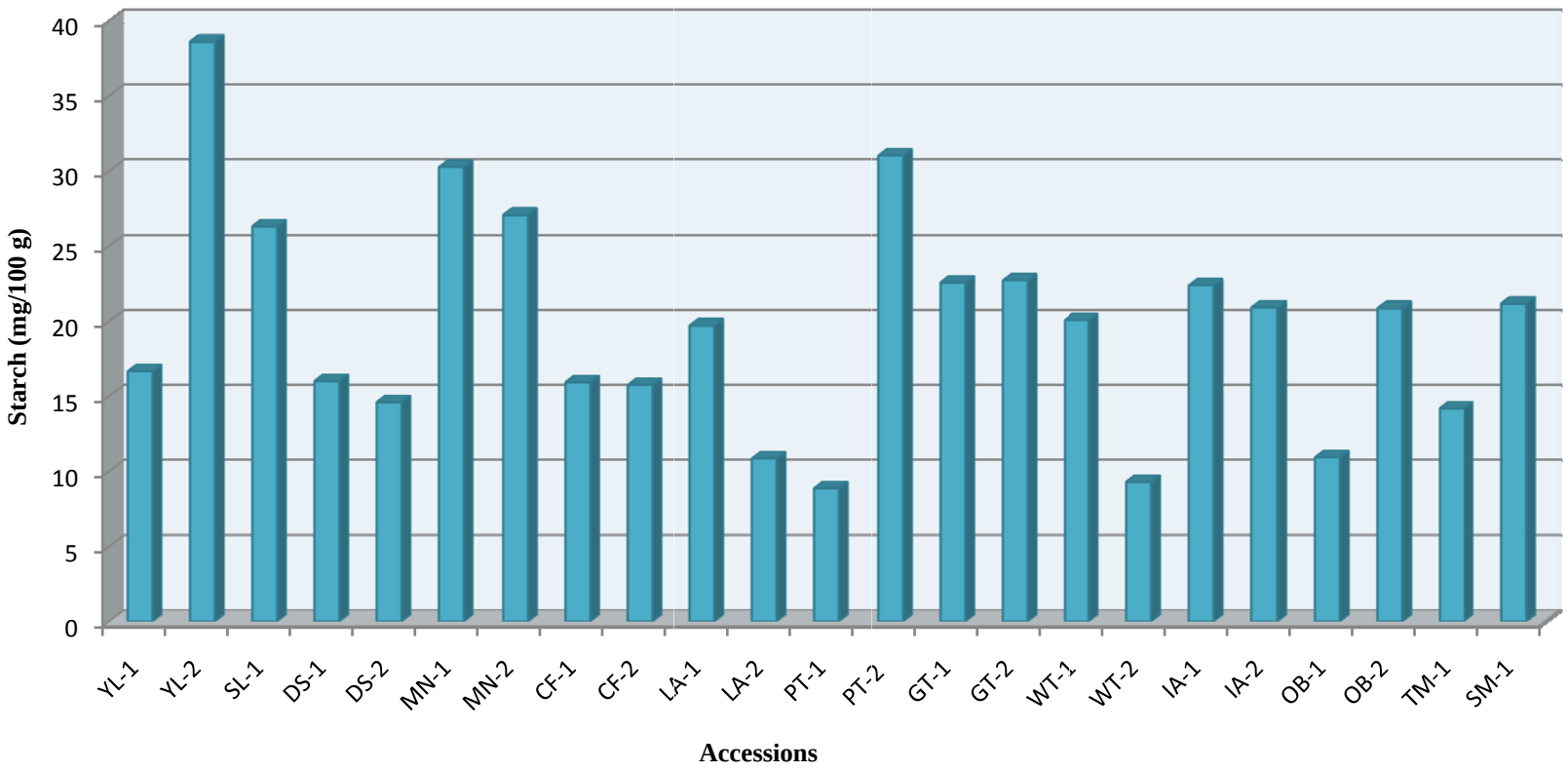


Fig 5.14 Starch content of fruit rind in unique nutmeg accessions

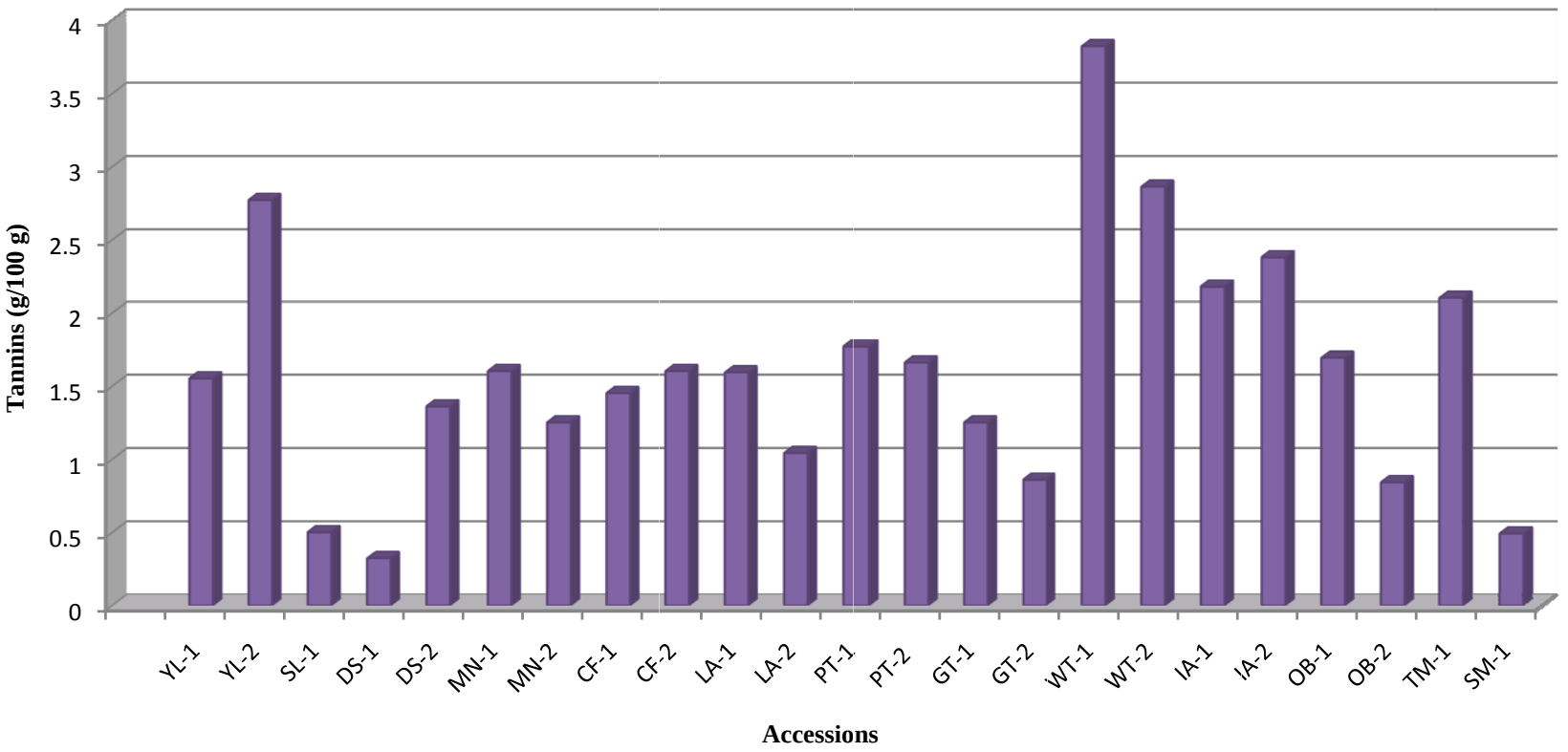


Fig 5.15 Tannin content of fruit rind in unique accessions of nutmeg

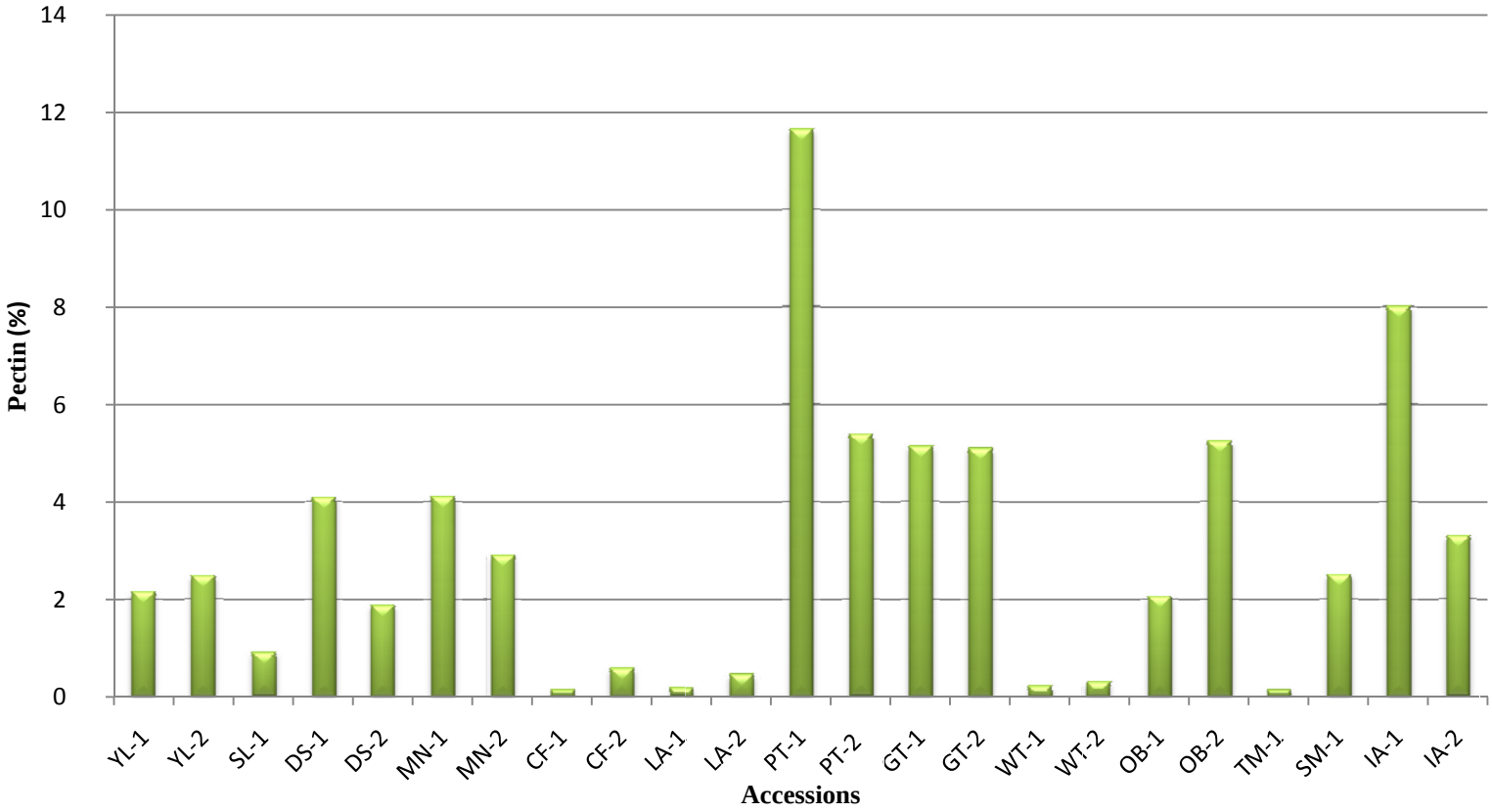


Fig 5.16 Pectin content of fruit rind in unique nutmeg accessions

observed were canopy shape, branching pattern, mature leaf shape, mature leaf colour and colour of flushes with respect to tree characteristics; season of flowering, inflorescence type, colour of perianth, colour of filament and colour of anthers with respect to flower characteristics; and fruit colour (fresh), mace colour (fresh and dry), nut colour (fresh and dry), kernel colour (dry), shape of mace, nature of fruit dehiscence with respect to fruit characteristics.

All the above said characteristics being qualitative in nature, the first step in the assimilation of these larger sets based on the similarities is through the qualitative clustering technique using NTSYS package 2.2. The result of such an analysis resulted in twelve clusters. The most frequently occurring characteristics within as also the clusters were pyramidal canopy shape, spreading branching pattern, elliptic shaped leaves with green colour on maturity and with greenish yellow flushes; with late flowering observed and the resultant flowers showed axillary raceme inflorescence type with creamy yellow coloured perianth. Seemingly creamy white filament colours with pale yellow coloured anthers were noticed in male flowers.

Nutmeg is an evergreen tree spice reaching up to a height of 10-20 m with spreading branches and oblong-ovate leaves (Shanmugavelu and Rao, 1977). Trees with narrowly pyramidal canopy will have less spread horizontally when compared with broadly pyramidal ones. The unique accessions PT-1 and PT-2 have much scope in high density planting system in the homesteads of Kerala. In an earlier study by Vikram (2016) reported that broadly pyramidal canopy shape dominates over other canopy shapes with regard to nutmeg. Canopy components like branching pattern, leaf and leaf characters eventually decide the fruit load on tree.

The economic resource of the trees namely fruit had light yellow colour with red coloured mace and yellow coloured mace at times. On drying and storage, the mace colour fades to scarlet red and yellow at times. Fresh nut colour was predominantly brown, which on drying turned light brown with the kernel also

following the same phenomenon. Yellow coloured mace with brown nut was a distinguishing one among the whole accession, a feature that other accessions achieve only on storage. Oval shaped mace was in common with triangular mace types noted at times. Nature of fruit dehiscence was normally two halved with a three halved and four halved dehiscence noticed at times in monoecious types and double seeded types respectively. Multiple splitting of rind on ripening is predominantly associated with monoecious and double seeded types than the normal types.

5.14.2.4 Summary statistics

A brief summary of the tree characteristics that were measured are: plant height (5m - 19 m),girth (18.8cm - 149.2cm), Canopy spread in N-S (4.3m -13.1m) and canopy spread in E-W directions (4.9m -14.7m), leaf length (8.6cm -14.28cm), leaf breadth (2.52cm - 5.68 cm), leaf area (15.05cm^2 - 49.09cm^2), number of orthotrops (0 - 45). The wide variation noticeable for all the characteristics is a pointer towards the extent of variability that has to be exploited for further crop improvement. In this context, wide variation that one may expect as regards the floral characteristics is only a natural conception assertive of the said hypothesis. The floral characters namely length and breadth of flower had variation as (6.35mm - 14.1 mm) and (4.20mm - 7.27mm). Similarly, the length and breadth of perianth (5.25mm - 10.51mm and 1.18mm -6.08mm).

The above discussions have vividly shown the variability of unique accessions with respect to the multi dimensional characters. If a one dimensional scale could be evolved at least based on a subgroup of characters, it will enrich the inference towards characterization. The ranking technique was evolved to achieve the above target. However, an accession namely SL-1 did not possess the features of the seed as the same was seedless in nature. This accession was christend as extra ordinary. The pooled rank scores of the rest 22 accessions ranged between 8.5 and 80.5. So a rational was developed using quartiles.

Based on the quartiles for the rank scores 4 more groups were added to the extra ordinary group and named as **Very good, Good, Moderate, Heteroscedastic** group according to their position in a linear scale; as those with a rank score below Q1, between Q1 and Q2, between Q2 and Q3, above Q3. Simple summary statistics namely Coefficient of Variation (CV) quantified the extent of variability of each and every characteristic for a particular group. The characteristic plant height was found varying among the constituent members for the **very good** and **good** group, whereas the girth at 140 cm height was totally inconsistent in all groups. Similarly much of the variation in canopy shape was noticed in the good group.

The studies especially relating to characterization of any member of the plant kingdom should be carried forward in a multi phased way as conservation of an endangered species, propagation of promising ones and exploitation of desirable characters through incorporation among the members already evaluated and characterized. The principles of breeding technique can definitely be applied in developing a genotype which may be robust in all characteristics, but in a phased manner. A concept diagram is drawn with these ultimate objectives.

5.15 Concept diagram

Twenty three accessions of nutmeg have been grouped mainly into five groups *viz* extraordinary, very good, good, moderate and heteroscedastic based on their pooled rank scores of fruit characteristics (Fig 5.17). Each group had their corresponding constituent members arranged in a linear scale. Accession SL-1 belonged to the Extraordinary group, which was highly unique among all the accessions due to its seedless nature. This rare accession needs to be conserved and also can be used for high mace oil recovery. As the acidity and tannin contents were significantly low among all the unique accessions, this could also be utilized for value addition of the rind. The reason for seedlessness has to be studied further.

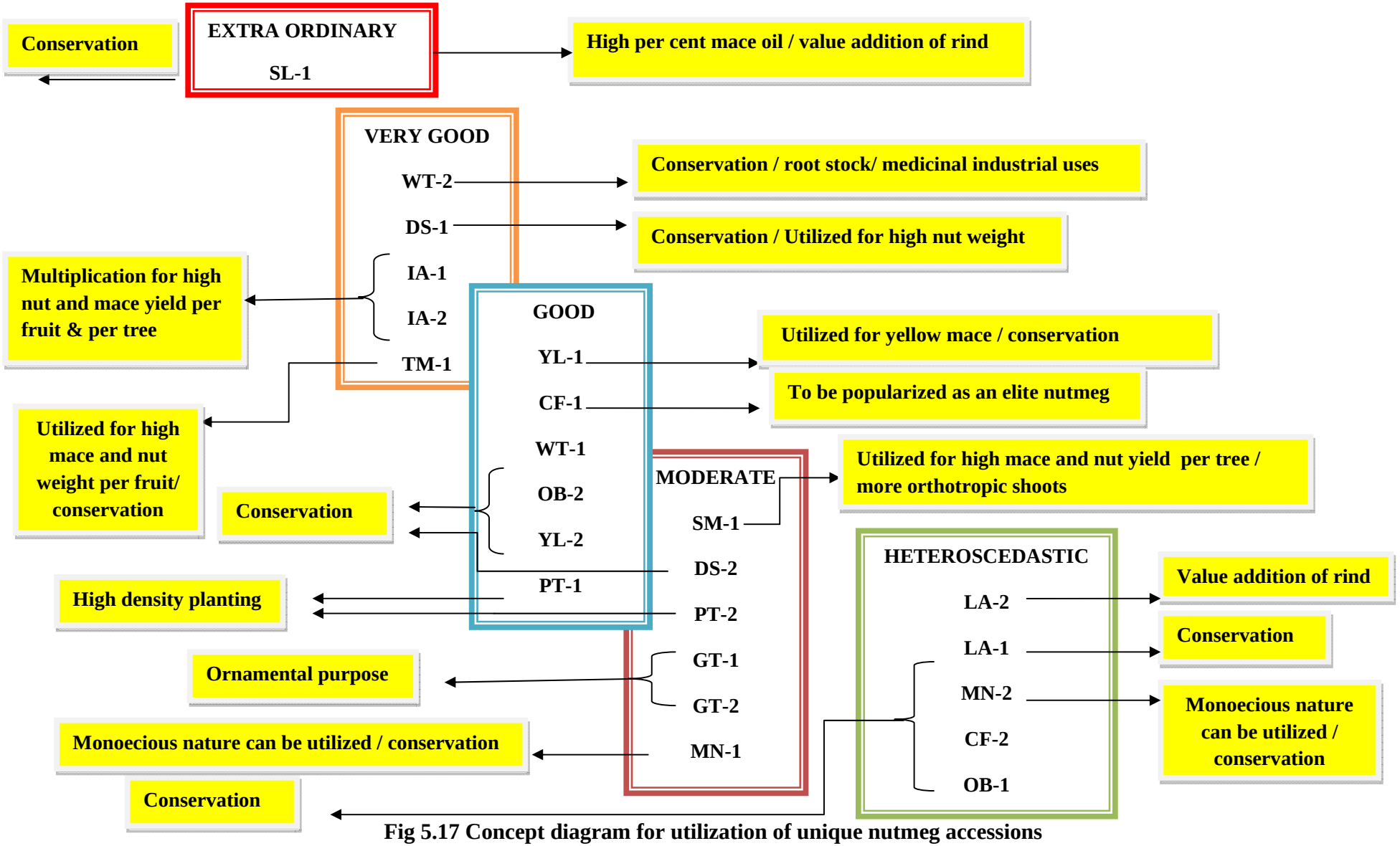
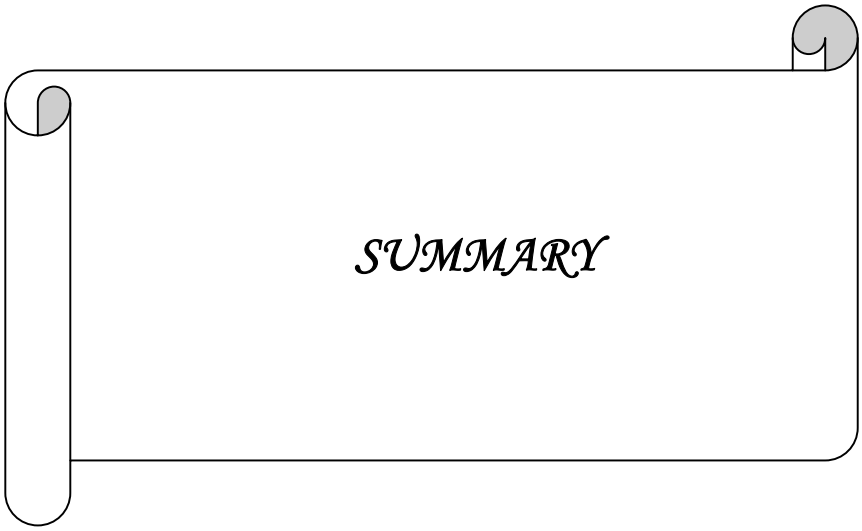


Fig 5.17 Concept diagram for utilization of unique nutmeg accessions

Very good group consists of WT-2, DS-1, IA-1, IA-2 and TM-1. Accession WT-2 (Wild type) can be used as hardy root stock which can perform well under water stress conditions and it can be conserved in the germplasm repository. Mace and kernel of WT-2 can be utilized by the ayurvedic medicine manufacturing industry and the paint industry. Double seeded nutmeg accession DS-1 needs improvement for obtaining high mace and nut yield per tree by increasing the number of fruits. Both the improved accessions IA-1 and IA-2 can be directly multiplied for cultivation due to their high mace and nut weight. Triangular mace nutmeg TM-1 characterized by high mace and nut weight per fruit could be improved.

Good group has six accessions *viz* YL-1, CF-1, WT-1, OB-2, YL-2 and PT-1. Yellow mace nutmeg accession YL-1 can be directly utilized for its yellow mace but for commercial cultivation the mace and nut yield per tree has to be improved. Accession CF-1 which possess all the desirable features of an elite nutmeg genotype can be commercially popularized as a variety. Accession WT-1, OB-2, YL-2 have only conservation value as they possess one or more of undesirable characters. Accession PT-1 is very much suitable for high density planting system, owing to its narrowly pyramidal (conical) canopy.

Moderate group consists of SM-1, DS-2, PT-2, GT-1,GT-2 and MN-1. Small leaf type accession SM-1 possessing high nut and mace yield per tree could be commercially utilized along with its high orthotropic shoot induction ability. Accessions DS-2 and MN-1 demand conservation. Both the grape type nutmegs fit well for ornamental purpose as they possess high number of small fruits. PT-2 characterized by narrowly pyramidal canopy can be utilized in high density planting system. The last group ie, heteroscedastic group consists of LA-2, LA-1, MN-2, CF-2 and OB-1. Low astringent accession, LA-2 with low acidity as well as tannin contents in the rind can be utilized for value addition purpose. The monoecious accession MN-2 could be utilized in breeding programme. Rest of the accessions MN -2, LA-1, CF-2 and OB-1 can be conserved in the gene pool.


SUMMARY

6. SUMMARY

The present study entitled “Collection and characterization of unique genotypes of nutmeg (*Myristica fragrans* Houtt.)” was carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara, Thrissur with the objective to collect and characterize the unique nutmeg accessions based on morphological and biochemical parameters. The survey spotted 21 unique nutmeg accessions that were having specific features as regards tree shape, sex, fruit type, astringency, mace, seed and wildness. The comparative evaluation of these 21 accessions has been achieved along with the observations on two most commonly spotted accessions, here in designated as improved accessions. The salient findings of the study are summarized as follows:

Unlike the normal nutmeg, two yellow maced accessions YL-1 and YL-2 were identified from Chalakudy and Kottayam districts. Yellow mace is a preferred characteristic that is rarely spotted in nutmeg and fetches premium price in the market.

Seedless nutmeg accession (SL-1) was spotted from Muniyara, Idukki district . Eighty per cent of fruits had rudimentary sterile seed inside the mace and rest twenty per cent fruits were totally seedless. The fruit colour in SL-1 was a peculiar yellowish light green with rough netted skin. SL-1 had a good measure of fresh mace content (9.74 g). The mace oil content in SL-1 was estimated to be 16.72 per cent, which was the highest among all the unique accessions. Chemo profiling of mace oil detected high myristicin and sabinene contents. Mace oleoresin was also significantly higher in SL-1. Rind of SL-1 possessed low acidity and tannin contents.

Two double seeded nutmeg accessions were collected from Pattikaad (DS-1) and Chalakudy (DS-2) regions respectively. Double seeded accessions possessed few male flowers also with large number of stamens borne in a central column.

Occurrence of abnormal flowers was noticed in these accessions. Fruit splitting was typically four halved in both accessions. GC-MS analysis of volatile mace oil revealed that one of the double seeded accessions contained highest amount of R-alpha pinene (DS-2).

The two monoecious nutmeg genotypes collected from Chalakudy and Pattikaad had umbellate cyme inflorescence with male and female flowers. Both the accessions had yellow coloured fruits which split open into three halves. Kernel oleoresin content was significantly higher in MN-1 among all the accessions. Few abnormal flowers were noticed in these accessions.

Clustering of fruits is not a common phenomenon in nutmeg. Two accessions CF-1 and CF-2 identified with cluster bearing habit were collected from Vaikkom and Chalampadam areas respectively. Accession CF-2 had few male flowers also. In CF-1 eighty per cent of the fruits were in three clustered, fifteen per cent two clustered and remaining 4-8 fruits per cluster. In CF-2, the clusters had 2-3 fruits per bunch on an average. CF-1 possessed all the desirable characters of an elite nutmeg and it was superior among all other unique accessions with respect to over all fruit characteristics and total yield.

The low astringent nutmeg accessions namely LA-1 and LA-2 were spotted from Chalakudy and Vazhikadavu regions. Considering the biochemical components of nutmeg rind, acidity and tannin contents were lower and pectin content was higher in LA-2, this could be used for value addition of rind. Even though LA-1 was initially designated as low astringent type, on final analysis it was other unique accessions which recorded comparatively lesser acidity and tannin contents than these low astringent types.

Two narrowly pyramidal nutmeg accessions PT-1 and PT-2 were collected from Chalakudy and Kallurkadu which can be utilized in high density planting system as it occupies less horizontal ground space coverage. Erect branching pattern

was observed in these accessions. Least canopy spread was noticed in these accessions in both directions. One of the accessions (PT-2) possessed highest nut to mace ratio among all the unique accessions.

The grape types GT-1 and GT-2 can definitely be exploited as ornamental types suitable for landscape horticulture. The grape nutmeg namely GT-1 and GT-2 were collected from Pattikkaad and Chalampadam areas. In accession GT-1, male flowers (4 per 10 cm^2) were also present in the inflorescence. The fruits were quite small when compared to the normal nutmeg, but number of fruits per tree was on the higher side. As the fruit itself is small, fresh mace and nut weight are also significantly low. Highest kernel volatile oil was recorded in GT-1 among all the unique accessions. Chemo profiling of mace and kernel oil revealed that kernel oil of accession GT-2 was devoid of sabinene and R-alpha pinene.

The two wild nutmeg accessions were collected from Pattikaad (WT-1) and Mullaringaad (WT-2) regions. The wild nutmegs identified as *M. malabarica* were monoecious in sex form. Another noticeable feature in wild nutmeg was the comparatively smaller flowers compared to normal nutmeg. Brown fruit colour with velvety texture was another typical feature of wild nutmeg, which on splitting exposed the attractive yellowish orange coloured oblong mace, tightly covering the brown coloured nut. Extraction of volatile oils from both kernel and mace in wild nutmeg accessions were very difficult, only a trace of non quantifiable oil layer was observed in accession WT-2.

Oblong shaped nutmeg is another interesting distinct feature due to the deviation in fruit shape from the normal nutmeg. Two oblong accessions namely OB-1 and OB-2 were collected from Chalakudy and Pattikkaad regions. Like wild nutmeg, oblong nutmeg also had oblong shaped nut and mace.

In nutmeg normally the mace shape varied among round, oval and oblong shapes, any deviation from this shape may be a distinct feature that needs attention.

Triangular shape of mace was much distinct when mace was attached to the nut. Triangular maced nutmeg labelled TM-1 was spotted from Koottala area. Mace and kernel oil recovery were comparatively less in TM-1. Chemo profiling of kernel oil revealed that sabinene content was second highest in TM-1 among all the unique accessions analyzed.

Small leaf type nutmeg labelled SM-1 spotted from Kanjirappally area had pyramidal tree canopy with spreading branching pattern. Even though the leaves were small, the foliage density was abundant. Leaf dimensions and area were only half that of normal nutmeg. However, the number of orthotropic shoots in SM-1 was found to be 45, which is a highly preferred characteristic for the vegetative propagation in nutmeg. Mace and nut yield per tree recorded were 3.13 kg and 11.09 kg respectively, which was comparable with improved accession. R-alpha pinene was absent in both mace and kernel oil of SM-1. Improved accessions from an earlier study namely IA-1 and IA-2 were collected from Chalakudy region and were used for a comparative analysis.

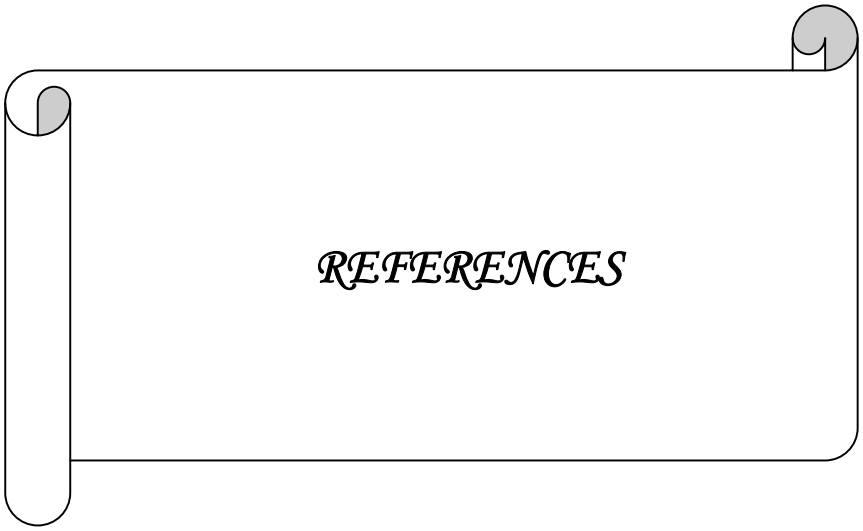
The data on fruit characteristics, which are indicators of the worthiness of any tree crop, was analyzed using Univariate General Linear model procedure. Accession DS-1 had high fruit weight, dry nut weight and dry kernel weight among all the unique accessions. Accession YL-2 possessed highest fresh pericarp thickness. Seedless nutmeg accession (SL-1) possessed high fresh mace weight and mace volume among all the unique accessions. Triangular mace nutmeg (TM-1) had highest shell thickness. Among all unique accessions, shelling percentage was highest in small leaf type nutmeg (SM-1). Narrowly pyramidal accession (PT-2) had high nut to mace ratio among all unique accessions. Wild type nutmeg possessed highest number of fruits. CF-1 was the best accession among all the unique nutmeg accessions belonging to *Myristica fragrans* Houtt.

After characterization of all the unique accessions, the similarity based on qualitative characters was achieved through UPGMA. Based on the dendrogram generated, 12 clusters were formed. The characteristics that were profusely spotted were pyramidal canopy shape, spreading branching pattern and elliptic green coloured mature leaves. Flush colour was predominantly greenish yellow. The season of flowering was mid and late. The most commonly noticed inflorescence type was axillary raceme with colour of perianth as creamy yellow mostly. Seemingly white filament colours were noticed with pale yellow colour anthers. The most economic part of most of the tree crops are their fruits. Fruit colour was predominantly light yellow with red coloured mace and yellow coloured mace at times. Brown coloured fruits with yellowish orange mace were noticed in wild nutmeg. On drying, the colour retention of mace was scarlet red in most cases with an exceptional one in yellow mace type. Nut colour was brown on drying.

As a contrast to the widely noticeable characteristics above, divergence in measure with respect to each and every characteristic was prominently noticed, asserting that a unique genotype can never slide to any other genotype. There was no definite pattern of concentration of most of the characteristics in one or the other of the unique accessions. Hence, a ranking method was adopted to evaluate the accessions on a rational scale. Ranks scores quantified the relative worthiness of the accession. The results based on the rank scores were further explored using the summary statistics, mainly quartiles. The quartiles of the data of the rank score were computed and the accession following under quartiles was designated as very good, good, moderate and heteroscedastic groups. In addition to this SL-1 was regarded as extraordinary due to its specific seedless nature. The result of this exploratory analysis pointed out the variability among different characteristics based on summary statistics of the different accessions. Highest variability in plant height, girth at 140 cm height, canopy spread in North -South and East- West direction among different groups were noticed in Good group. In case of leaf characteristics, moderate group

possessed highest coefficient of variation. Length of flower showed high variability in very good group. Breadth of flower varied significantly in good group. Length of perianth varied significantly in heteroscedastic group. Breadth of perianth varied significantly in good group.

A concept diagram was drawn ultimately with all these characters. From the concept diagram the unique accessions can be selected for utilization in different aspects according to their characteristics as indicated in the diagram. According to the concept diagram, all unique accessions are worthy of conservation. SL-1 and LA-2 can be utilized for value addition of rind. Accession GT-1 and GT-2 are best suitable for ornamental purpose. WT-2 can be utilized for water scarce areas as root stock. Double seeded nutmeg accession DS-1 needs improvement for obtaining high mace and nut yield per tree by increasing the number of fruits. Both the improved accessions IA-1 and IA-2 can be directly multiplied for cultivation due to their high mace and nut weight. Triangular mace nutmeg TM-1 characterized by high mace and nut weight per fruit could be improved. Yellow mace nutmeg accession YL-1 can be directly utilized for its yellow mace, but for commercial cultivation the mace and nut yield per tree has to be improved. Accession PT-1 and PT-2 characterized by narrowly pyramidal canopy can be utilized in high density planting system. Small leaf type accession SM-1 possessing high nut and mace yield per tree with its high orthotropic shoot induction ability could be popularized commercially. Accession CF-1 which possesses all the desirable features of an elite nutmeg genotype can be commercially popularized as a variety.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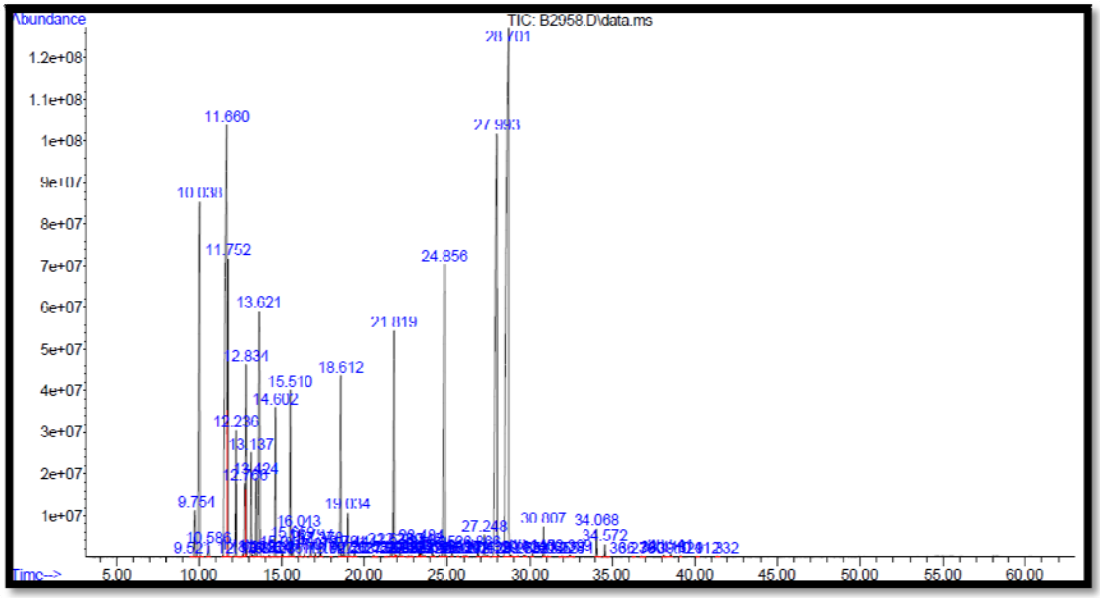
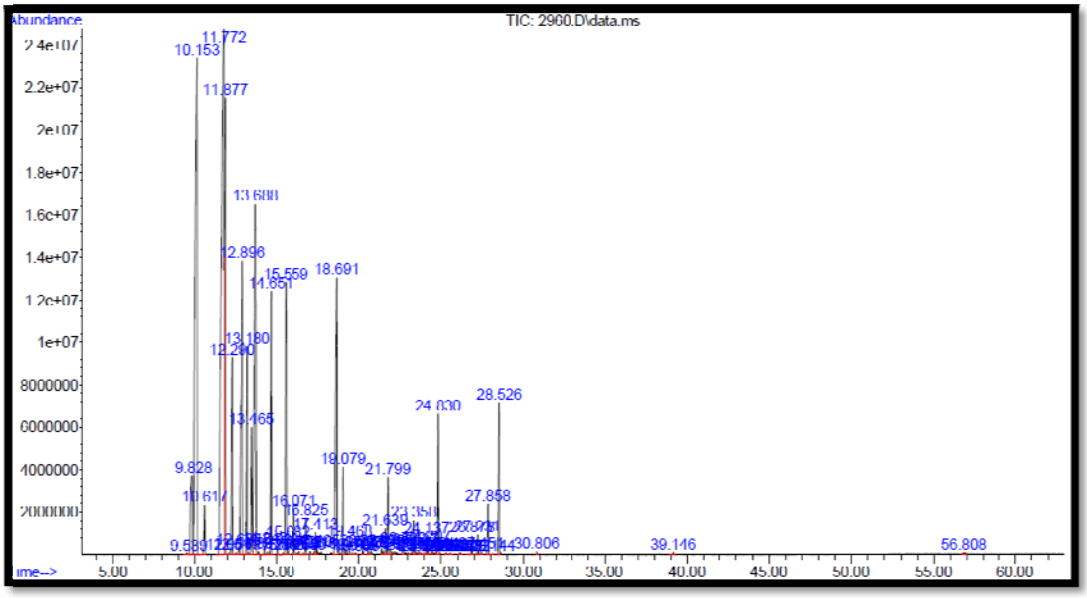
APPENDICES

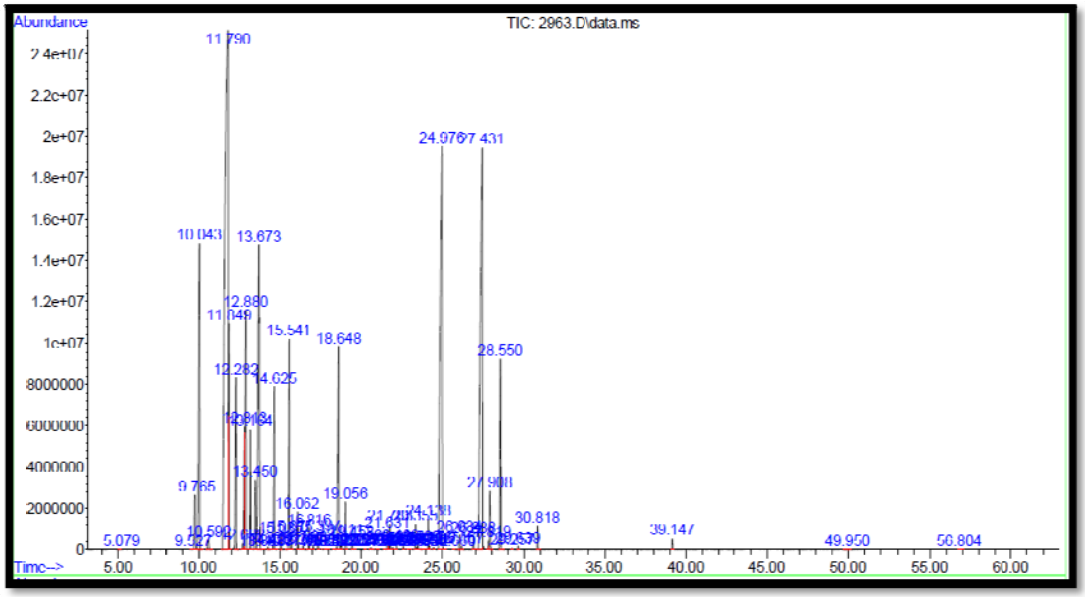
APPENDIX I : GC-MS profiles of mace volatile oil



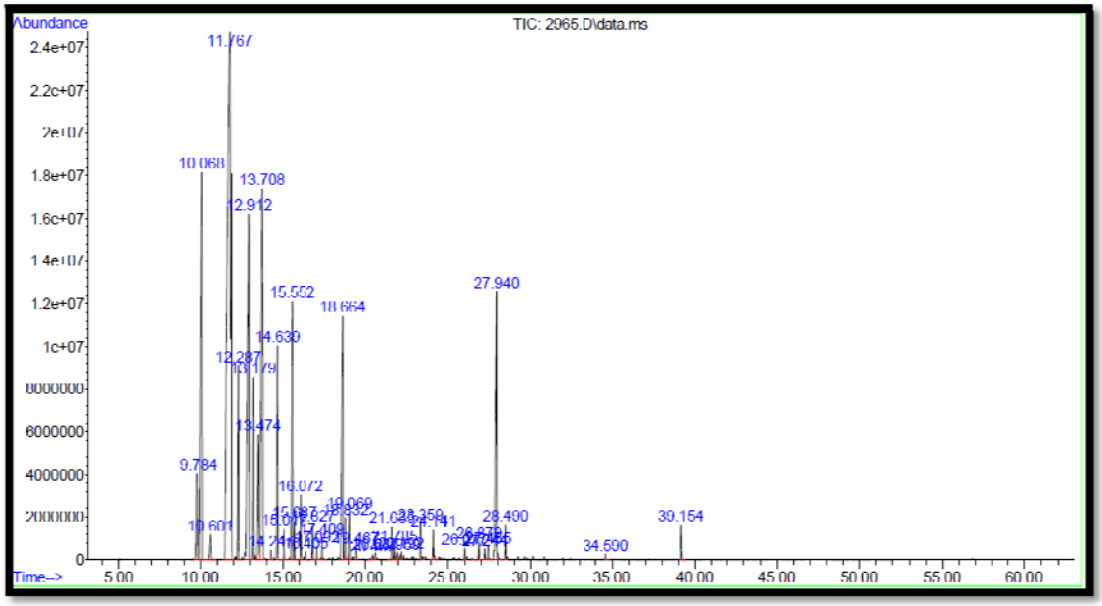
A1. GC-MS profile of mace volatile oil of MN-1



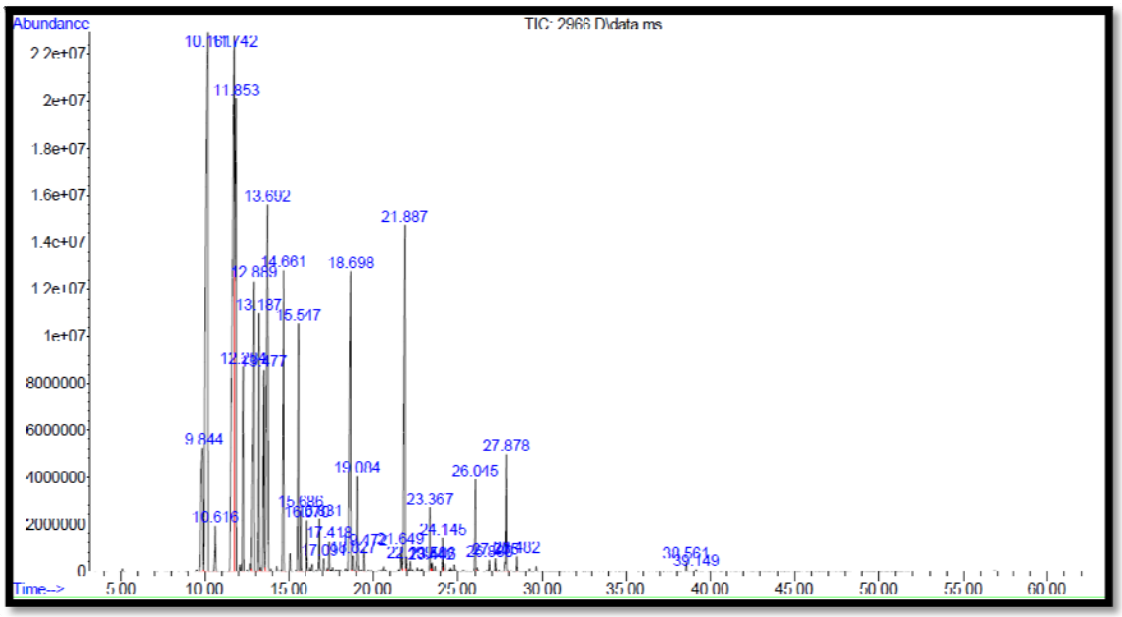
A2.GC-MS profile of mace volatile oil of YL-1



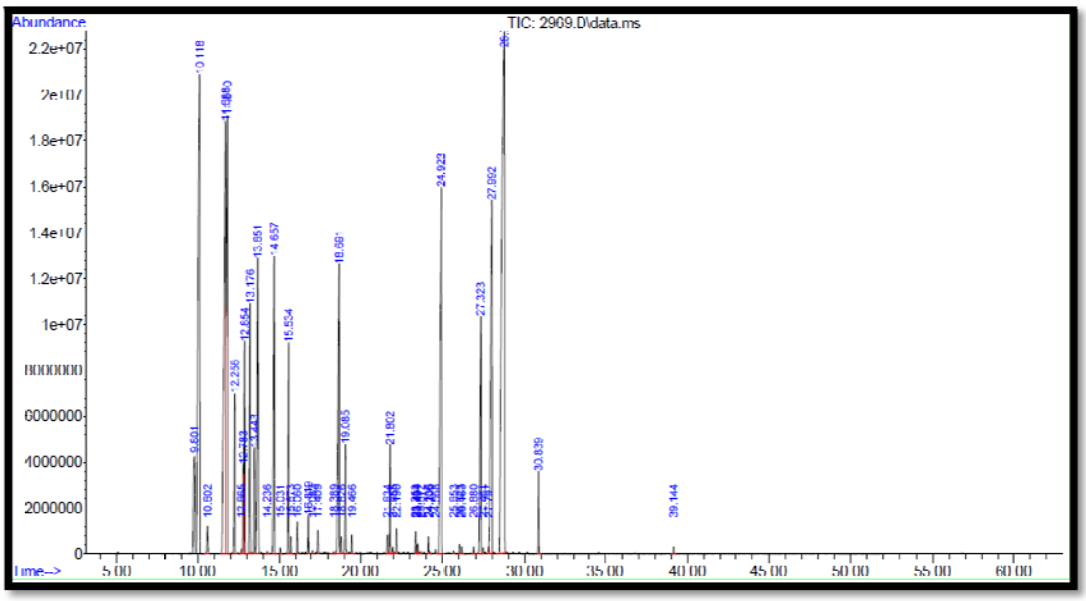
A3.GC-MS profile of mace volatile oil of SM-1



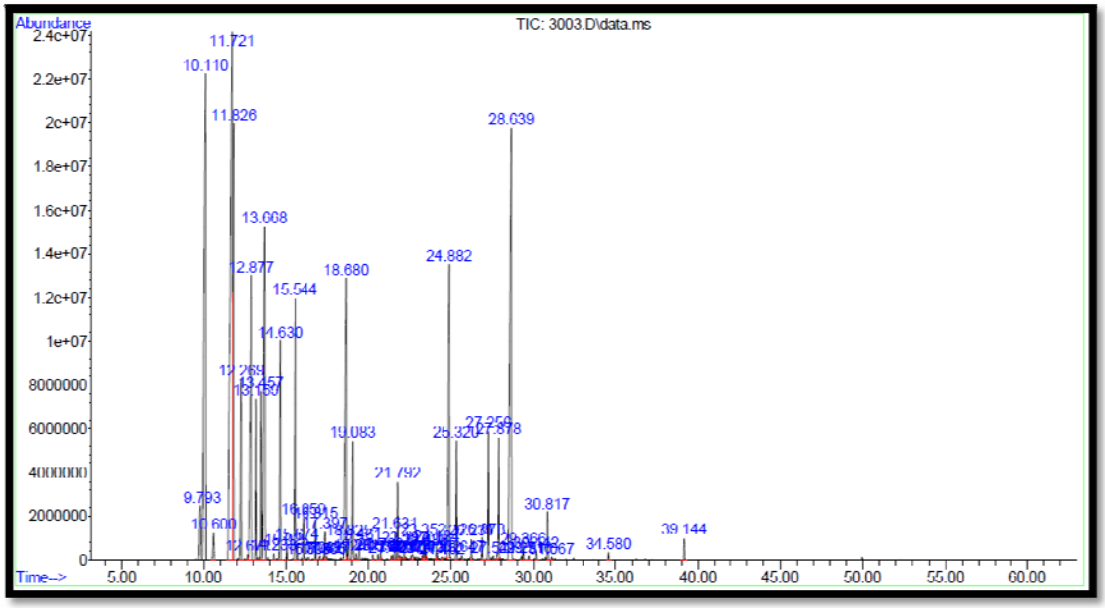
A4.GC-MS profile of mace volatile oil of OB-2



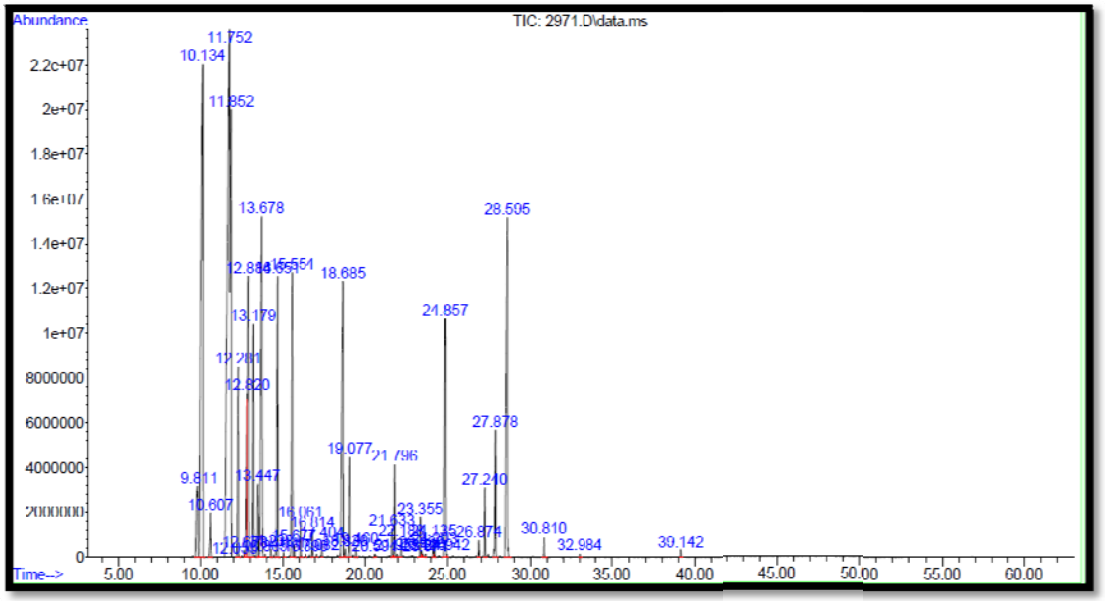
A5. GC-MS profile of mace volatile oil of CF-1



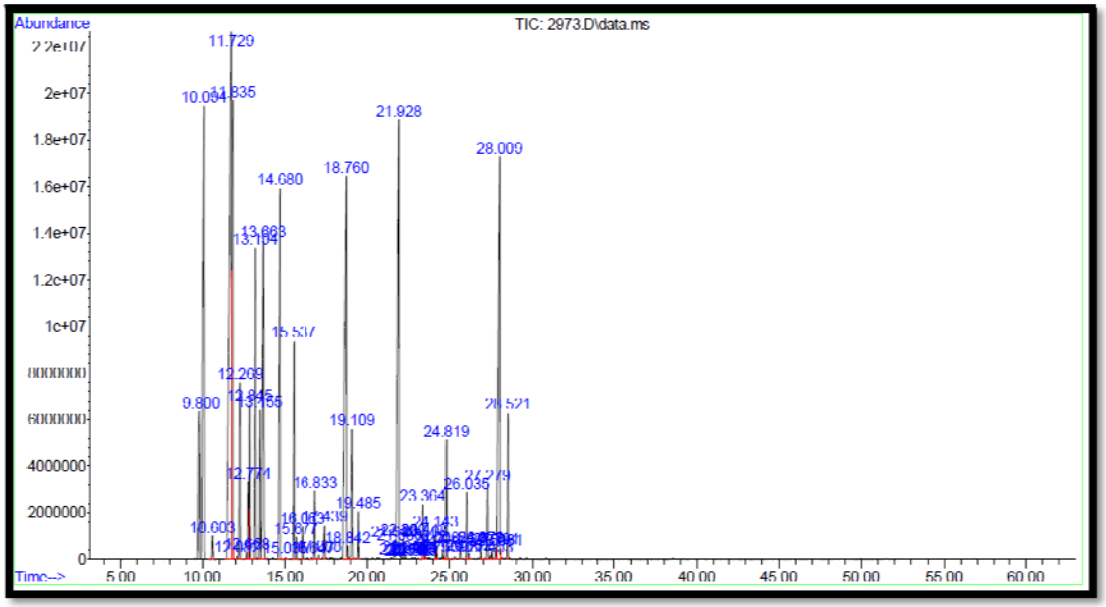
A6. GC-MS profile of mace volatile oil of PT-2



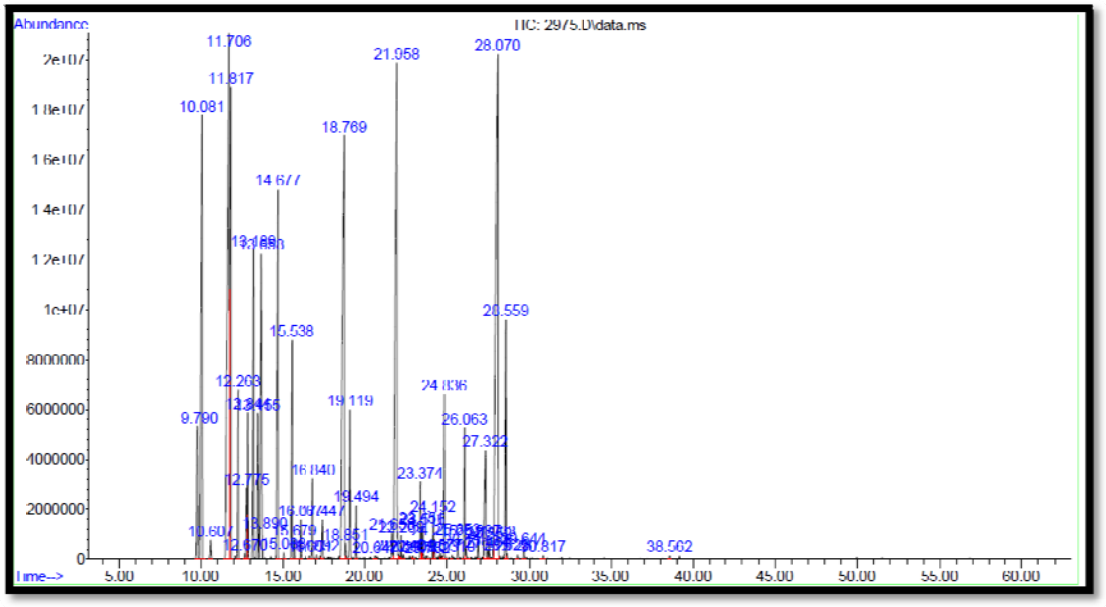
A7. GC-MS profile of mace volatile oil of IA-1



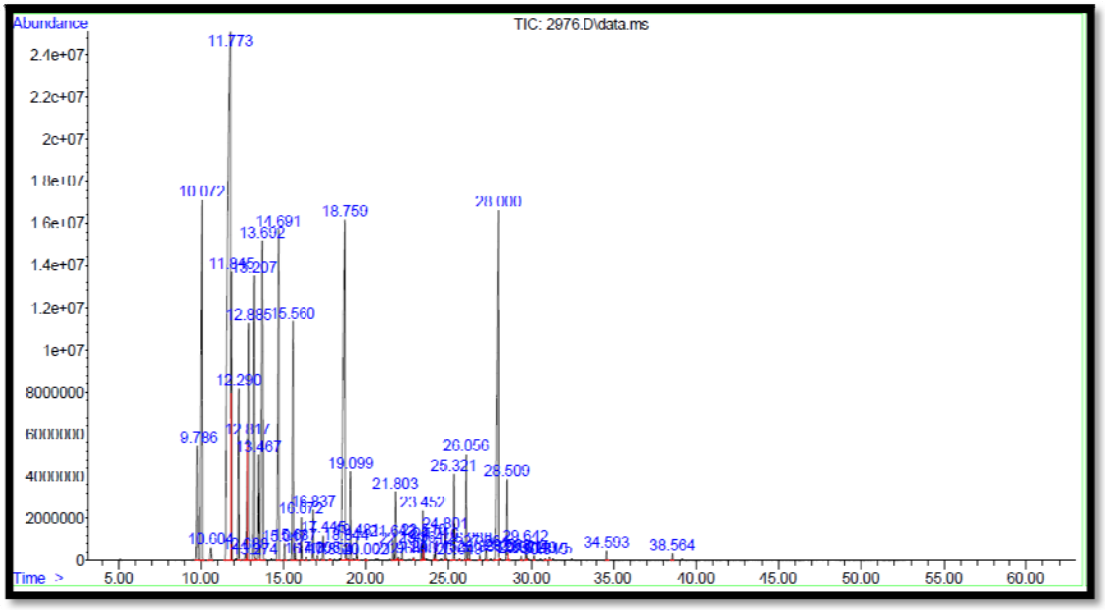
A8. GC-MS profile of mace volatile oil of YL-2



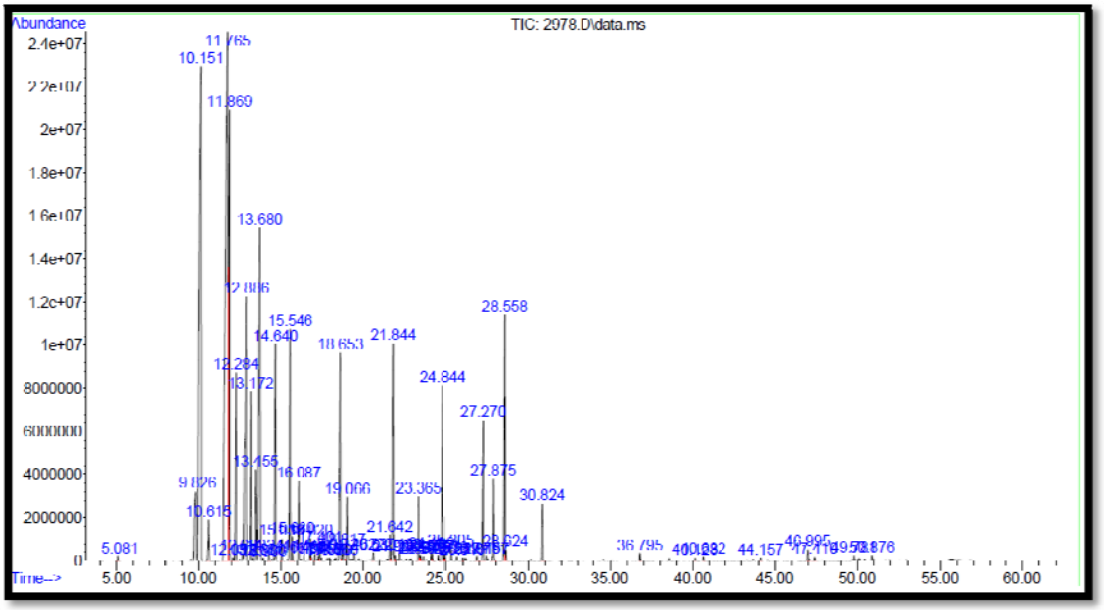
A9. GC-MS profile of mace volatile oil of LA-2



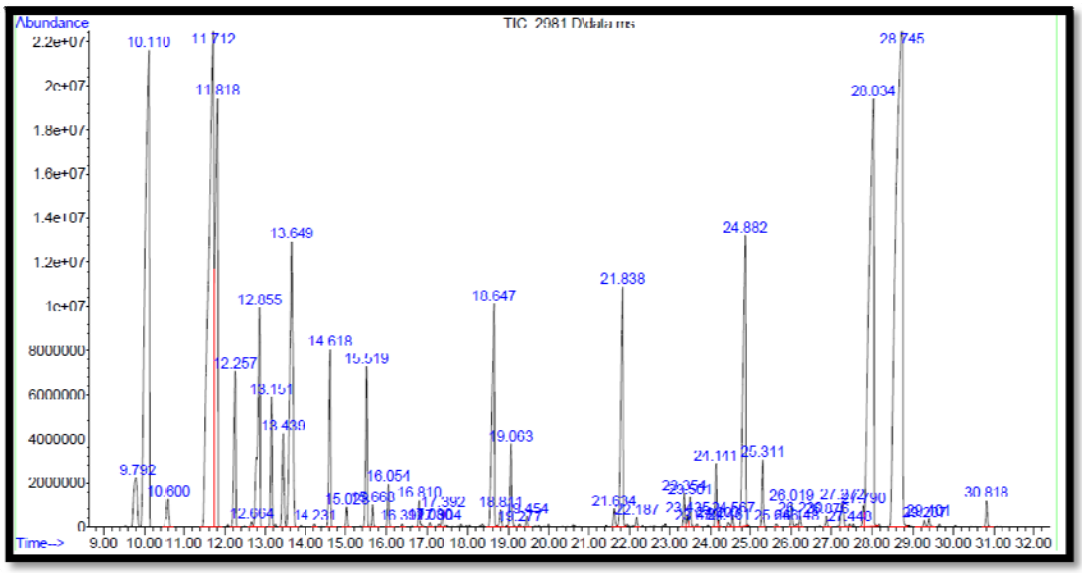
A10. GC-MS profile of mace volatile oil of LA-1



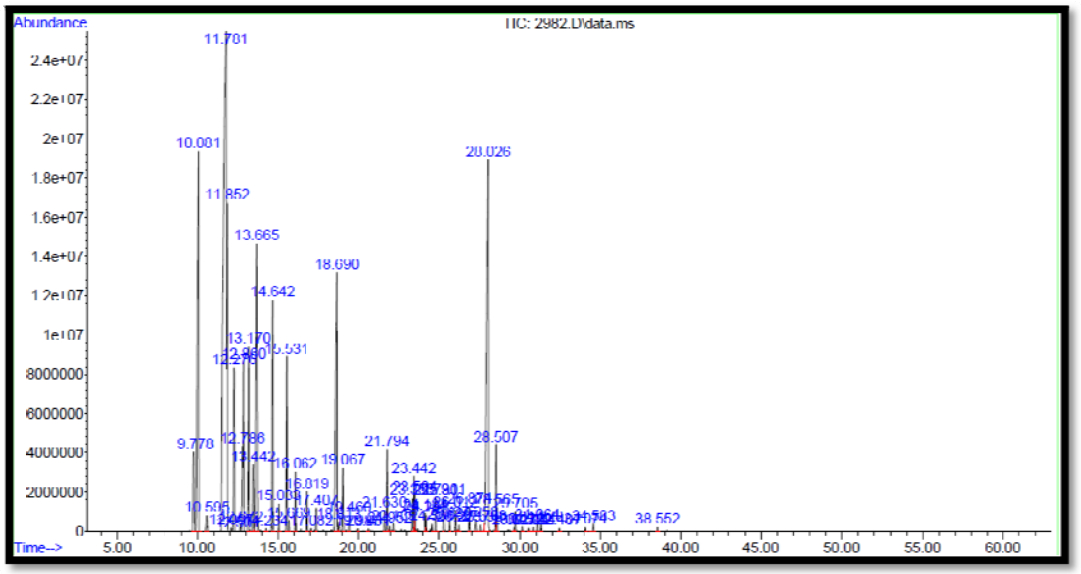
A11. GC-MS profile of mace volatile oil of DS-1



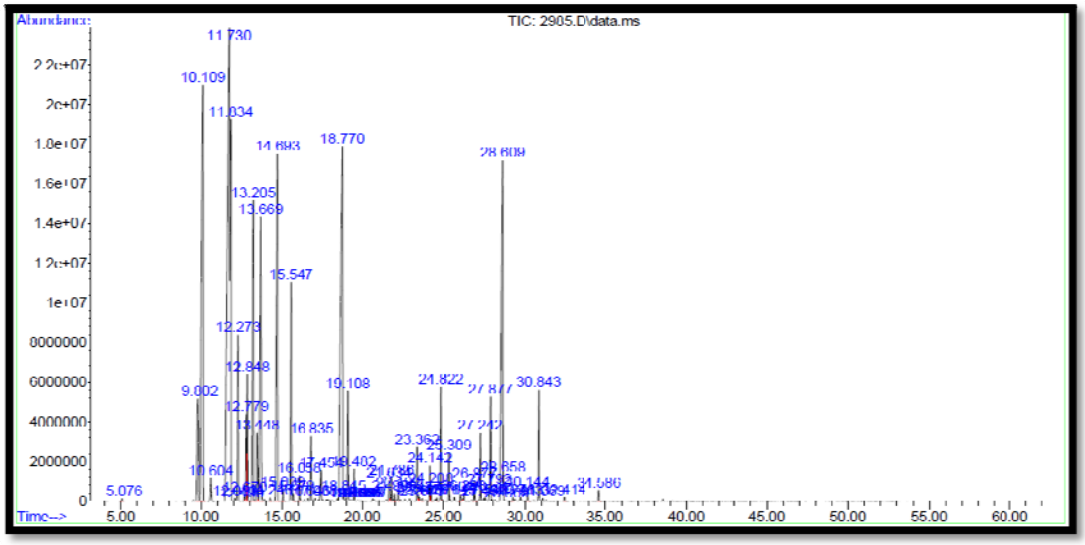
A12. GC-MS profile of mace volatile oil of DS-2



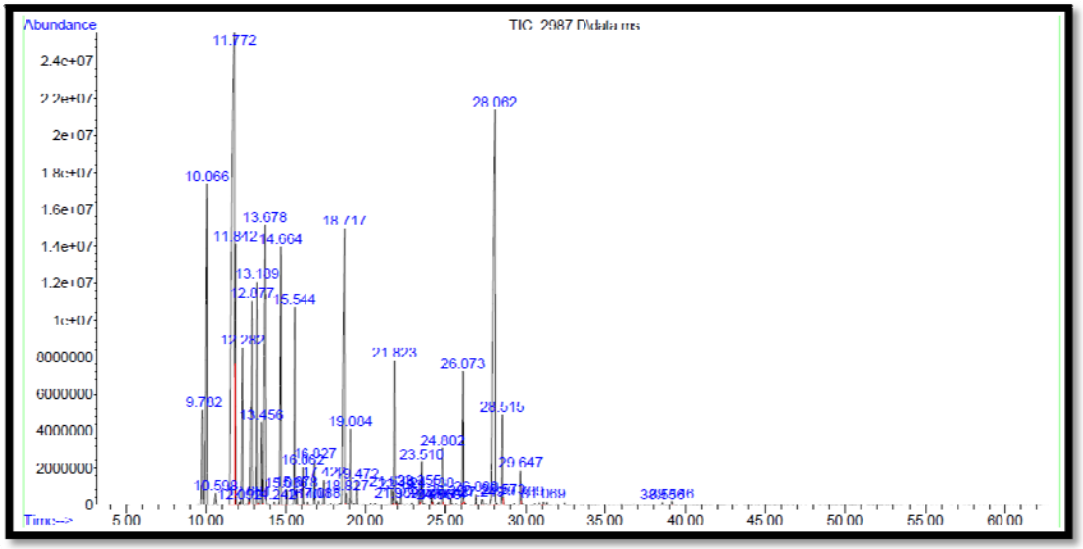
A13. GC-MS profile of mace volatile oil of PT-1



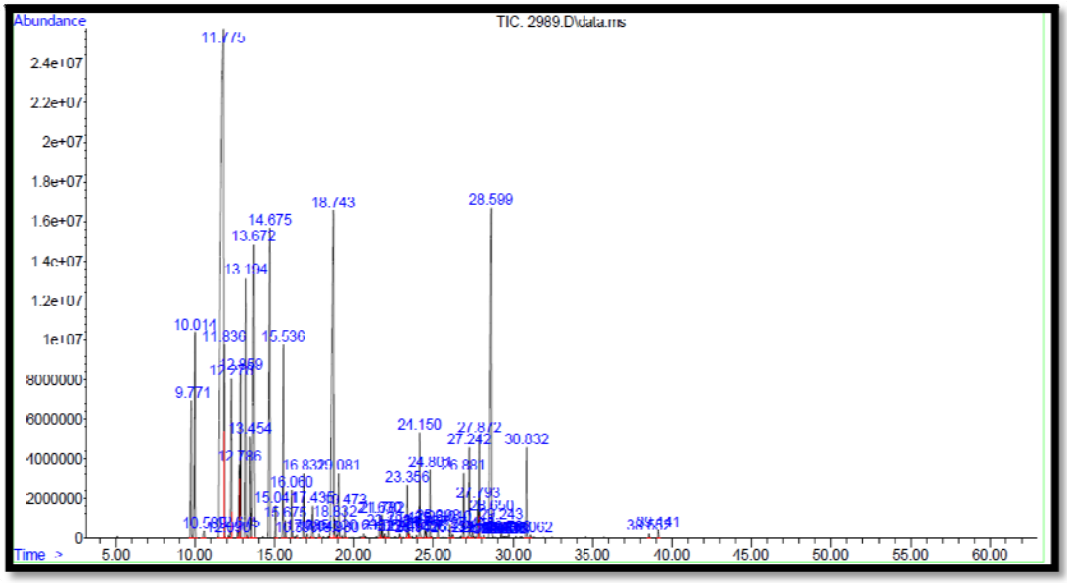
A14. GC-MS profile of mace volatile oil of MN-2



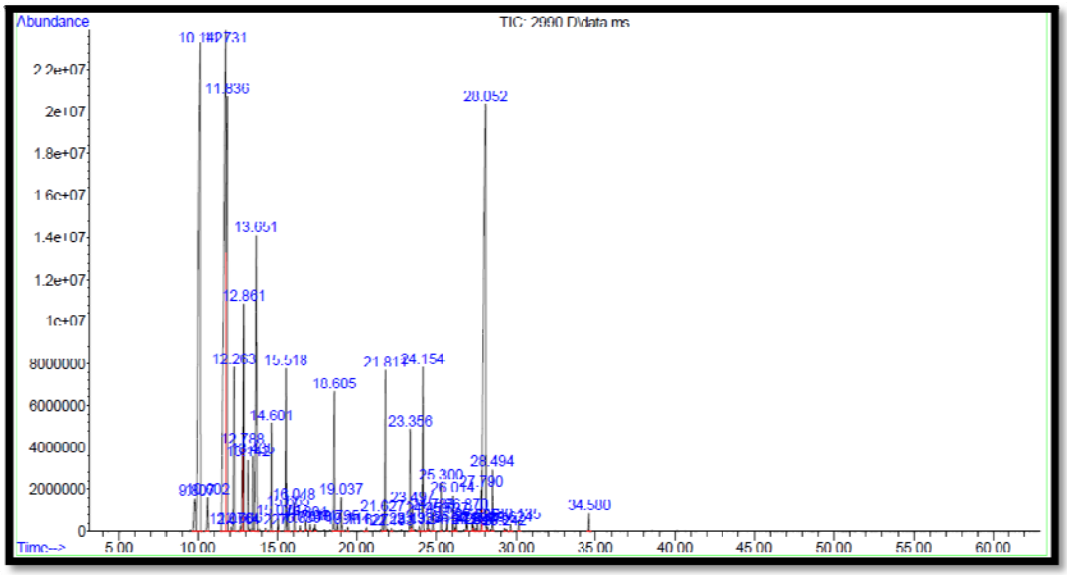
A15. GC-MS profile of mace volatile oil of OB-1



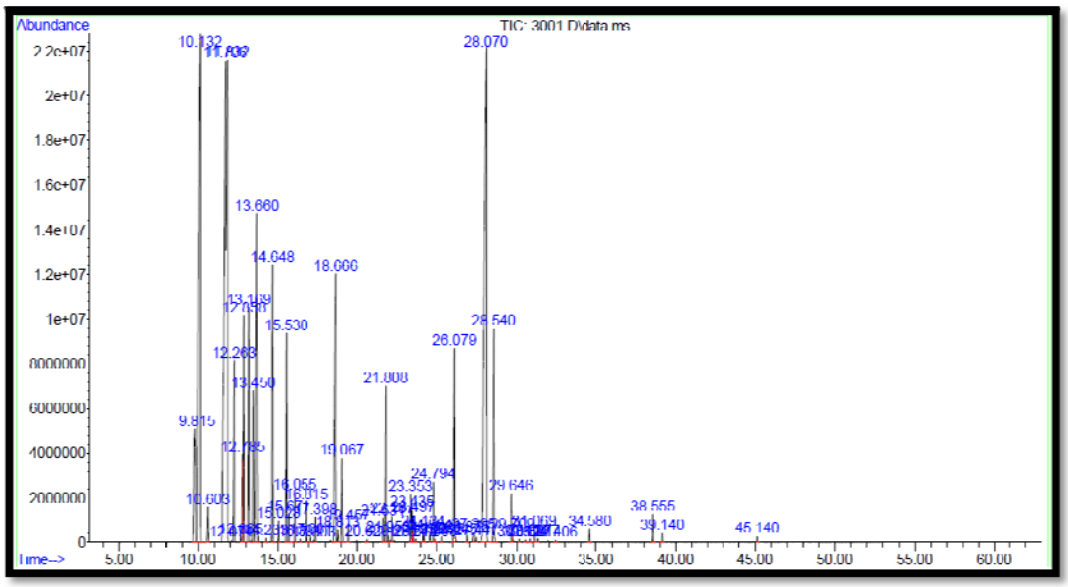
A16. GC-MS profile of mace volatile oil of LA-1



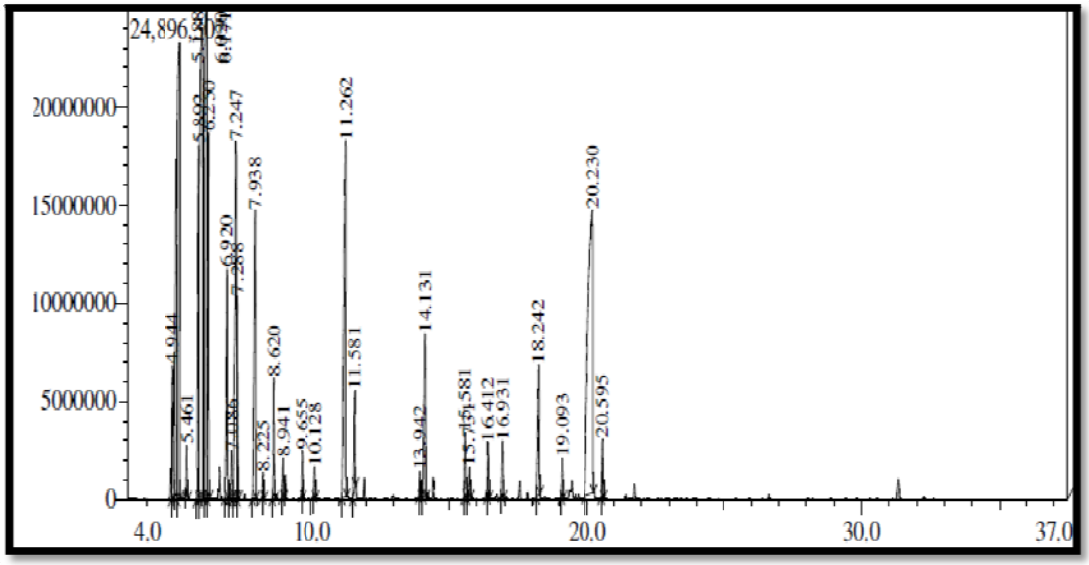
A17. GC-MS profile of mace volatile oil of TM-1



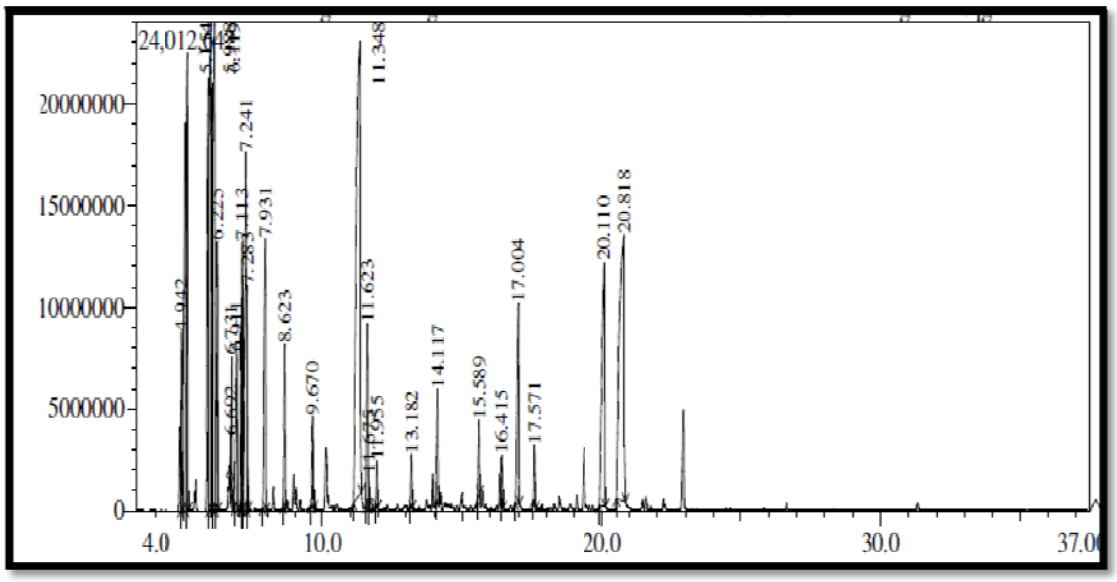
A18. GC-MS profile of mace volatile oil of SL-1



A19. GC-MS profile of mace volatile oil of GT-1

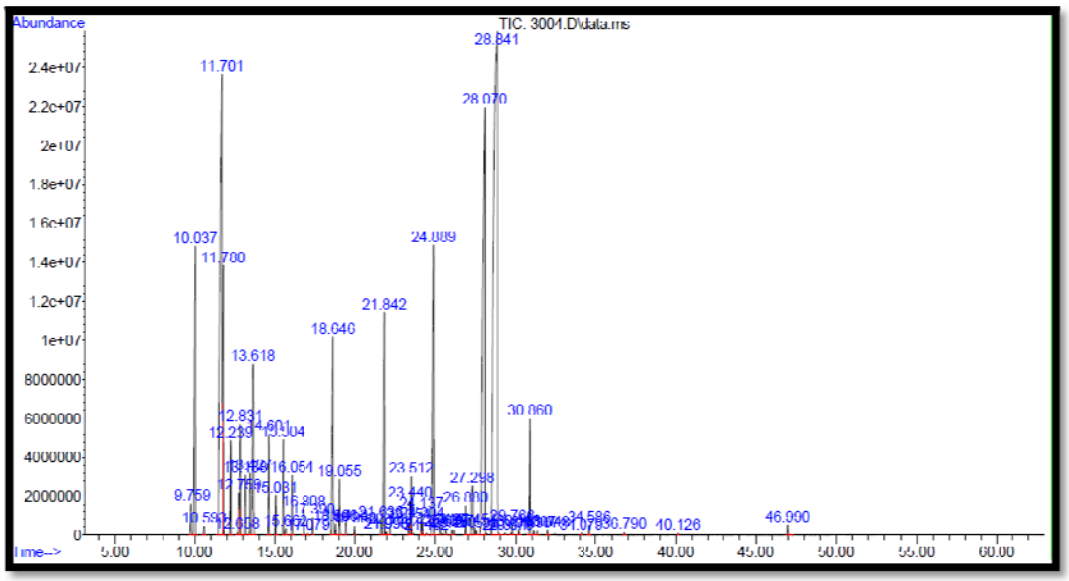


A20. GC-MS profile of mace volatile oil of CF-2

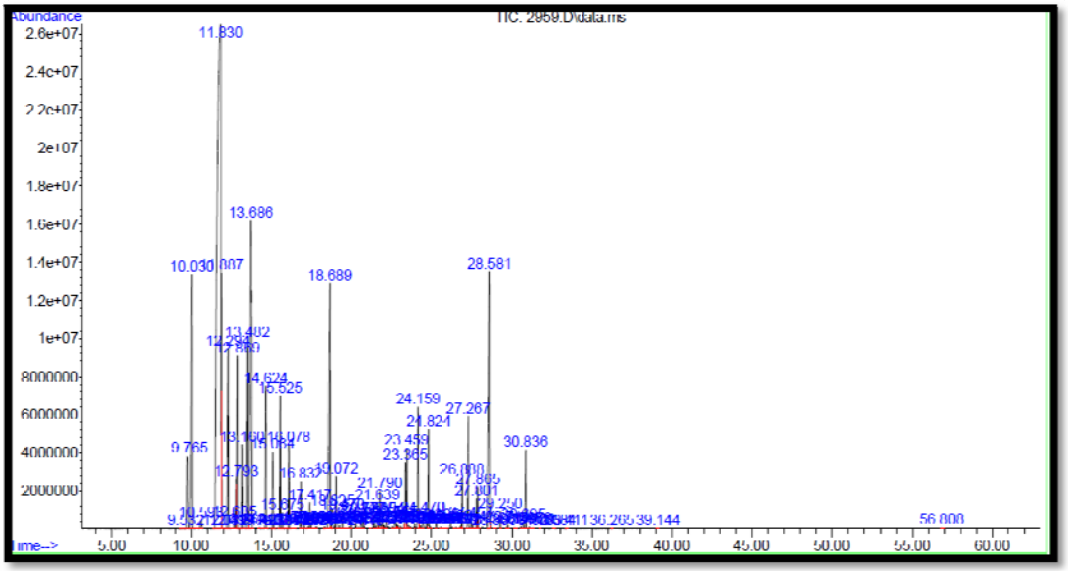


A21. GC-MS profile of mace volatile oil of IA-2

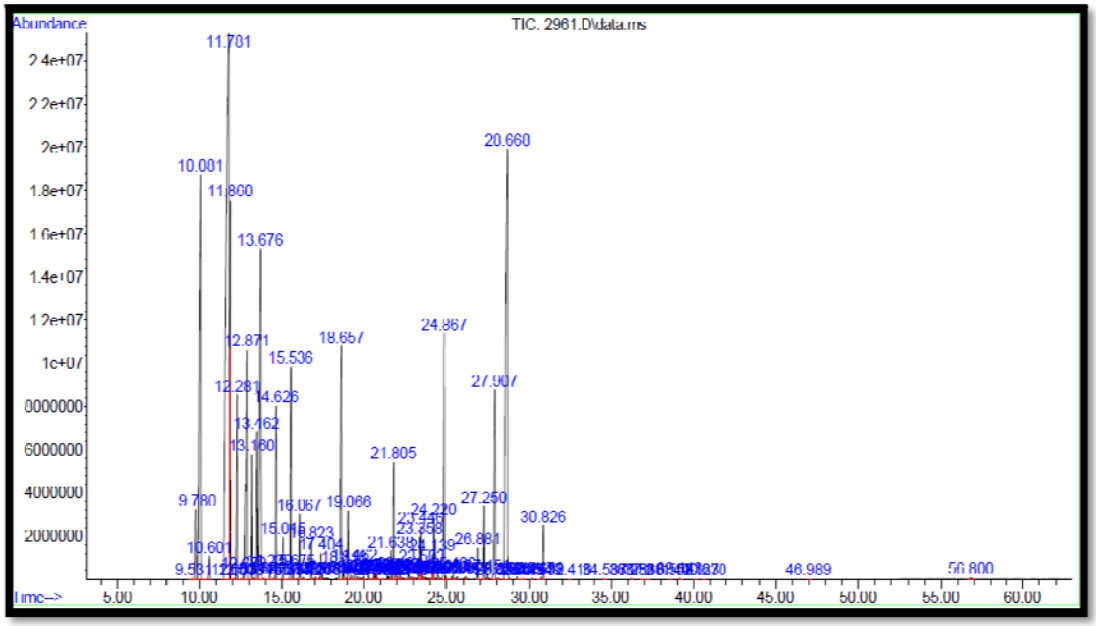
APPENDIX II:GC-MS profile of kernel volatile oil



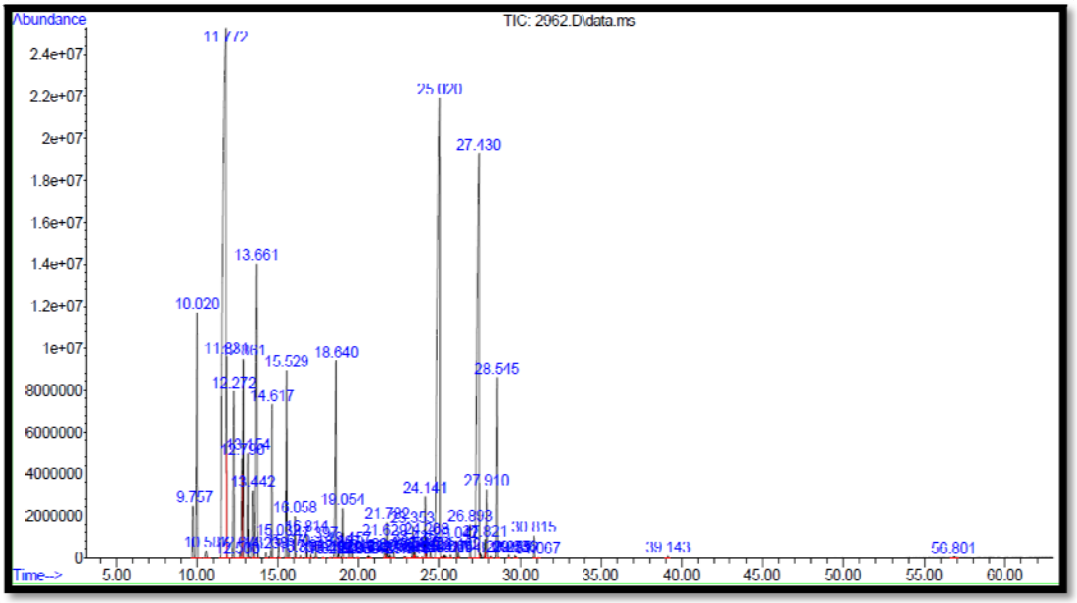
A1. GC-MS profile of kernel volatile oil of MN-1



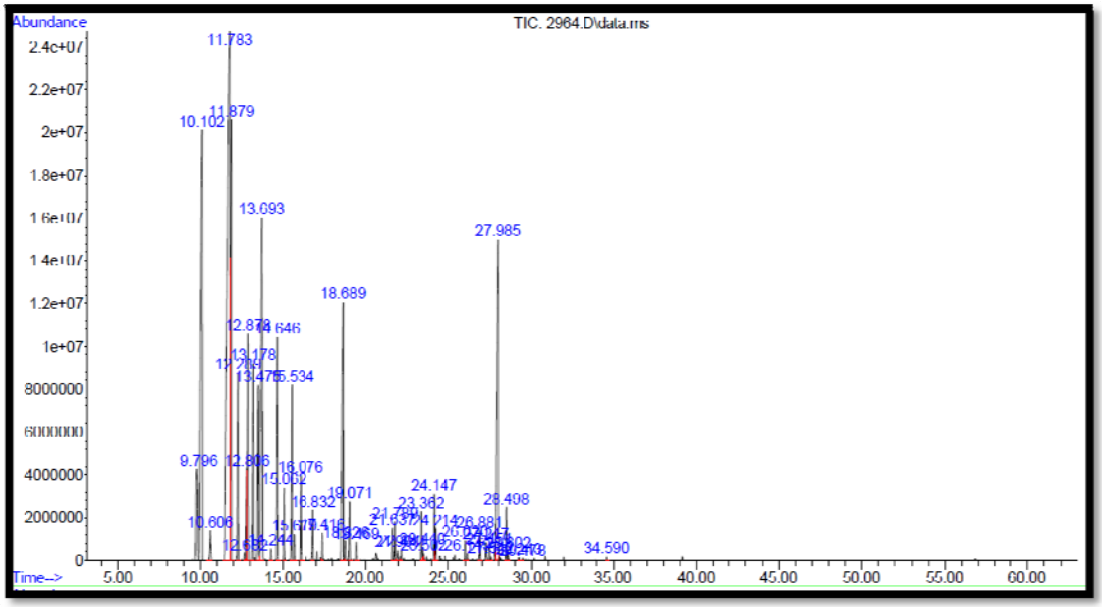
A2.GC-MS profile of kernel volatile oil of GT-2



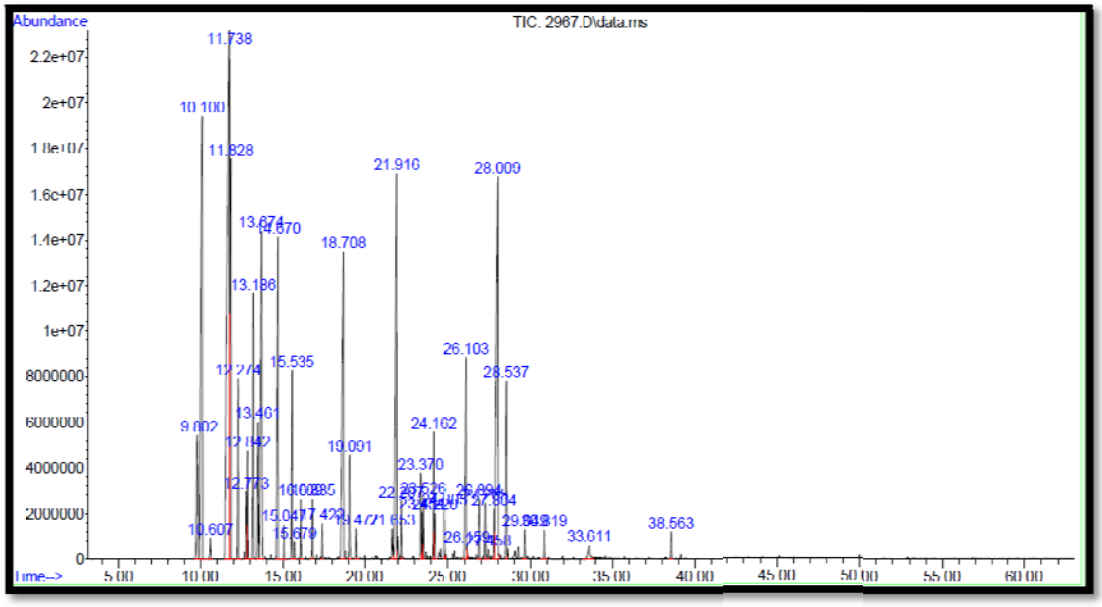
A3. GC-MS profile of kernel volatile oil YL-1



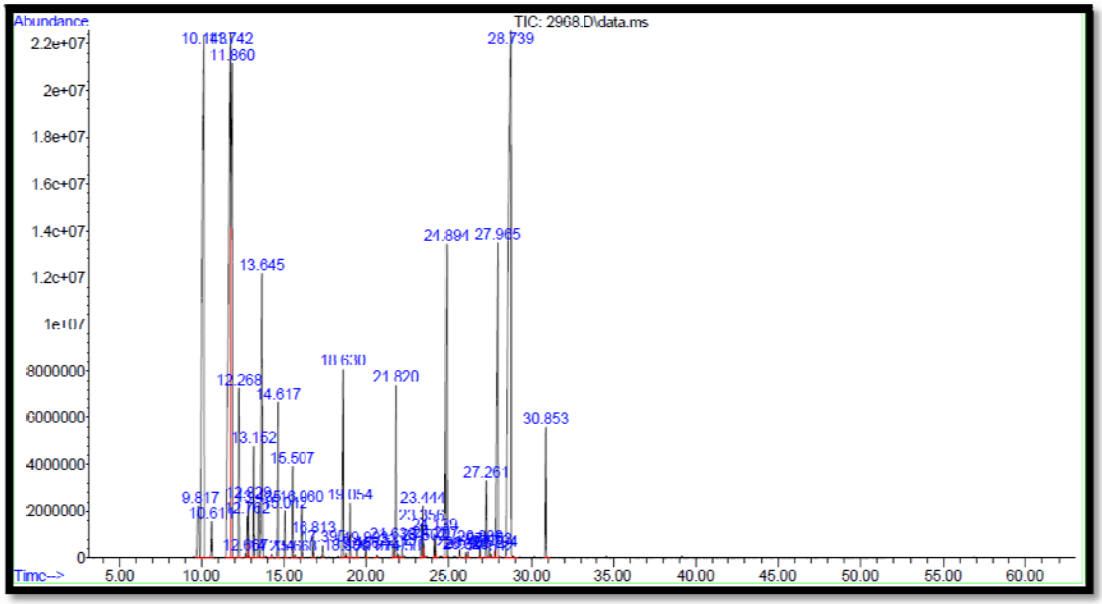
A4. GC-MS profile of kernel volatile oil SM-1



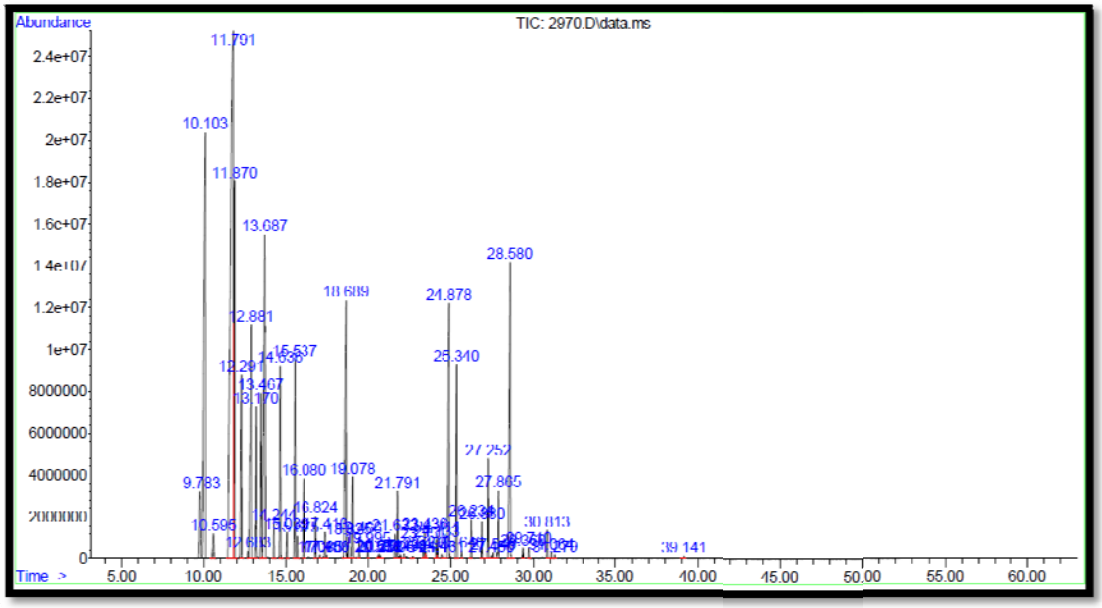
A5. GC-MS profile of kernel volatile oil OB-2



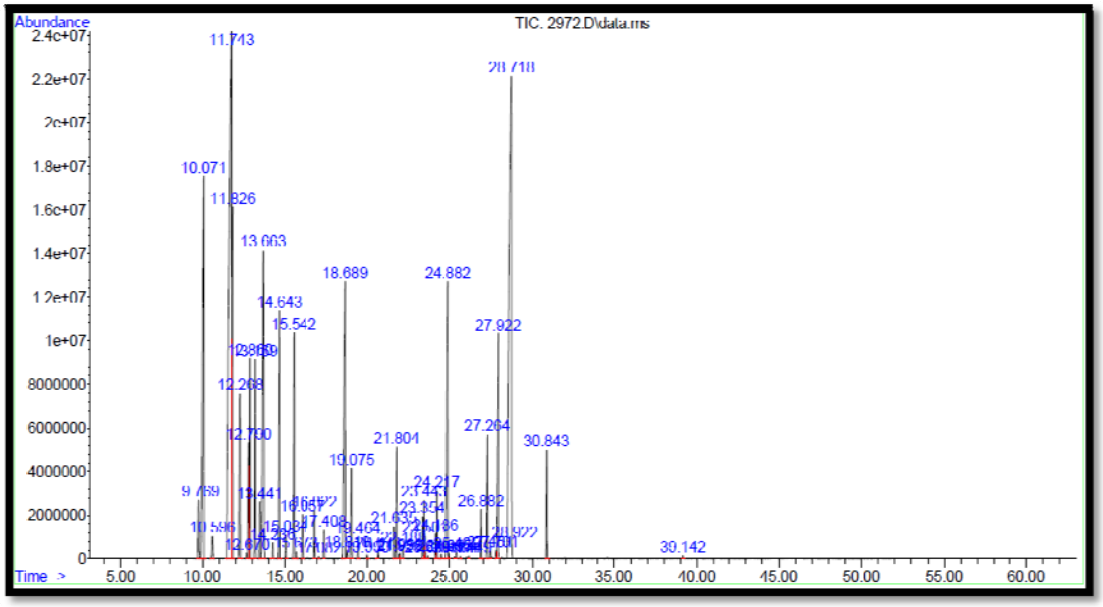
A6. GC-MS profile of kernel volatile oil CF-1



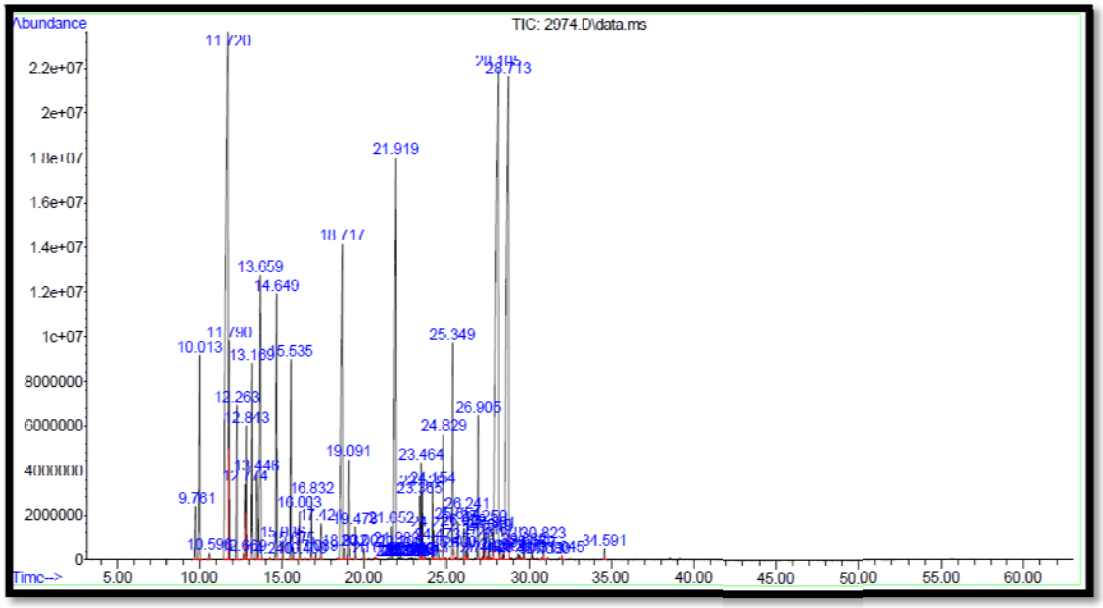
A7. GC-MS profile of kernel volatile oil PT-2



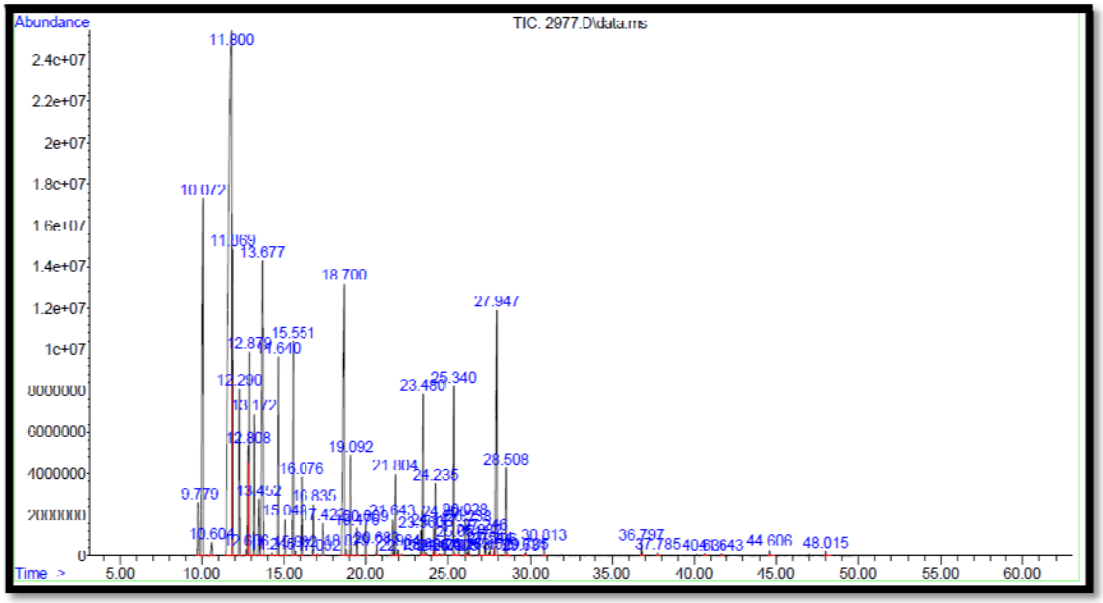
A8. GC-MS profile of kernel volatile oil IA-1



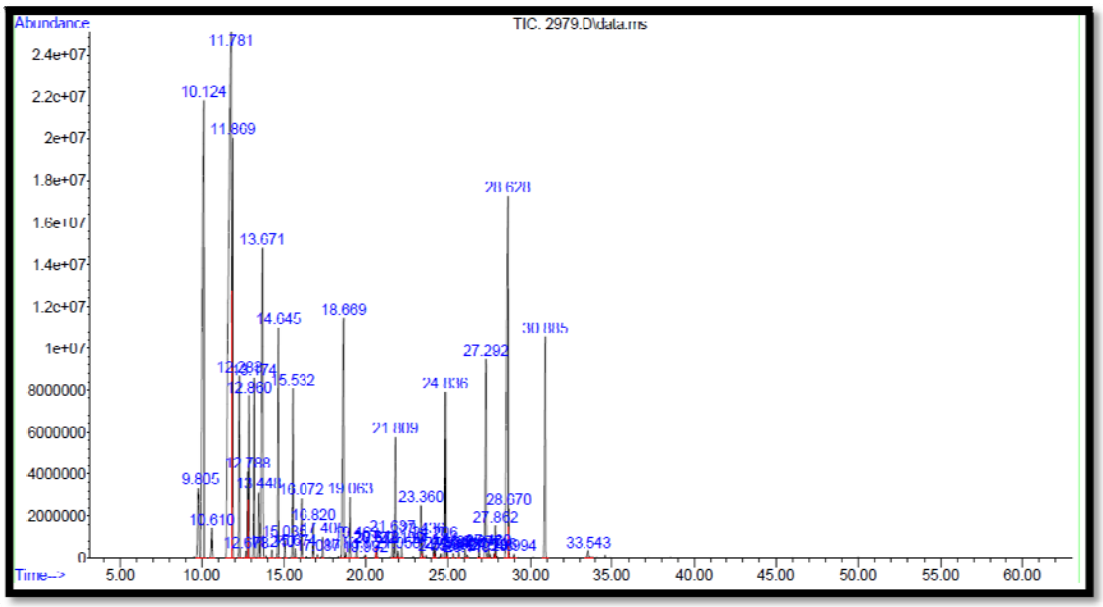
A9. GC-MS profile of kernel volatile oil YL-2



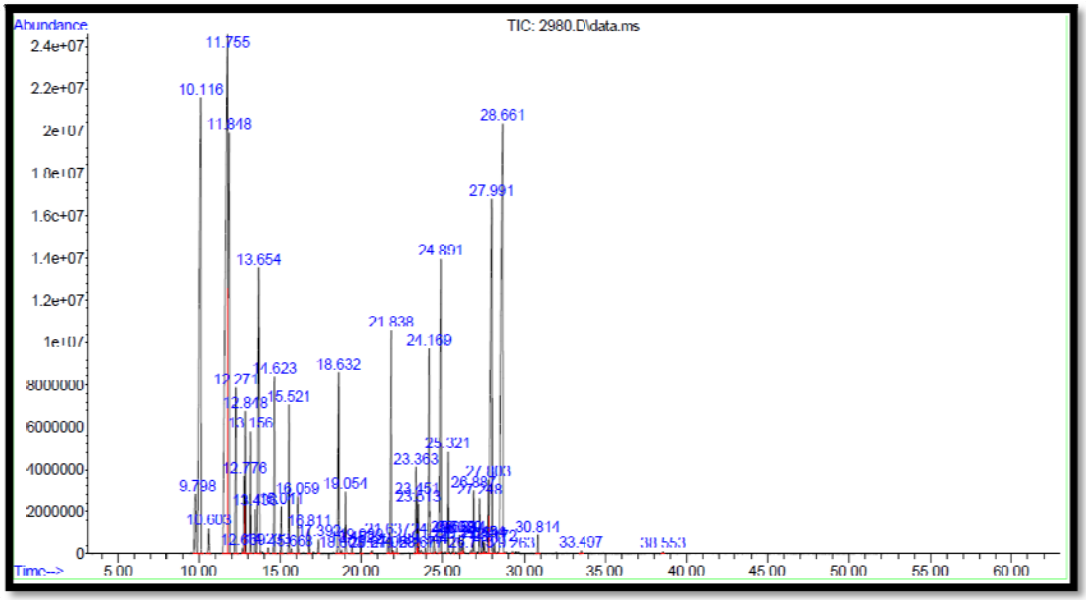
A10. GC-MS profile of kernel volatile oil LA-2



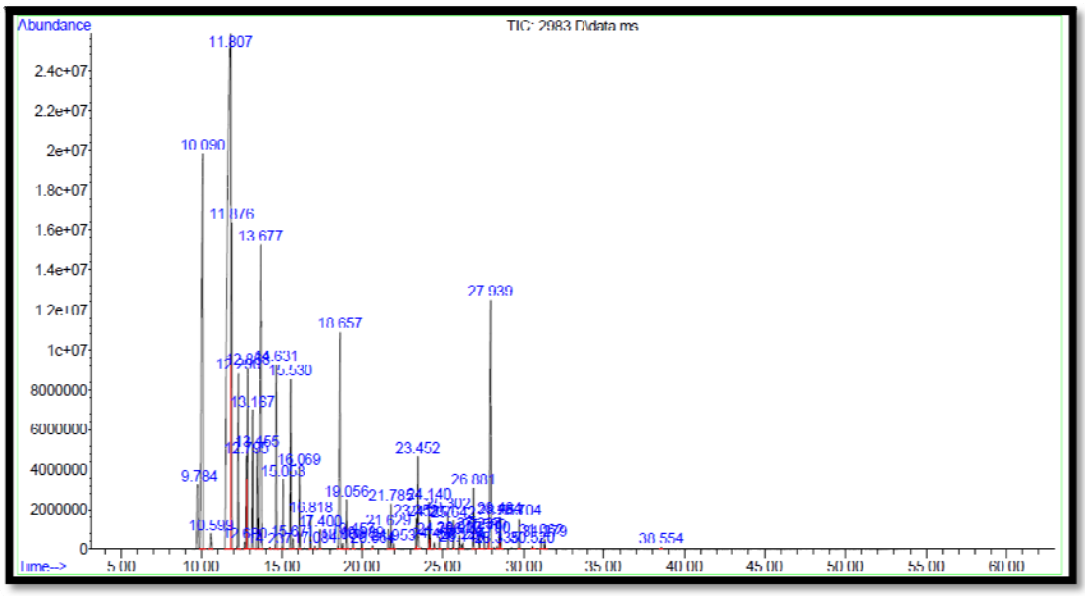
A11. GC-MS profile of kernel volatile oil DS-1



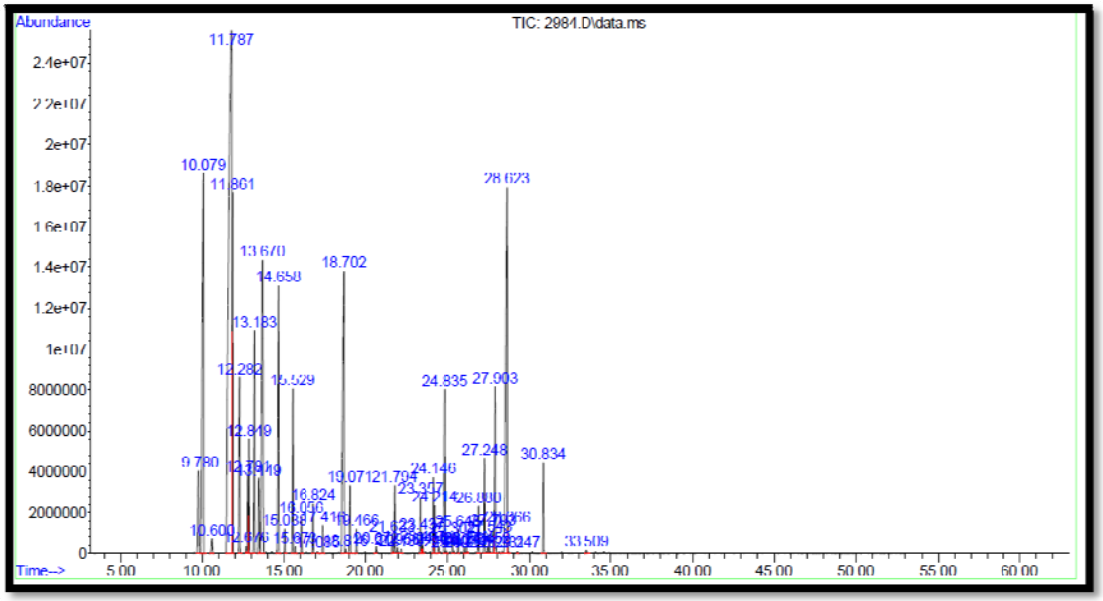
A12. GC-MS profile of kernel volatile oil DS-2



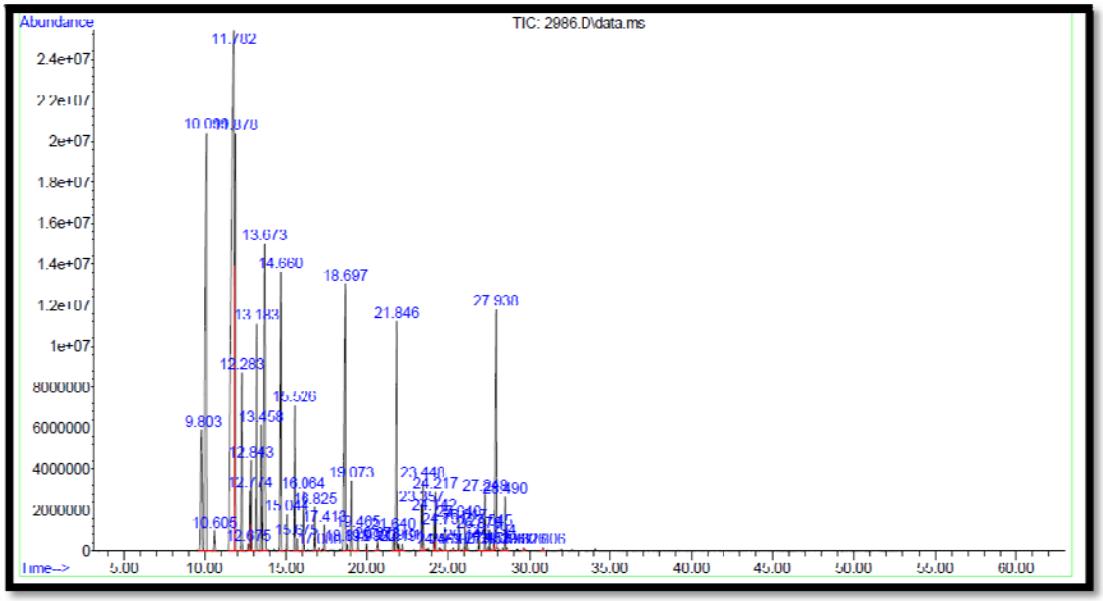
A13. GC-MS profile of kernel volatile oil PT-1



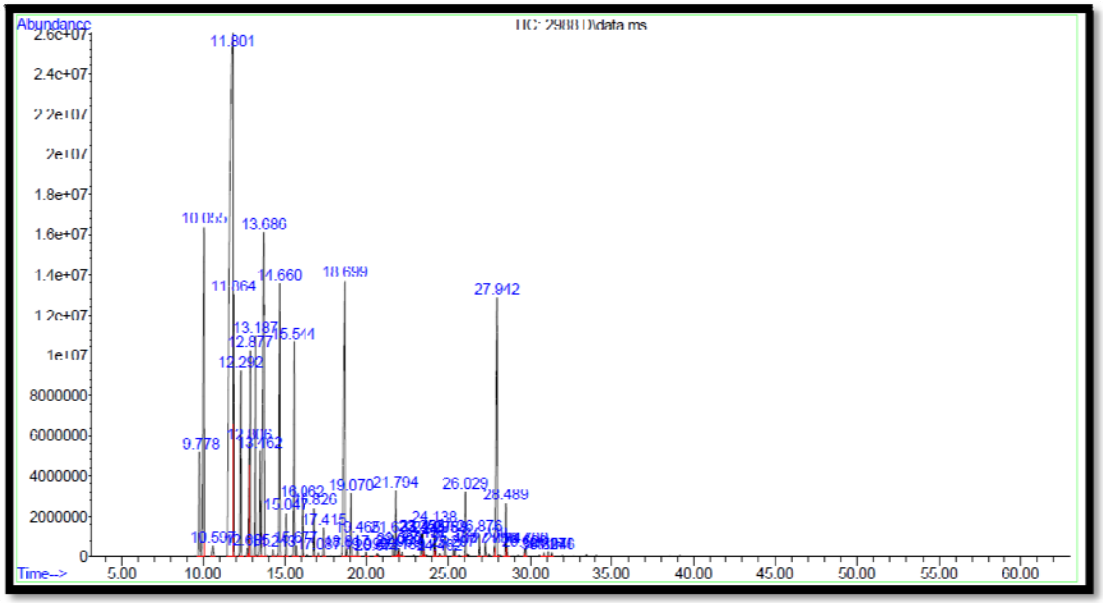
A14. GC-MS profile of kernel volatile oil MN-2



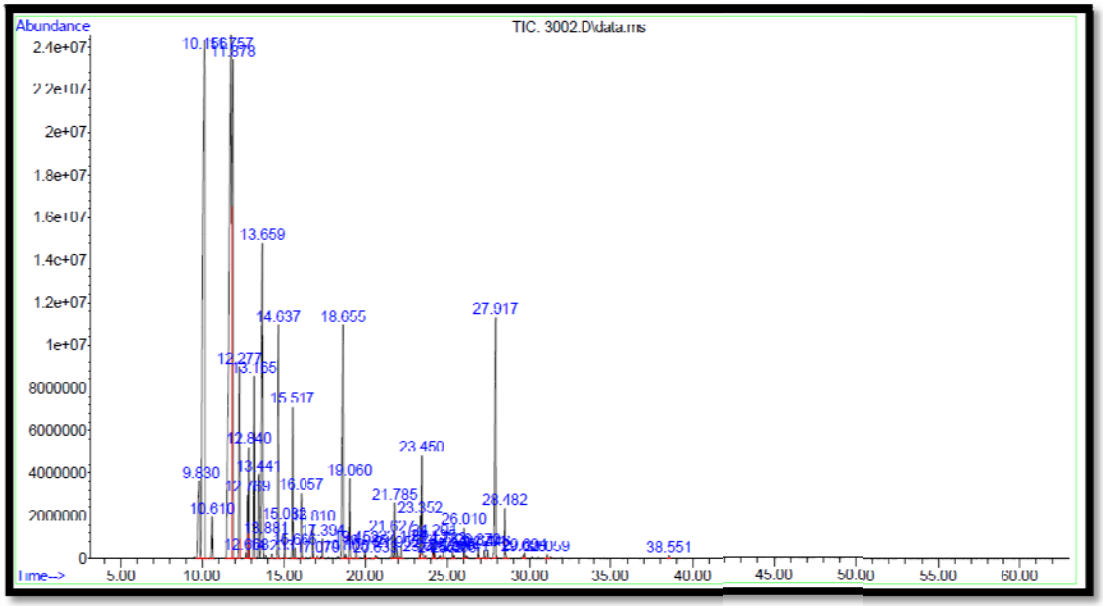
A15. GC-MS profile of kernel volatile oil OB-1



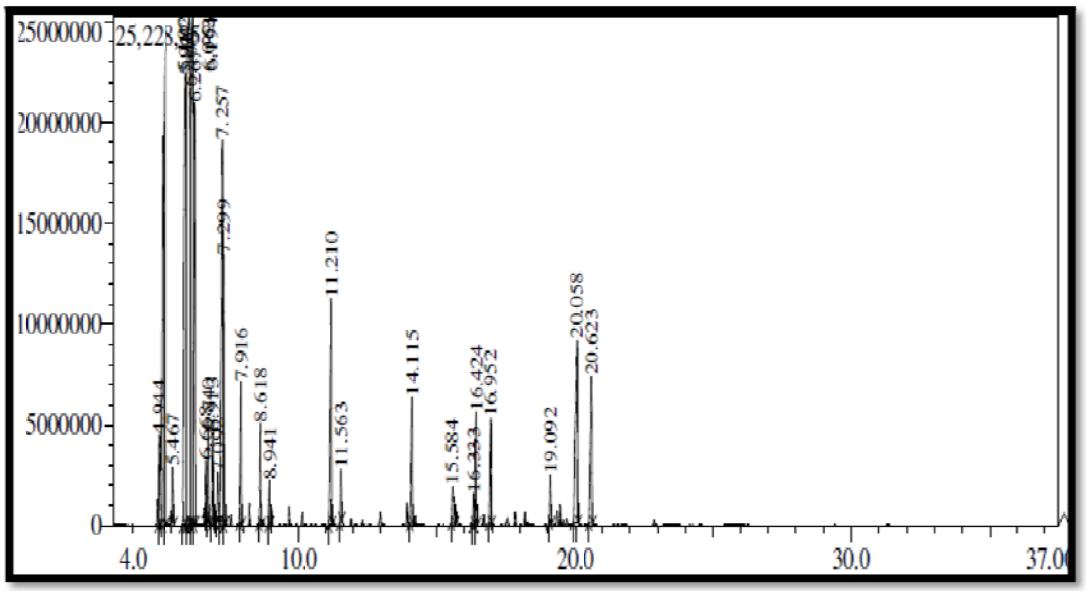
A16. GC-MS profile of kernel volatile oil LA-1



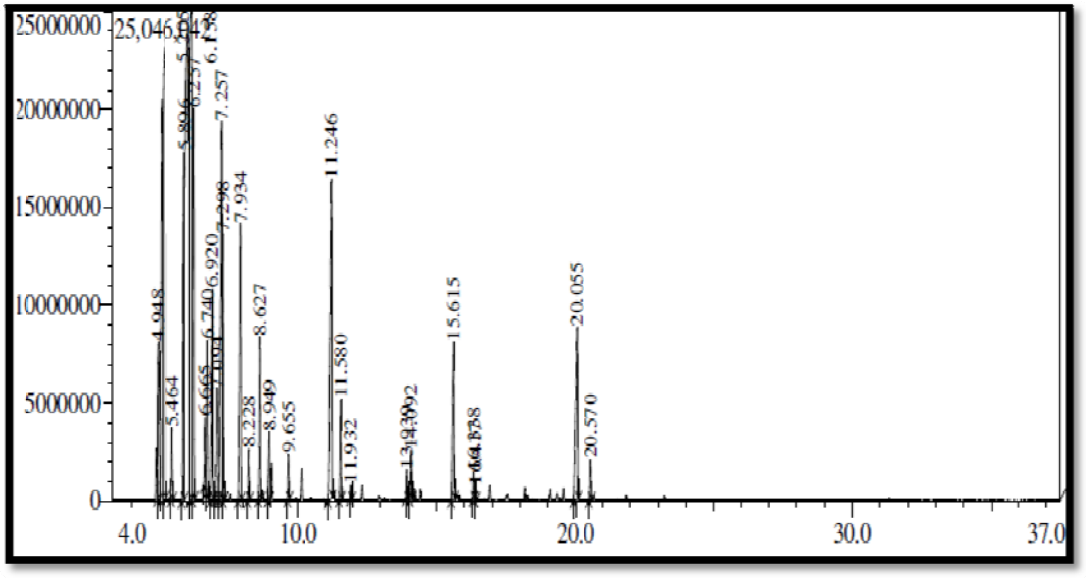
A17. GC-MS profile of kernel volatile oil TM-1



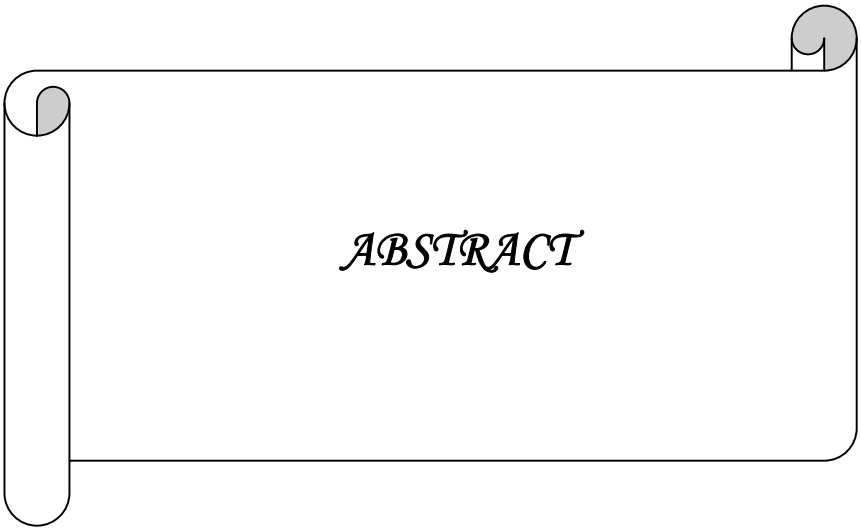
A18. GC-MS profile of kernel volatile oil GT-1



A19. GC-MS profile of kernel volatile oil CF-2



A20. GC-MS profile of kernel volatile oil IA-2


ABSTRACT

COLLECTION AND CHARACTERIZATION OF UNIQUE GENOTYPES OF NUTMEG (*Myristica fragrans* Houtt.)

By

PRIYANKA S. CHANDRAN
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ABSTRACT OF THE THESIS

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Faculty of Agriculture

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COLLEGE OF HORTICULTURE
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ABSTRACT

The present study entitled “Collection and characterization of unique genotypes of nutmeg (*Myristica fragrans* Houtt.)” was carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara, Thrissur with the objective to collect and characterize the unique nutmeg accessions based on morphological and biochemical parameters.

Twenty one unique accessions possessing 13 unique characters were identified and collected through an extensive survey throughout the nutmeg growing tracts of Kerala. The unique accessions collected from diverse locations of Kerala along with the two improved accessions identified in an earlier study formed the material for the present study. Morphological characterization was attempted using 18 qualitative and 31 quantitative parameters as per the nutmeg descriptor. Fruits collected from these unique accessions were subjected to biochemical estimation of constituents in rind, nutmeg and mace. The mace and kernel volatiles were subjected to GC MS analysis.

The unique germplasm included yellow maced nutmegs which is a desirable characteristic that is rarely spotted in the nutmeg family. Another unique tree with rudimentary sterile seed / seedless nature was located which is an exception from the normal. This accession had a good measure of fresh mace (9.74 g), mace oil and oleoresin content. The double seeded nutmeg accessions DS-1and DS-2 may be regarded as accessions having aberration in terms of mace and nut characteristics, with a typical four halved fruit splitting nature and two seeds per fruit. Monoecy is a very much preferred characteristic as nutmeg is usually classified as dioecious in nature, but the two monoecious accessions namely MN-1 and MN-2 had very less number of fruits with average quality mace and nut. The two cluster fruited accessions had on an average three fruits per bunch and four to eight fruits per cluster occasionally. Accession CF-1 had high nut and

mace yield per tree among all the unique nutmeg accessions collected in the present study. Among the low astringent nutmeg accessions, LA-2 adds robustness towards further research in nutmeg for use in the value addition of rind. The narrowly pyramidal (conical) shaped nutmeg accessions PT-1 and PT-2 had the least canopy spread. Grape nutmeg is another interesting unique accession with numerous small sized fruits, thin mace and small nut.

Wild nutmeg (*Myristica malabarica*) accessions had large brown velvety fruits with yellowish orange coloured oblong mace. Oblong shaped nutmeg was another interesting distinct feature noticed in the study. Another accession with triangular shaped mace was spotted which possessed the second highest sabinene content in kernel oil among all unique accessions. Small leaf type nutmeg labelled SM-1 possessed dark green coloured narrow elliptic leaves with a nut and mace yield comparable to cluster fruited nutmeg.

Among the quantitative tree characteristics, canopy spread was lowest in narrowly pyramidal accessions. Small leaved nutmeg had smallest leaf dimensions and leaf area was only half that of the normal nutmeg. Monoecious nutmeg had significantly high measure of flower characteristics. Biochemical characterization of volatile and nonvolatile contents of mace and nut revealed that accession GT-1 had high kernel oil recovery, MN-2 had high kernel oleoresin recovery and CF-1 had high fat content in kernel. Chemo profiling of the mace and kernel volatiles identified thirty eight constituents. Among these, myristicin ,elemicin, sabinene and safrole were the major constituents. Highest myristicin, sabinene, safrole and elemicin in mace oil were observed in GT-1, OB-2, LA-1and MN-1. Highest myristicin, sabinene, safrole and elemicin in kernel oil were observed in LA-2, MN-2, CF-1 and MN-1.

Based on qualitative tree, flower and fruit characters, twenty three unique nutmeg accessions were grouped into twelve clusters at 60% similarity. The similarities noticed were assessed through UPGMA (unweighted pair group method with arithematic mean) method and a dendrogram was drawn accordingly using NTSYS package 2.2. Similarity studies among unique accessions revealed

that majority of accessions had broadly pyramidal canopy architecture with spreading branching pattern. Inflorescence noticed was axillary raceme in majority, with the exceptional umbellate cyme inflorescence in monoecious, double seeded and grape nutmeg accessions. Mid and late season flowering nature was exhibited by majority of the unique accessions. Mace colour was predominantly red with exceptions to yellow maced and wild nutmeg. Monoecious nutmeg had three halved fruit splitting nature, while double seeded nutmeg had four halved fruit splitting nature.

All the fruit characteristics were measured and put to analysis for identification of sub groups among the 23 accessions using Univariate General Linear model. Further a ranking technique was adopted to bring the different characteristics present in the unique accessions into a comparative scaled value in one dimension. Based on the pooled rank scores, five groups namely extra ordinary, very good, good, moderate and heteroscedastic groups were obtained. The measured variability for each of the characteristics that were prominent among the five groups were listed as: leaf length (19.13%) and length of flower (24.81%) in 'Very good' group; plant height with a CV of 45.12%, girth with a CV of 75.27%, canopy spread N-S (38.98%)and E-W (40.39%), breadth of flower with a CV of (11.74%) and breadth of perianth (36.36%) in 'Good' group; leaf breadth (23.56%) and leaf area (26.99%)in 'Moderate' group; length of perianth in 'Heteroscedastic' group. A concept diagram was developed for utilizing the unique nutmeg accessions. All the unique accessions are worthy of conservation. Apart from conservation value, SL-1 and LA-2 offer scope for value addition of rind. Accession GT-1 and GT-2 are best suitable for ornamental purpose. Accession CF-1 which possesses all the desirable features of an elite nutmeg genotype can be commercially popularized as a variety.