CHARACTERIZATION AND EVALUATION OF NUTMEG (*Myristica fragrans* Houtt.) ACCESSIONS

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CHARACTERIZATION AND EVALUATION OF NUTMEG (*Myristica fragrans* Houtt.) ACCESSIONS

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THESIS

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KERALA, INDIA
2016

DECLARATION

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

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CERTIFICATE

Certified that this thesis entitled **"CHARACTERIZATION AND EVALUATION OF NUTMEG (*Myristica fragrans* Houtt.) ACCESSIONS"** is a record of research work done independently by Mr. Vikram H. C. (2012-22-108) 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 him.

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Introduction

1. INTRODUCTION

Nutmeg (*Myristica fragrans* Houtt.) belonging to the family Myristicaceae, is unique among the spice crops, as the donor of two distinct spices; nutmeg and mace. The name *Myristica* is derived from the Greek word Myron, a sweet liquid distilled from the plant (Everett, 1981). Nutmeg, indigenous to Moluccas Islands in Indonesia, was introduced to India by the British during the 18th century.

Indonesia is the largest producer and exporter of nutmeg and mace in the world market, followed by Graneda (Anandraj, 2015). In India, area and production of this perennial tree spice is steadily increasing over the past few years (Krishnamoorthy *et al*., 2012). As of now, the nutmeg is mainly cultivated in Southern India particularly in all the districts of Kerala and certain parts of Tamil Nadu and Karnataka. The present area under this crop in India is 18730 ha with an annual production of 12730 tonnes (Spices Board, 2014).

Nutmeg has tremendous potential in the spice industry, as flavour in food and also as ingredient in many value added products. Due to medicinal properties, it is used immensely in the pharmaceutical industry. Oil, oleoresin and fixed oil are extracted from dried nutmeg as also mace. Myristicin, elemicin, sabinene and safrole constitute 80 per cent of the oils, thus extracted. Very high variability exists in the composition as also proportion of these components of oils extracted from nutmeg and mace (Rema *et al*., 2015).

Nutmeg is normally dioecious**,** though other sex forms occur sporadically (Krishnamoorthy, 2000). Research on identification of male and female plants at the seedling stage through morphological characters (Krishnamoorthy *et al*., 1996), specific molecular markers and biochemical markers **(**Sudhamayee *et al*., 2014) have yielded only limited positive results.

Nutmeg is a cross pollinated crop. High amount of variability has been reported throughout the nutmeg growing tracts with respect to the growth rate and flushing pattern (Nazeem, 1979), productivity, size and shape of the leaf, flower size and shape of the fruit and nut (Haldankar *et al*., 2004; Sasikumar, 2009;

Senthilkumar *et al.*, 2010; Miniraj *et al*., 2015a). A few monoecious as also other elite types of nutmeg have been reported from Kerala (Miniraj *et al*., 2012; Sasikumar *et al*., 2014) and Coastal Karnataka (Rema *et al*., 2015).

One of the major constraints in nutmeg cultivation is the lack of improved varieties. The work on crop improvement of nutmeg is sparingly few. Indian Institute of Spices Research, Kozhikode has released two improved varieties suited for cultivation in Kerala. At present, there are many number of elite types identified and popularized by progressive farmers in Kerala which excel the released varieties in terms of yield as well as quality. The average yield ranges from 500 to 3000 fruits per tree per year. Systematic crop improvement work in this tree spice is yet to gain momentum. An understanding of the variability existing in the crop is very essential for formulating crop improvement programmes.

Genetic diversity is normally distinguished through morphological characters. However, such morphological characters could be affected by environmental influences, developmental stage of the plant, type of planting material used etc. This necessitates a highly reliable and precise method for assessment of genetic variability, to bring into characterization, the volatility for the environment. Biochemical markers such as isozyme and DNA markers like RAPD (Williams *et al*., 1990) and ISSR (Zietkiewicz *et al*., 1994) are most suitable for exact genetic diversity analyses, as these markers are not altered for the environment. The task yet to be shouldered is multifaceted.

The present study entitled "Characterization and evaluation of nutmeg (*Myristica fragrans* Houtt.) accessions" was taken up exclusively with the specific objectives to characterize nutmeg accessions based on morphological, biochemical and molecular parameters so as to scale the variability in a multidimensional way.

Review of literature

2. REVIEW OF LITERATURE

Considerable research attention is being attached very recently to the nutmeg crop as it has become one of the important spice commodities in domestic as well as international markets. Multifarious strategic research efforts have been initiated in this tree spice for utilizing the existing diversity for crop improvement programmes and value addition.

In this chapter, the available information on morphological, biochemical and molecular studies on nutmeg is presented under various subtitles. Wherever literature on nutmeg is lacking, relevant research works on other tree spices are also presented.

2.1 Diversity in *Myristica* spp.

Nutmeg (*Myristica fragrans* Houtt., 2n=44) belongs to the small, primitive family Myristicaceae of the order Laurales. *Myristica* is the largest genus with 120 species. Family Myristicaceae has about 18 genera and 300 species. Members of the family are pantropical, being associated with the rainforests of Asia, Africa, Madagascar, South America and Polynesia (Sinclair, 1958).

India has four genera namely *Horsfieldia, Gymnocranthera, Knema and Myristica*, and altogether 15 species. These members occur in the evergreen forests of Andaman and Nicobar Islands, Meghalaya and the Western Ghats (Krishnamoorthy *et al.*, 1997). *Gymnocranthera canarica* and *M. fatua* are exclusively grown in swamps. *M. malabarica* is occasionally noticed in swamps and is more frequent in the evergreen forests. *Gymnocranthera, M. fatua* var. *magnifica* and *M. malabarica* are endemic to the Western Ghats. *M. fragrans* is of commercial importance and is the widely cultivated species. *M. prainii, Knema andamanica* and *M. andamanica* are endemic to the Andaman and Nicobar Islands and are used in tribal medicine (Sharief, 2007). Nutmeg is a native of Moluccas Islands in Indonesia. Semi domesticated gene pools of *Myristica* do not exist as most of the species occur in the wild. Most of the species are endangered,

endemic or threatened, available only in the vulnerable hotspots and are used either in medicine or as spice.

The oldest nutmeg populations in India are in Kalady and Pala of Kerala, and are reported to be more than 150 years old. Nutmeg is usually grown in river banks as it grows luxuriously in silts deposited by rivers.

2.2 Botany of *Myristica fragrans* Houtt.

Nutmeg is a dioecious or occasionally monoecious evergreen aromatic tree, grows 10-20 m in height (Shanmugavelu and Rao, 1977) with spreading branches which carry oblong-ovate leaves, acute at apex and base, 5-15 cm long and 2-7 cm wide, dark green and lustrous (Vergheese *et al.*, 1990). Nazeem (1979) observed the shoot growth in nutmeg as cyclical, a period of growth followed by quiescence. Six flushes were observed in an year. All the flushes were not seen in all the shoots, which indicate continuous growth of the tree. Two peak flushes are observed during the month of May-June and September. The branched inflorescence produced by male is an axillary raceme (Joshy, 1946). In female, it is simple cyme (Joseph, 1980). Flowers are drooping, creamy yellow and fragrant. Though nutmeg is dioecious, five different types of trees *viz*., pure male, pure female and bisexual male, bisexual female and hermaphrodite were also reported (Krishnamoorthy, 2000). The perianth receives ten vascular traces and is postulated with pentamerous origin. The androecium consists of a solid column or androphore, to which 14-22 bilocular anthers are attached. The single pistil is flask shaped with a short to non-existent style and bilobed stigma. The ovule is single (Nair and Bahl, 1956). Fruit is pear shaped and yellow in colour. The pericarp is fleshy when the fruit matures, it splits into two, exposing the deep red coloured net like aril covering the shining black seed.

Flowering pattern of male and female trees differ. In female trees, flowering continued to seven months, whereas in male trees flowering was observed throughout the year. Highest flowering in both the cases was in July followed by October. The female flowers took 154 days for complete

development. Male flowers took only about half the period taken by the female flowers to develop. Anther dehiscence occurred about 24 hours prior to anthesis. The stigmatic receptivity lasted for six days after anthesis; it was highest during the first three days. The chief agent of pollination is wind. The percentage of fruit set varied among the trees and for different aspects. Highest fruit set was observed in trees on Western and Eastern aspects (Nazeem, 1979). Fruits attained maturity in 206 to 237 days after fruit set. The developing fruits followed a sigmoid growth pattern (Nazeem and Nair, 1981).

2.3 Variability in morphological characters of nutmeg

Variability may be defined as the amount of variation present among the members of a population or species for one or more characters at genotypic or phenotypic levels. The phenotypic characters of the plants are highly influenced by environmental conditions. Different environments may cause variation in morphological characters and thus make difficulties in the genotype identification. Presence of variability among the genotypes is a prerequisite for any crop improvement programme.

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

In another study involving seedling progenies of sixteen elite mother trees of nutmeg, correlation was examined by Krishnamoorthy *et al.* (1996). They observed that phenotypic coefficient of variation was more than the genotypic coefficient of variation for different characters, which indicated environmental expression of these traits.

Shigvan and Kore (2003) reported that artificial pollination in nutmeg would be beneficial over open pollination, which helps in increasing fruit set and yield. Same authors further observed the fruit development and fruit drop in nutmeg, it takes eight months for fruit development and maximum fruit drop was observed after three months of fruit set.

Haldankar *et al*. (2003) recorded yield in nutmeg genotypes for continuous six years under Konkan region of Maharashtra. Twenty year old trees grown in coconut plantations showed variation in yield (number of fruits and nut weight) attributed by two components *i.e*., genotype and season.

The relative amount of heritable portion of total variation was found out with the help of heritability estimates and genetic advance. Shinde *et al.* (2006) observed high heritability, genetic advance and genetic gain for fruit characters in nutmeg, which indicated good scope for exploiting variation by selection on per se performance basis.

Genetic divergence studies with 34 genotypes of nutmeg grouped them into 12 clusters. One of the clusters had genotypes with characters of large fruits and nuts and high nutmeg butter yield. Another cluster was superior for fruit length, total weight of fruits, nut length, pericarp weight and shell weight (Haldankar *et al*., 2007).

A good amount of variability has been reported in growth rate, productivity, size and shape of the leaf, flower size and shape, fruit set and size of the fruit and seed in nutmeg (Krishnamoorthy *et al.*, 1996; Haldankar *et al*., 2004; Haldankar *et al.*, 2006; Sasikumar, 2009).

Haldankar *et al.* (2009) studied thirty nutmeg genotypes under Maharashtra conditions and differentiated them into early (225 days), mid (250-275 days) and late (300 days) duration types.

Performance evaluation of twenty three nutmeg accessions was carried out by Senthilkumar *et al.* (2010) for fruit and yield characters under high altitude

region of Kodagu in Karnataka. Highest variability was recorded for fresh seed yield per tree and medium to high variability was recorded for mace characters. Minimum variability was recorded for the leaf and seed characters.

Parthasarathy (2010) has reported that selection in nutmeg will be effective, if trees are selected with optimum fruit number and moderately good seed weight.

Das *et al*. (2012) estimated the genetic diversity of nutmeg species in North Moluccas. The accessions showed 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.

In its native country *i.e.* Moluccas Islands, flowering season of nutmeg was observed during month of March or May and continued upto December. Fruit harvesting starts in March or April and continued up to December or January for eight to ten months. The average fruit set percentage of nutmeg ranged from 22.63 to 47.53 as observed by Sangadji *et al.* (2015).

An extensive survey was carried out by Miniraj *et al.* (2015) in the nutmeg growing areas of Kerala and the collected accessions are maintained in nutmeg germplasm centre at Kerala Agricultural University, Thrissur. Morphological and biochemical characterization of these collected accessions revealed monoecious types, unique types with yellow mace and other superior types with excellent nut and mace characters as well as quality parameters. A few monoecious, rare and other elite types of nutmeg have been reported from Kerala and Coastal Karnataka (Miniraj *et al*., 2012; Rema *et al*., 2015; Sasikumar *et al*., 2014).

2.3.1 Other tree spices

Krishnamoorthy *et al.* (1998) observed high genotypic coefficient of variation for fresh and dry weight of bark, bark oleoresin, leaf and bark oil, leaf size index and per cent recovery of bark in cinnamon.

Mathew *et al.* (1999) identified two seedling variants of all spice that showed distinct morphological variation with respect to canopy shape, branching habit, internodal length, leaf characters and plant height (dwarf/semi-dwarf).

Kore *et al.* (2005) carried out investigation to study the genetic variability of twenty two *Garcinia* genotypes, which exhibited high magnitude of PCV and GCV for the fruit characters. They have also recorded high heritability and genetic advance for length, weight and volume of fruit, fresh rind weight, pulp weight and dry seed weight.

Genetic diversity analysis of *Garcinia* spp. collected from different growing locations of Karnataka exhibited wide range of variation and also showed distinct fruit characters. But these species had similar values for biochemical parameters (Nalini and Chimmad, 2005).

Abraham *et al.* (2006) collected and characterized fifty six accessions of Malabar tamarind from Western Ghat regions of Karnataka and Kerala. Accessions exhibited maximum variability for fruit colour, fruit shape, fruit size, nature of branching, canopy shape, fruit weight, fruit girth, rind weight and rind thickness.

Kumar *et al.* (2006) observed high heritability coupled with high genetic advance as percentage of mean for length of fruit beak, weight of individual fruit, volume of individual fruit, weight of fruit rind, weight of flesh, rind:flesh ratio, weight of dry fruit rind and index of seed in *Garcinia*. High heritability coupled with moderate genetic advance as percentage mean was observed for hydroxy citric acid in *Garcinia*. Genotypic association analysis of *Garcinia* revealed that hydroxy citric acid content was significantly and positively associated with fruit weight, fruit volume, fruit girth, rind thickness, weight of wet and dry fruit rind, recovery percentage of rind and number of seeds per fruit.

Manjunatha *et al*. (2009) observed significant variation among the accessions of gambodge from Coastal districts of Karnataka with respect to fruit characters, seed, seedling characters and seed oil content.

Performance analysis of fifteen cassia genotypes was carried out under the high rainfall and high altitude region of Karnataka by Senthilkumar *et al.* (2009). Results revealed significant variation among the genotypes for fresh and dry bark yield per plant at seventh year after planting.

Mansyah *et al.* (2010) observed variation among different collections of *Garcinia* for morphological characters *viz*., canopy shape, mature leaf colour, number of flowers and fruits per cluster, pedicel length, fruit shape, stigma lobe shape, size and thickness, number of fruit segments, and rind thickness. Based on morphological characters, *Garcinia* collections were grouped into seven clusters.

2.4 Diseases of nutmeg

Die back disease of nutmeg caused by *Diplodia* sp. was first reported in Trivandrum district, Kerala, India by Wilson and Sathiarajan (1974).

Skaria *et al.* (2000) noticed a new disease, horse hair blight in nutmeg caused by *Marasmius equicrinis* in Ernakulam, Kerala, India. Symptoms of the disease are formation of mycelial hairs and drying of leaves and branches leading to a bird's nest appearance.

Phytopthora is one of the important pathogens causing disease in many plantation and spice crops of Kerala *viz*., coconut, arecanut, rubber, cocoa, black pepper, cardamom and vanilla. Mathew and Beena (2012) for the first time described the leaf fall and shoot rot of nutmeg in Kerala, caused by *Phytophthora* sp. Mathew and Miniraj (2013) reported severe outbreak of leaf fall of nutmeg during monsoon season (mid June to end of June) in central Kerala *viz*., Ernakulam, Kottayam and Thrissur districts.

2.5 Biochemistry of nutmeg

The pericarp, nut and mace possess various constituents of economic importance.

Maya *et al.* (2006) evaluated *Myristica* species for biochemical characters in nut, mace and leaf. Among the species, nuts of *M. fragrans*, *M. beddomeii* and *M. prainii* were rich in myristic acid and mace of *M. fragrans* had high content of palmitic acid. *M. fragrans* leaf contained very high phenyl alanine. *K. andamanica* leaf was rich in amino acids alanine and threonine.

Abdurrasheed and Janardanan (2009) estimated the chemical composition of nutmeg collected from Tellicherry and Kannur, Kerala. Both nut and mace were chemically analysed and the constituents reported were starch (28.20 and 38.10% respectively), protein (8.30 and 6.10% respectively), fat (33.80 and 26.40% respectively), K (0.62 and 0.88% respectively), Ca (0.12 and 0.11% respectively), Fe (98 and 111 ppm respectively), Cu (13 and 21 ppm respectively), Zn (16 and 15 ppm respectively), Mn (41 and 23 ppm respectively), P (150 and 112 ppm respectively), essential oil (8.7 and 15.8 % respectively).

A study undertaken by Raphael *et al.* (2010) investigated the proximate and mineral composition in African nutmeg and revealed its composition *viz*., moisture (13.15±2.73%), total ash (3.90±1.05%), crude fat (27.7±2.57%), crude protein (10.13±1.95%), crude fibre (23.38±4.45%), carbohydrate (21.20%) and fatty acids (23.28%). Minerals (mg/g) included Mg (86.96±4.01), Ca (416.01±1.42), K (869.64±4.03), P (112.03±4.45), Mn (1.05±0.35), Fe (21.71±0.52), Na (17.66±0.32), Cu (0.19±0.02), Al (4.98±0.68) and Zn (1.52±0.11).

Thomas and Krishnakumari (2015) studied proximate and mineral composition of dried nutmeg seeds and reported the presence of high moisture content (51.03±0.25 mg/g), fibre (10.91±0.3 mg/g) and calcium (30.95±1.25). The study indicated high nutritive value and mineral composition of nutmeg.

Nutmeg rind constitutes 80 to 85 per cent of its whole fruit weight and at ripe stage, it has an acidic astringent taste with aromatic flavour. Teena (2015) estimated the proximate composition of fresh rind of nutmeg, as moisture (88.45%), acidity (1.43%), total phenol (35.20 mg/100g), tannin (33.80 mg/100g),

total soluble solid (3.38 ^{0}B), total sugars (2.69%), protein (1.25 g/100g), crude fibre (2.55 %), pectin (0.78 %) and iron (607.80 mg/kg).

2.5.1 Volatile oil, oleoresin and fixed oil

Apart from nutmeg and mace, volatile oil, oleoresin and fixed oil are derived from *M. fragrans*. These value added products find use in the food, medicine and perfumery industries. Nutmeg volatile oil is colourless or yellow liquid having the characteristic odour and taste. The oil is insoluble in water, but soluble in alcohol. It keeps best in cool, tightly closed containers protected from light. The oleoresin of nutmeg and mace are used almost entirely in the flavouring of processed foods. 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 (consistency of butter at room temperature). The crude fixed oil contains essential oil upto 10-12 per cent. The major component of fixed oil is trimyristin. Fixed oil consists of mainly saturated fats (90%) with 10 per cent unsaturated fats (Leela, 2008).

Significant work on nutmeg volatile oil has been carried out in different parts of India and it is observed that chemical composition of nutmeg oil was influenced by location (Evans, 2003).

Omobuwajo *et al.* (2003) analysed the African nutmeg, *Monodora myristica* and recorded the biochemical constituents *viz*., moisture content of 7.67 per cent, volatile oil (v/w) of 3.02 per cent and oleoresin content of 21.30 per cent.

Maya *et al*. (2004) reported that the essential oil content in South Indian nutmeg ranged from 3.90 to 16.50 per cent, whereas in mace it varied from 6.00 to 26.10 per cent. The major constituents in oil recorded were sabinene, myristicin, elemicin and safrole. The chief flavour contributing components, namely myristicin and elemicin were present in low concentrations.

A study was conducted by Kumar (2012) to evaluate the comparative analysis of volatile oil of nutmeg samples collected from North East India and

Kerala. The results revealed that volatile oil yield of samples from North East India and Kerala were 8.50 and 7.25 per cent, respectively.

2.5.2 Profiling of volatiles

The volatile oil of nutmeg consists of major classes of compounds: monoterpene hydrocarbons (61-88%), oxygenated monoterpenes, aromatic ethers, sesquiterpenes, aromatic monoterpenes, alkenes, organic acids and miscellaneous compounds (Leela, 2008). Myristicin, sabinene, safrole and elemicin constitute about 80 per cent of the alkenylbenzene derivatives (Olaleye *et al*., 2006). Bioactive compounds including camphene, eugenol, isoelemicin, isoeugenol, methoxyeugenol and elemicin were identified as the main constituents of *M. fragrans* seed essential oil (Chirathaworn *et al*., 2007).

Mallavarapu and Ramesh (1998) studied the chemical composition of essential oils from nutmeg and mace and found that Indian nutmeg oils were intermediate in quality, compared to East Indian (Indonesian) and West Indian (Grenada) nutmeg oils.

Krishnamoorthy and Zachariah (2002) studied the stored mace powder for volatile oil and chemical transformations. Among the major components of oil, alpha-pinene and sabinene concentrations increased on storage, whereas concentration of safrole, myristicin and elemicin decreased on storage.

Oganwande *et al.* (2003) found that essential oil isolated from nutmeg kernel contains sabinene (49.09%), alpha-pinene (13.19%), alpha-phellandrene (6.72%) and terpinen-4-ol (6.43%) as major constituents. The presence of myristicin and elemicin in nutmeg is often related to the hallucinogenic action, while safrole has been suspected to be carcinogenic (Taketa *et al*., 2004).

Maya *et al.* (2004) reported that, among the 65 accessions of nutmeg, A9/71 and A9/95 had high sabinene and low hallucinogens (myristicin, elemicin and safrole) in both nutmeg and mace oils. Alkaloids, saponins, anthraquinones,

cardiac glycosides, flavonoids and phlobatanins were also detected in the aqueous extract of nutmeg (Olaleye *et al*., 2006).

Krishnamoorthy and Mathew (2006) reported that monoterpene hydrocarbons together with smaller amount of oxygenated monoterpenes and aromatic ethers are the major constituents of both nutmeg and mace oil.

Biochemical profiling of leaf volatile oil of three *Myristica spp*. was done by Zachariah *et al*. (2008). Among the species, *M. fragrans* dominated in monoterpenes (91%) followed by *M. beddomei* which contained mono(48%) and sesquiterpenes (35%), whereas *M. malabarica* was dominated by sesquiterpenes (73%). They further recorded that α-pinene (19.59%), t-caryophyllene (14.63%) and β-pinene (12.46%) were more in *M. beddomei*. *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*.

According to Bakkali *et al.* (2008), myristicin (5-allyl-1-methoxy-2, 3 methylenodioxy benzene) is the principle component of nutmeg volatile oil which is present in both nut and mace.

Nutmeg leaves yield 0.41 to 0.60 per cent of light brown volatile oil with a pleasing spicy odour on water distillation. Steam distillation of dried leaves gave 1.58 per cent of colourless volatile oil containing alpha-pinene (80%) and myristicin (10%). Oil of mace resembles nutmeg oil in odour, flavour and composition and no distinction is made between them in the trade. Like nutmeg oil, mace oil also becomes viscous on storage due to absorption of oxygen. Old mace yields more viscous oil than the fresh one (Anon, 2008).

Abdurrasheed and Janardanan (2009) studied the GC profile of nut and mace oil collected from Tellicherry and Kannur, Kerala and revealed the compounds such as alpha-pinene (9.3 and 10.0 % respectively), sabinene (37.1

and 19.7% respectively), safrole (4.8 and 3.3% respectively), myristicin (12.5 and 22.0 respectively) and elemicin (27.2 and 30.2% respectively).

Aroma compounds of nutmeg are mainly monoterpenes (87.5%), monoterpene alcohols (5.5%) and other aromatics (7.0%). The principle 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).

In a study, Soni (2012) compared the volatile oil constituents of nutmeg collected from North East India and Kerala. Chemoprofiles of North East Indian nutmeg oil constituted majorly, a-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 a-pinene (15.21 %), sabinene (38.79%), limonene (4.51 %), terpineol (4.15 %), safrole (6.34 %) and myristicin (6.15 %).

Dried nutmeg seeds collected form Iraqi markets yielded volatile oil content 4.7-7.5 g/100g by steam distillation method. The oil has spicy odour, slightly pungent taste and colourless to pale yellow colour. Specific gravity (0.89 gm/ml), refractive index (1.48) and optical rotation (+22˚) were recorded in this sample oil. When subjected to gas chromatography they identified the presence of 49 volatile compounds (Al-Jumaily and Al-Amiry , 2012).

Myristicin, elemicin, sabinene and safrole constitute 80 per cent of both nutmeg and mace essential oils. Gas chromatography and mass spectrometry analysis of 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%), terpene-4-ol (9.60%) and several other minor components (Kapoor *et al.*, 2013).

Leela *et al*. (2013) chemoprofiled oil from nutmeg leaves, mace and kernel. Major components of leaf volatiles were α-pinene (59.7-67.1%), sabinene (45.7-63.1%) and β-pinene (32.6-50.7%). Nut volatiles were rich in myristicin and mace volatiles high in safrole and sabinene content.

Nutmeg germplasm A9/71, collected from a farmers field in Kallar, Ooty, Tamil Nadu, yielded high level of sabinene in nut and mace volatile oil (45.0 and 41.9 %, respectively). Other constituents of oil were low to medium (Rema *et al.*, 2013).

GC-MS analysis of nutmeg rind oil exhibited 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 (Teena, 2015).

2.5.2.1 Other tree spices

Eighteen compounds were identified in leaf oil of *Cinnamomum pauciflorum* comprising 98.80 per cent of the total volatile oil. Cinnamaldehyde (94.0%) was the predominant one (Baruah and Nath, 2006).

Rao (2006) studied the effect of storage of cinnamon leaves on content and chemical composition of essential oil. The results revealed that neither essential oil content nor chemical composition of oil such as eugenol, eugenyl acetate, linalool and benzyl benzoate was affected during the storage of leaves up to 15 months.

Rao *et al.* (2007) isolated the volatile oil from cinnamon petiole for the first time and when subjected to GC-MS analysis, it yielded 25 compounds (87.31% of the total volatile oil). (E)-Cinnamaldehyde (33.04%), eugenol (17.32%), linalool (16.85%) and (E)-cinnamyl acetate (11.78%) were the major compounds.

Kamaliroosta *et al.* (2012) reported that cinnamon volatile oil and total phenolic compounds from bark constituted 1.3 per cent and 5.77 mg/g, respectively. Gas chromatography-mass spectrometry profiling of cinnamon volatiles showed predominant constituent as cinnamaldehyde.

2.5.4 Isozyme analysis

Isozymes have been the most frequently used biochemical markers for the study of perennial crops, because they show co-dominant expression, lack epistatic and pleiotropic interactions and are consistently expressed despite environmental conditions. They have been utilised extensively to differentiate cultivars, to identify parents and characterize progeny following controlled pollinations and to construct genetic linkage maps (Moore and Durham, 1992).

In a gender identification study of nutmeg using the peroxidase biochemical marker, Angadi *et al*. (2006) observed that both the peroxidase banding pattern and enzyme activity of female was greater than that of male plants.

Sudhamayee *et al.* (2014) used biochemical markers for identification of female plants in nutmeg at early stages of growth. The biochemical marker assay of acid phosphatase did not record any polymorphism for male and female trees. But glutamate oxaloacetate transaminase showed low intensity polymorphic amplicons in females. However, repeatability of these results has to be ascertained for further confirmation.

2.5.4.1 Other tree spices

Diversity analysis conducted using isozyme markers namely, peroxidase, polyphenol oxidase, esterase and superoxide dismutase in cambodge population collected from Western Ghats revealed that most of them were from similar geographic locations. The mean percentage of polymorphic loci was 52.50 (Parthasarathy *et al.,* 2010).

Genetic diversity analysis in genus *Garcinia* spp. was studied using the peroxidase marker by Wittayawannakull *et al.* (2010). The study revealed eight polymorphic amplicons with polymorphism information content of 0.79.

2.6 Molecular studies

Molecular markers are proved to be highly heritable and exhibit enough polymorphism to discriminate genotypes of different crops (Kumar, 1999). They can be very useful in identification of accessions and varieties at early stages of growth and to characterise the genotype comprehensively. Application of molecular markers as complementary approach for genetic characterisation has been reported in many crops (Karp *et al.*, 1998).

2.6.1 Isolation of plant genomic DNA in nutmeg

Nutmeg is very rich in polyphenol and other secondary metabolites in the tissues making it very difficult for isolation of quality DNA from the genus *Myristica*. This difficulty is often characterized in the form of lower levels of DNA recovery and poor absorbance ratios due to RNA and protein contaminations, while analyzed in a spectrophotometer. These high metabolites in the tissues also negatively interfere with reactions such as DNA restriction, amplification and cloning (Bryant, 1997).

For the first time, suitable protocols for the recovery of high quality DNA in different species of nutmeg was standardised by Sheeja *et al.* (2008). They developed an efficient protocol for isolation of DNA from wild and related genera of Myristica rich in polysaccharides and poly phenols. The protocol utilized CTAB (3%), 1.5 per cent PVP and 0.3 per cent β-mercaptoethanol for isolation and RNase and phenol chloroform extraction for purification. In another study, Sudhamayee (2010) suggested that third leaf from the shoot tip and emerging pale green leaves can be used to yield good quality DNA in nutmeg. Deshpande and Lele (2011) perfected an efficient protocol for the isolation of high molecular weight DNA from powder of traded nutmeg seeds. This technique helps in tracing the geographical origin of the commodity.

Suitable protocols for the recovery of high quality DNA from nutmeg through different methods were evaluated by Divyasree *et al.* (2014). Among the different methods studied, CTAB protocol given by Doyle and Doyle (1987) was

found to yield the best in terms of DNA quantity and quality. The DNA recovery from the tender leaf tissues was high in the mean range of 83.55 µg/g with a purity of 1.79. They also suggested that emerging pale green leaf is best for the isolation, yielding a high quality of DNA with negligible protein and RNA contamination. This finding had been supported by Swapna *et al*. (2013) and Swapna *et al*. (2014).

2.6.1.1 Other tree spices

Swetha *et al.* (2014) have standardised the isolation of genomic DNA from the dried cinnamon bark. Results revealed that good quality of DNA ranged from 5 to 8.1 µg g^1 of dried bark and absorbance values at 260 nm and 280 nm gave a ratio higher than 1.8 indicating good quality of DNA. Electrophoresis amplification also confirmed the quality of isolated DNA.

2.6.2 RAPD and ISSR markers

Randomly amplified polymorphic DNA (RAPD) is one method of indentifying polymorphism that can be used to elicit information on molecular differences among individuals of a population between lines or accessions or any breeding materials (Welsh and McClelland, 1990). RAPD markers are generated by the use of short (10-mer) synthetic oligonucleotides in a single primer (Williams *et al*., 1990). Compared to other DNA based markers, RAPD maker is simple, fast and cost effective, it can be done with small amount of DNA. RAPD method is a universal set of random primers, used for genomic analysis of any organism (Welsh and McClelland, 1990).

Inter simple sequence repeats (ISSR) techniques is a PCR based method, which involves amplification of DNA segments present at an amplifiable distance in between two identical microsatellite repeat regions, oriented in opposite direction. ISSR markers usually 15-25bp long, as primers in a single primer PCR reaction, targeting multiple genomic loci to amplify mainly the inter sequence of different sizes. ISSR markers have high reproducibility, due to the use of longer

primers (16-25 mers) which permits the subsequent use of high annealing temperature leaning to higher stringency (Gupta *et al*., 1994).

Sheeja *et al*. (2006) analysed the clonal and seedling progenies of a high yielding selection of nutmeg for detecting the variations among the populations and uniqueness using RAPD profiles. Total of 405 amplified products were scored with twenty polymorphic loci. The similarity index within the clones and seedlings were ranging from 96-100 per cent and 76-100 per cent. The Jaccard's Similarity Index values ranged from 0.72 to 1.00 showed the close relatedness among the progenies and mother.

Mansyah *et al*. (2013) investigated the genetic variation in apomictic *Garcinia* from West Sumatra collections using RAPD technique. Molecular analysis revealed variation among the *Garcinia* accessions and similarity index ranged between 42 and 100 per cent.

RAPD and ISSR markers were employed to investigate the phylogenetic relationships among the ten different species and related genera of *Myristica*. The 20 RAPD and 16 ISSR primers produced the 497 and 262 amplified DNA fragments. The mean polymorphism information content (PIC) was 0.34 and 0.35 for RAPD and ISSR markers respectively. The combined cluster analysis of RAPD and ISSR markers generated the species broadly into two major groups comprising of *M. praini* in the first and remaining species including *M. fragrans* in second group. These similarities at molecular level were close to either morphological or geographical locations (Sheeja *et al.*, 2013).

Aniedi *et al*. (2014) analysed the genetic diversity in twenty one African nutmeg accessions from South Eastern regions of Nigeria by employing the RAPD markers. Total of seventy seven bands were generated, ranging from 3 (OPB 17) to 13 (OPT 07), all were polymorphic. The mean polymorphic information content and genetic diversity were 0.763 and 0.697, respectively. Cluster analysis depicted four groups and showed high genetic variation among the accessions.

Sudhamayee *et al*. (2014) have reported sex specific molecular marker in nutmeg for the identification of female plants in the early stages of growth. The study revealed that RAPD marker, OPK 01 generated specific fragments to female plants. However, repeatability of these results has to be ascertained for confirmation.

2.6.2.1 Other tree spices

Abeysinghe *et al.* (2009) used the nucleotide sequence of different cpDNA regions and an ITS region of the rDNA to assess genetic distinctiveness and relatedness of nine *Cinnamomum* spp. in Sri Lanka. The molecular analysis has not clearly revealed the genetic distinction among the species, but study would greatly facilitate identification of species correctly at DNA level.

Wittayawannakull *et al.* (2010) analysed the genetic variability in twenty two *Garcinia* accessions by using the RAPD technique. They obtained the sequence specific amplification and recorded high polymorphic value indicating a wide genetic diversity among the accessions. Cluster analysis of twenty two *Garcinia* accessions directed to five groups at mean similarity coefficient 0.54.

Morphological and molecular diversity analysis among the kokum genotypes collected from four different locations of Maharashtra was assessed by Thatte *et al*. (2012) using the RAPD and ISSR markers. Morphological traits showed very high variation for all the four locations with respect to plant height and leaf characters. Molecular analysis showed maximum genetic diversity for two locations such as Sawantwadi and Diveagar.

Thatte and Deodhar (2012) examined morphological and biochemical parameters for detecting the difference between the male and female trees in *Garcinia indica* with no positive results. The positive results have been obtained by using DNA markers. The sequence specific primers such as OPW 05 and OPW 08 of RAPD markers and UBC 881 of IISR markers showed the polymorphic bands in female and male plants respectively.

Field evaluation of apomictic *Garcinia* populations in its native habitat recorded high variability for tree shape, fruit shape and petal colour. Morphological variations also showed conformity with the molecular level variability using RAPD, Amplified Fragment Length Polymorphism (AFLP) and ISSR markers (Sobir *et al.*, 2013).

Gangaprasad *et al.* (2013) used twenty eight RAPD primers to assess the genetic relatedness and genetic diversity of thirteen Indian tamarind collections. Molecular analysis showed that all the primers used revealed clear distinction among genotypes and they generated a total of 131 scorable amplicons of which 116 were observed polymorphic with 88.54 per cent of polymorphism. Genetic similarity coefficient values indicated that a wide genetic base of genotypes was used for the study.

Morphological and molecular diversity analysis between *Garcinia* and their close relative species was carried out by Sulassih *et al*. (2013) using ISSR technique. They obtained a total of 212 polymorphic amplicons and genetic similarity between the *Garcinia* species were 0.78 and 0.63. Based on cluster analysis performed following UPGMA method, the accessions were grouped into three clusters.

Materials and Methods

3. MATERIALS AND METHODS

The present investigation "Characterization and evaluation of nutmeg (*Myristica fragrans* Houtt.) accessions" was carried out at the Department of Plantation Crops and Spices, College of Horticulture, Kerala Agricultural University, Thrissur during 2012-2015. Details of the materials used and methods followed in the study are described in this chapter.

3.1 STUDY AREA

Fifty accessions of nutmeg collected from diverse locations of Kerala and assembled at two private plantations in Chalakkudy in central Kerala lying between latitude 10.30^0 N and longitude 76.33^0 E formed the material for the study. The plantations belong to two professional budders namely, Mr. Joby C. Peenickaparamban and Mr. Antony C. Peenickaparamban. A distinct feature of these two plantations is that a core collection of nutmeg germplasm procured from the major nutmeg growing tracts of Kerala is readily available (Plate 3.1). The trees are intercropped with coconut and are under good management. These accessions belong to the same age of fifteen years as per farmers registers. Fifty nutmeg accessions were selected from these plantations together after thorough visual evaluation and these selected accessions comprised of forty two females, four monoecious and four males. Two trees per accession were readily available for recording observations. The details of the select nutmeg accessions are presented in Table 3.1. The standard procedure of recording the qualitative and quantitative characters was followed by taking samples from all the four sides of the tree.

3.2 CHARACTERIZATION OF NUTMEG ACCESSIONS

All the 50 accessions were subjected to morphological, biochemical and molecular characterization.

Experimental trees

Advisory committee

Farmer with the researcher

Plate 3.1 Study plot in the Chalakkudy river basin

Table 3.1. Details of nutmeg accessions included in the study

Sl. No.	Accession	Sex form	Nature of tree
1	Acc.1	Female	Budded on *M. fragrans*
2	Acc. 2	Female	Budded on *M. fragrans*
3	Acc. 3	Female	Budded on *M. fragrans*
4	Acc. 4	Female	Budded on *M. fragrans*
5	Acc. 5	Female	Budded on *M. fragrans*
6	Acc. 6	Female	Budded on *M. fragrans*
7	Acc. 7	Female	Budded on *M. fragrans*
8	Acc. 8	Female	Budded on *M. fragrans*
9	Acc. 9	Female	Budded on *M. fragrans*
10	Acc. 10	Female	Budded on *M. fragrans*
11	Acc. 11	Female	Budded on *M. fragrans*
12	Acc. 12	Female	Budded on *M. fragrans*
13	Acc. 13	Female	Budded on *M. fragrans*
14	Acc. 14	Female	Budded on *M. fragrans*
15	Acc. 15	Female	Budded on *M. fragrans*
16	Acc. 16	Female	Budded on *M. fragrans*
17	Acc. 17	Female	Budded on *M. fragrans*
18	Acc. 18	Female	Budded on *M. fragrans*
19	Acc. 19	Female	Budded on *M. malabarica*
20	Acc. 20	Female	Budded on *M. beddomei*
21	Acc. 21	Female	Budded on *M. fragrans*
22	Acc. 22	Female	Budded on *M. fragrans*
23	Acc. 23	Female	Budded on *M. fragrans*
24	Acc. 24	Female	Budded on *M. fragrans*
25	Acc. 25	Female	Budded on *M. fragrans*
26	Acc. 26	Female	Budded on *M. fragrans*
27	Acc. 27	Female	Budded on *M. beddomei*
28	Acc. 28	Female	Budded on *M. fragrans*
29	Acc. 29	Female	Budded on *M. fragrans*
30	Acc. 30	Female	Budded on *M. fragrans*
31	Acc. 31	Female	Budded on *M. fragrans*
32	Acc. 32	Female	Budded on *M. fragrans*
33	Acc. 33	Female	Budded on *M. fragrans*
34	Acc. 34	Female	Budded on *M. fragrans*
35	Acc. 35	Female	Budded on *M. fragrans*
36	Acc. 36	Female	Budded on *M. fragrans*
37	Acc. 37	Female	Budded on *M. fragrans*
38	Acc. 38	Female	Budded on *M. fragrans*
39	Acc. 39	Female	Budded on *M. fragrans*
40	Acc. 40	Female	Budded on *M. fragrans*
41	Acc. 41	Female	Budded on *M. fragrans*
42	Acc. 42	Female	Budded on *M. fragrans*
43	Acc (H). 1	Monoecious	Seedling
44	Acc (H). 2	Monoecious	Seedling
45	Acc (H). 3	Monoecious	Seedling
46	Acc (H). 4	Monoecious	Seedling
47	Acc (M). 1	Male	Seedling
48	Acc (M). 2	Male	Seedling
49	Acc (M). 3	Male	Seedling
50	Acc (M). 4	Male	Seedling

3.2.1 Morphological characterization

3.2.1.1 Qualitative and quantitative parameters

Observations on qualitative as also quantitative parameters recorded from the selected accessions were utilised for the morphological characterization. In total fifty one qualitative parameters and thirty eight quantitative parameters were recorded through the various phenophases of the tree. Fruit and yield observations were recorded during the peak period of harvest. The first platform to be stepped into towards characterization of nutmeg is a broad based standard descriptor which is yet to be built up. The first venture was to create such a platform. Towards this end, the observations were recorded on various parameters and the mode of recording the observation for each parameter was decided based on expertise as also expert opinion and the said parameters along with method of recording are presented in Table 3.2. Floral measurement methods followed in the study is depicted in Plate 3.2.

3.2.1.2 Incidence of pests and diseases

Preliminary scorings of the accessions were done as and when disease and pest incidence was noticed during the course of study. Scoring was done for major diseases of nutmeg *viz.*, *Phytopthora* leaf fall, *Colletotrichum* leaf spot, *Colletotrichum* fruit rot, *Marasmius* thread blight and *Lasiodiplodia* die back. Leaf diseases and fruit rot severity were scored (0- 5 scale) by collecting forty samples randomly from the four sides of the tree, while thread blight and die back severity were scored (0- 5 scale) by fixing the quadrats (1 m^2) randomly on the four sides of canopy of tree. Results were expressed as per cent disease incidence.

The grading is tabled below.

Grade	Per cent infected leaves/area	Category
0	No infection	Zero
1	< 10 %	Very minute
2	> 10 – 25 %	Medium

Table 3.2. Morphological parameters of nutmeg and methods of recording

Sl. No.	Character	Mode/time/stage of recording
A – Qualitative characters		
1	Sex form	Bearing trees
2	Stem colour	Bearing trees stem
3	Branching pattern	Growth stage
4	Canopy shape	Fully mature trees
5	Foliage density	Fully mature trees
6	Flushing pattern	Growth stage
7	Colour of flushes	New flush emergence
8	Shape of mature leaf	Measured on the 5th leaf from bud at the tip of the branch
9	Colour of mature leaf	Matured leaf
10	Leaf margin	Visual observation
11	Shape of leaf apex	Visual observation
12	Season of flowering	Peak flowering season
13	Periodicity of flowering	Visual observation throughout the growth cycle
14	Inflorescence type	Visual observation at peak flowering
15	Number of flowers per cluster	Peak flowering season
16	Frequency of flower clusters	During flowering season
17	Nature of fruit bearing	Visual observation during peak season
18	Periodicity of fruit bearing	Observed bearing trees which have attained stability in yield
19	Number of fruits per cluster	Flowering season
20	Frequency of fruit clusters	Visual observation during peak season
21	Density of fruit bearing	Peak bearing season
22	Nature of fruit dehiscence	Stage of falling of fruits
23	Bearing season	Bearing trees that has stable yield
24	Colour of pedicel	Observed on simultaneously during flowering season
25	Colour of perianth	Peak flowering
26	Shape of perianth	Peak flowering
27	Colour of filament	Peak flowering
28	Colour of anther	Peak flowering
29	Colour of pistil	Peak flowering
30	Colour of immature fruit	Green fruit stage

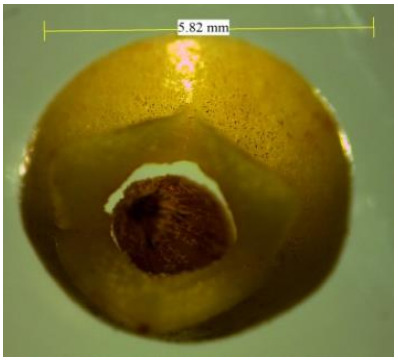
31	Colour of mature fruit	Recorded at mature fruit stage
32	Shape of fruit	Visual observation during peak bearing season
33	Shape of fruit base	Visual observation during peak bearing season
34	Shape of fruit apex	Visual observation during peak bearing season
35	Fruit pubescence	Visual observation during peak bearing season
36	Number of seeds per fruit	Visual observation during peak bearing season
37	Nature of fruit splitting	Observed in mature and split opened fruits
38	Number of splits in split fruits	Observed in mature and split opened fruits
39	Shape of mace	Visual observation of split opened fruits
40	Colour of mace (fresh)	Visual observation of split opened fruits
41	Colour of mace (dry)	Visual observation of dried mace
42	Nature of mace	Recorded in mature fruits
43	Beakness of mace	Visual observation of fresh harvested mace
44	Attachment of mace to nut	Split opened fresh fruit
45	Shape of nut	Split opened fresh fruit
46	Colour of nut (fresh)	Split opened fresh fruit
47	Colour of nut (dry)	Observed in dried nut
48	Grooves on nut	Observed in mature nut
49	Nature of groove on nut	Observed in mature nut
50	Colour of kernel (dry)	Deshelled nuts after drying
51	Kernel wrinkles	Deshelled nuts after drying
Section B- Quantitative characters		
52	Tree height (m)	The length from ground level to top most portion of the canopy
53	Girth at 140 cm height (cm)	The circumference of the tree trunk measured at 140 cm height form the base
54	Canopy spread (N-S & E-W)- (m)	Recorded simultaneously with tree height as the maximum canopy diagonal from E-W and N-S direction
55	Internodal length (cm)	Recorded as distance between three consecutive internodes from base of tree
56	Number of orthotrops	Recorded by counting of orthotropes present in the tree at the time of growth observations
57	Leaf length (cm)	Recorded as mean length of 5^{th} leaf from bud from the base to tip of the leaf blade, 10 leaves each on four sides of the tree.
58	Leaf breadth (cm)	Recorded on the same leaves used for measurement of leaf length. Width is measured at the widest portion of the leaf

59	Leaf area (cm^2)	Recorded on the same leaves used for measurement of the length and breadth of leaf. Leaf area is measured for the whole leaf
60	Chlorophyll content	Recorded using chlorophyll meter
61	No .of flowers/10cm^2	Recorded by counting number of flowers in 10 cm^2 area of tree canopy on all the four sides of the tree
62	Length of flower (mm)	Recorded the mean length of 10 flowers. Length is recorded from base to tip of flower
63	Breadth of flower (mm)	Recorded on the same flowers used for measuring the length of flower. Breadth recorded at the widest portion of flower
64	Length of tepal (mm)	Recorded the mean length of 10 flowers. Length is recorded from base to tip of tepal
65	Breadth of tepal (mm)	Recorded simultaneously on same tepal used for measuring tepal length. Breadth recorded at the widest portion of tepal
66	Length of pistil (mm)	Recorded on the female flower. Pistil length measured from base to tip of pistil
67	Breadth of pistil (mm)	Recorded on the same female flower. Pistil breadth measured from base to tip of pistil
68	Fruit set percentage	Number of fruits set in marked flowers were counted and expressed in percentage
69	Fruit weight (g)	Recorded the mean weight of 25 randomly collected ripe fruits
70	Fruit length (mm)	Recorded mean length of 25 ripe fruits randomly harvested
71	Fruit breadth (mm)	Recorded on the same fruits used for measuring the fruit length. Breadth is recorded at the widest portion of the fruit
72	Thickness of pericarp (mm)	Measured using vernier calliper in the split open fruits
73	Mace weight (fresh and dry)- (g)	Mace was separated from 25 randomly collected split open fruits, weighed fresh and oven dried to take dry weight
74	Nut weight (fresh and dry)- (g)	Nut was separated from the 25 randomly collected split open fruits. Weighed fresh and then oven dried to record dry weight
75	Shell thickness (mm)	Recorded after deshelling and measured using vernier calliper. Mean weight of 25 shells recorded
76	Kernel weight (g)	Kernel weight was recorded after deshelling of nut. The mean weight of 25 kernels recorded
77	Fruit volume (cm^3)	Recorded by water displacement method. Expressed as cm^3

78	Nut volume (cm^3)	Water displacement method. Expressed as cm^3
79	Mace volume (cm^3)	Water displacement method. Expressed as cm^3
80	Kernel volume (cm^3)	Water displacement method. Expressed as cm^3
81	Nut length (mm)	Mean length of 25 nuts randomly collected
82	Nut breadth (mm)	Recorded on the same nuts used for measuring the nut length. Breadth was recorded at the widest portion of the nut
83	Ratio of nut to mace	Proportion of nut and mace weight was recorded and calculated as ratio of nut to mace
84	Shelling percentage	Recorded as ratio of kernel to dried nut weight. Expressed in percentage
85	No. of fruits/m^2	Recorded by fixing $1m^2$ quadrats during peak bearing season on all the four sides of the tree
86	Number of fruits per tree	Recorded by counting fruit number regularly during peak harvesting period. Observation was made during two consecutive bearing seasons, 2013 and 2014
87	Fresh and dry nut yield (kg/tree/year)	Estimated from average nut weight and number of fruits per tree
88	Kernel yield (kg/tree/year)	Estimated from average kernel weight and number of fruits per tree
89	Fresh and dry mace yield (kg/tree/year)	Estimated from average mace weight and number of fruits per tree

Length of flower

Breadth of flower



Breadth of flower

` Length of tepal

Breadth of tepal

Anther lobe

Length of anther lobe

Length of pistil

Plate 3.2. Floral measurement methods in nutmeg

3	>25 – 50 %	Moderate
4	>50 – 75 %	Severe
5	>75 %	Very severe

Per cent Disease Severity (PDS) was calculated using the formula suggested by Wheeler (1969).

$$PDS = \frac{\text{Sum of total numerical ratings}}{\text{Total number of leaves/area observed} \times \text{Maximum disease grade}} \times 100$$

3.2.1.3 Statistical analysis

Qualitative parameters were subjected to cluster analysis. Genetic association among the accessions were estimated by Jaccard's similarity coefficients (Jaccard, 1908) using NTSys pc version 2.1 (Rohlf, 1992). Based on the similarity matrix, cluster analysis was performed and dendrogram was constructed by unweighted pair-group method (UPGMA) for the female and bisexual accessions (Sneath and Sokal, 1973).

All the thirty eight quantitative characters were initially subjected to analysis of variance as Completely Randomised Design (CRD). Out of the thirty eight observations recorded, twenty six characters were selected based on statistical significance and economic importance for further analysis.

Descriptive statistics *viz.*, mean, range, standard deviation, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (H^2), genetic gain (GG), kurtosis and skewness were estimated. The genotypic and phenotypic correlation coefficients and genotypic path coefficient analysis among the characters were also worked out. Grouping of the accessions was done and the genetic divergence was computed for forty six accessions

(female and monoecious) following the D^2 statistics developed by Mahalanobis (1936).

3.2.2 Biochemical characterization

3.2.2.1 Estimation of contents of volatile oil, oleoresin and fixed oil

The selected forty six nutmeg accessions (female and monoecious) were evaluated for contents of oil and oleoresin in both the kernel and mace. Fixed oil content was estimated only from kernel. The procedures followed for estimation of contents of volatile oil, oleoresin and fixed oil are furnished below.

3.2.2.1.1 Volatile oil content of kernel and mace

The essential oil was isolated by hydro distillation method using the Clevenger's apparatus (Clevengers, 1982). Known quantity of the dried and powdered samples of both kernel and mace were separately subjected to boiling with appropriate proportion of water for three hours. Oil content was measured and expressed in weight by volume basis as per cent of oil.

3.2.2.1.2 Oleoresin content of kernel and mace

The dried powdered samples of both kernel and mace were used for extracting oleoresin. Oleoresin was determined by solvent extraction method with petroleum benzene (60-80^0 C boiling range) using soxhlet apparatus for three hours. The results were expressed as percentage (ASTA, 1968).

3.2.2.1.3 Fixed oil content of kernel

The dried powdered sample of kernel was subjected to fixed oil content estimation by cold percolation method using glass column. Fixed oil content was estimated by using acetone after three washings and results were expressed as percentage (AOAC, 1975).

3.2.2.1.4 Statistical analysis of oil, oleoresin and fixed oil contents

The oil, oleoresin and fixed oil contents in the samples were calculated and expressed in percentage. Analysis of variance was estimated for the characters. Jaccard's similarity coefficients were worked out for all the forty six accessions using NTSys pc version 2.1 (Rohlf, 1992). Based on the similarity matrix, cluster analysis was performed and dendrogram constructed by UPGMA by Sneath and Sokal (1973).

3.2.2.2 Chemoprofiling of volatiles using GC MS

Volatile oils of seventeen distinct nutmeg accessions (both kernel and mace) were subjected to Gas Chromatography Mass Spectroscopy (GC MS) analysis.

GC MS of the oil samples was recorded in a Shimadu GC MS QP-2010 equipped with capillary column RTX-5 of 0.25µ × 0.32 mm × 30 m dimension. Pure volatile oil (1 µl) was fed to the injector with a port temperature 220^0 C, interface temperature was 240^0 C, and detector temperature was 240^0 C. Carrier gas used was helium with a linear velocity of 48.1 cm /sec split ratio 50. Iodination energy 70 eV and mass range 40-650 amu. Major compound peaks were analysed by comparing its mass fragment pattern with the standard spectra available in the data base.

Quartile cumulative frequency analysis was calculated for grading components of oil. Further, accessions were ranked based on values in the three category (as Q_1, Q_2 and Q_3) based on major components of the volatile oil.

Volatile oil of select nutmeg accessions which yielded higher quantities of major components like myristicin, elemicin, sabinene and safrole were again subjected to GC-MS analysis after storing the oil for one year. The comparison of fresh and stored volatile constituents was done and change in constituents was calculated and expressed in percentage.

3.2.2.3 Biochemical analysis of nutmeg pericarp

Pericarp separated from seventeen distinct nutmeg accessions was subjected to biochemical analysis following standard analytical procedures (Table 3.3). Freshly collected split opened nutmeg pericarp was used for the analysis.

Analysis of variance was performed for all the biochemical data with completely randomised design. Multiple comparisons among the treatments were done using Duncan's Multiple Range Test. Accessions were grouped into high and low based on significance of the values.

3.2.2.4 Isozyme profiling

Seventeen distinct nutmeg accessions from the different clusters were subjected to isozyme profiling. Tender leaves were collected early in the morning. One gram of leaf tissue from each accession was homogenised in 0.75 ml extraction buffer solution containing antioxidants and protease inhibitors. Isolation was carried at 4^0 C with pre chilled mortar and pestle.

The extracts were then centrifuged at 1500 rpm for 15 min at 3^0 C. The clear supernatant was collected and used as the enzyme source for electrophoresis. Forty to fifty microgram protein equivalent of the supernatant was loaded on the 10% poly acrylamide gel along with loading dye. Electrophoresis was carried out at 20 mA for a period till the tracking dye migrated to the lower end of the separating gel.

Peroxidase isozyme was stained using benzidine as the donor. The gel was stained with sodium acetate buffer (pH 5.1) containing 3% H_2O_2 and 0.1% benzidine in a dark condition for 30 min. The isoforms of peroxidase were visualized and documented (Nazeem *et al*., 2008)

Polyphenol oxidase was detected by enzymatic browning. The gel was equilibrated for 30 min in 0.1% p-phenylene diamine in 0.1 M potassium phosphate buffer (pH 7) followed by 10mm catechol in the same buffer. The addition of catechol was followed by gentle shaking which resulted in the

Table 3.3. Biochemical estimation of nutmeg pericarp

Sl. No.	Component	Analytical method	Reference
1	Moisture (%)	Oven dry method	Ranganna (1986)
2	Acidity (titrable acidity)- (%)	Titration method	Ranganna (1997)
3	Ascorbic acid (mg/100g)	Volumetric method	Sadasivam and Manickam (2010)
4	Pectin (as % Calcium pectate)	Gravimetric method	Ranganna (1986)
5	Protein (g/100g)	Lowry's method	Sadasivam and Manickam (2010)
6	Starch (g/100g)	Colorimetric method	Sadasivam and Manickam (2010)
7	Total phenol (mg/100g)	Floin ciocalteau reagent Method	Sadasivam and Manickam (2010)
8	Tannin (mg/100g)	Folin-Denis method	Sadasivam and Manickam (2010)
9	Total minerals (%)	Volatilizing organic matter method	Ranganna (1986)
10	Crude fibre (%)	Acid – alkali digestion method	Chopra and Kanwar (1978)

appearance of dark brown distance protein bands (Sadasivam and Manickam, 2010).

The stained gel was used for scoring data. The bands were scored visually for their presence (1) or absence (0) of enzyme amplification. The characteristic matrix base in the score was used for construction of dendrogram using the NTSys pc software version 2.0 (Rohlf, 1992).

3.2.2.5 Enzyme activity

3.2.2.5.1 Activity of peroxidase

Fresh leaf tissue (1g) samples collected in the morning were homogenized by grinding with 0.1 M phosphate buffer (pH 7.0) containing protease inhibitors. Homogenates were centrifuged at 10000 rpm for 15 min at 4^0C and the supernatant was used as the enzyme source. Peroxidase activity was measured spectrophotometrically and the absorbance was read at 470nm. Assay system containing 30 ml of 0.1M phosphate buffer (pH 7) at 25^0C was added to 50 µl of 20 mm guaiacol solution, 100 µl of enzyme extract and 30 µl of 0.0042 % H_2O_2 in cuvette. The enzyme activity was expressed in units/µl (Sadasivam and Manickam, 2010).

3.2.2.5.2 Activity of polyphenol oxidase

The leaf tissue (1 g) was homogenised by grinding with 50 mm tris HCl (pH 7.2), 0.4 M sorbitol and 10 mm Nacl. The homogenate was centrifuged at 12000 rpm for 10 min at 4^0C and the supernatant was used for the assay. 2.5 ml of 0.1 M sodium phosphate buffer (pH 6.0) was added to 0.5 ml of 0.01 M catechol and 100 µl of enzyme extract. All the components were mixed well and absorbance read at 495 nm in spectrophotometer (Sadasivam and Manickam, 2010).

3.2.3 Molecular characterization using RAPD and ISSR markers

Seventeen distinct nutmeg accessions from the clusters were subjected to molecular characterization. RAPD and ISSR markers were employed for screening and, to characterize and estimate the genetic diversity among the accessions at DNA level.

3.2.3.1 Isolation of genomic DNA

Nutmeg leaf is rich in secondary metabolites like phenols, which interfere with the isolation and purification of DNA. Phenol contamination can also cause problems in downstream processing and amplification by inhibiting certain enzymes in PCR. Young tender leaves (third leaf from the shoot tip) were collected from the trees and stored in ice until isolation. The DNA extraction was done following the Cetyl Trimethylammonium Bromide (CTAB) protocol (Doyle and Doyle, 1987) with modifications, as descried below.

The leaf tissue (0.2 g) was ground into fine powder with liquid nitrogen in the presence of 1.5% PVP (Polyvinyl pyrrolidone) and 0.3% β-mercaptoethanol. Transferred the sample into 2ml centrifuge tube containing 1ml of pre warmed extraction buffer (5%). The contents were incubated at 65^0C for 30 minutes with occasional mixing. An equal volume of chloroform: isoamyl alcohol mixture (24:1) was added and mixed by inversion for 10 minutes at room temperature, and then centrifuged at 10000 rpm for 15 minutes at 4^0C. Carefully transferred the aqueous phase, added 2/3rd volume of ice cold isopropanol and incubate tubes at -20^0C for 20 minutes. The DNA was precipitated in ice cold isopropanol and it was centrifuged at 10000 rpm for 15 minutes at 4^0C. Discarded the supernatant, wash the pellet with 70 per cent ethanol and then contents were centrifuged at 10000 rpm for three minutes at 4^0C. Pellets were air dried and dissolved in 50 µl of autoclaved distilled water.

The DNA which had RNA as contaminant was purified by RNase treatment and incubated at 37^0C for 30 minutes. Add equal volume of chloroform: isoamyl alcohol (24:1) mixture and gently mixed by repeated inversions.

Precipitate was centrifuged once again at 10000 rpm for 15 min at 4^0C. DNA pellets were washed with 70% ethanol, air dried and stored at -20^0C after completely dissolving in 50 µl autoclaved distilled water.

The purity of the DNA sample was determined by measuring the absorbance at A260 nm in a NanoDrop^R ND-1000 spectrophotometer and A260/A280 ratio was evaluated. Molecular weight and concentration of the DNA was estimated using agarose gel electrophoresis on 0.8 per cent agarose and visualized by ethidium bromide staining. The DNA and gel loading dye (8:2 ratio) mixture were thoroughly mixed by pipetting up and down several times. The contents were loaded carefully using micropipette. The gel was run at 70 volts until the tracking dye reached 2/3rd length of the gel. Gel documentation was done with the gel documentation system (Alpha Imager, USA). The quality of DNA was ascertained through restriction digestion using EcoRI/Hind III double digestion. DNA dilutions were made with autoclaved distilled water to a final concentration of 30ng/µl and stored in -20^0C (Sheeja *et al.,* 2008).

3.2.3.2 Polymerase chain reaction and analysis of markers

Forty three RAPD and eighteen ISSR markers were used for the analysis. The list of RAPD and ISSR primers used and their sequence are given in Table 3.4 and 3.5 respectively.

Composition of the reaction mixture for PCR (0.5 ml PCR tubes)

a. Template DNA (30ng/ul) : 1.00 µl
b. 10X reaction buffer A : 2.00 µl
c. $MgCl_2$ (20 mM) : 0.50 µl
d. dNTP's (10 mM) : 0.50 µl
e. Taq DNA polymerase (3U/ µl) : 0.30 µl
f. Primer (10 pM) : 1.50 µl
g. Autoclaved distilled water : 14.20 µl

Total volume : 20.00 µl

Table 3.4. List of decamer primers used for screening DNA samples from nutmeg accessions

Sl. No.	Primer name	Primer sequence	Annealing temperature (^{0}C)
1	OPA 01	5'CAGGCCCTTC3'	37
2	OPA 07	5'GAAACGGGTG3'	37
3	OPA 08	5'GTGACGTAGG3'	37
4	OPA 09	5'GGGTAACGCC3'	37
5	OPA 10	5'GTGATCGCAG3'	37
6	OPA 11	5'CAATCGCCGT3'	37
7	OPA 12	5'TGGGCGATAG3'	37
8	OPA 14	5'TCTGTGCTGG3'	37
9	OPA 16	5'AGCCAGCGAA3'	37
10	OPA 17	5'GACCGCTTGT3'	37
11	OPA 18	5'AGGTGACCGT3'	37
12	OPB 20	5'GGACCCTTAC3'	37
13	OPC 02	5'GTGAGGCGTC3'	37
14	OPC 12	5'TGTCATCCCC3'	37
15	OPC13	5'AAGCCTCGTC3'	37
16	OPE 03	5'CCAGATGCAC3'	37
17	OPE 12	5'TTATCGCCCC3'	37
18	OPE 14	5'TGCGGCTGAG3'	37
19	OPE 15	5'ACGCACAACC3'	37
20	OPE 16	5'GGTGACTGTG3'	37
21	OPB 08	5'GTCCACACGG3'	37
22	OPB 09	5'TGGGGGACTC3'	37
23	OPB 14	5'TCCGCTCTGG3'	37
24	OPB 19	5'ACCCCCGAAG3'	37
25	OPB 07	5'GGTGACGCAG3'	37
26	OPA 02	5'TGCCGAGCTG3'	37
27	OPC 05	5'GATGACCGCC3'	37
28	OPC 07	5'GTCCCGACGA3'	37
29	OPC 08	5'TGGACCGGTG3'	37
30	OPD 07	5'TTGGCACGGG3'	37
31	OPD 08	5'GTGTGCCCCA3'	37
32	OPL 12	5'GGGCGGTACT3'	37

Table 3.4. Continued….

33	OPP 13	5'GGAGTGCCTC3'	37
34	OPL 18	5'ACCACCCACC3'	37
35	OPX 17	5'GACACGGACC3'	37
36	OPY 02	5'CATCGCCGCA3'	37
37	OPY 17	5'ACCCCCGAAG 3'	37
38	OPC 17	5'TTCCCCCCAG3'	37
39	OPB 10	5'CTGCTGGGAC3'	37
40	OPB 06	5'TGCTCTGCCC3'	37
41	OPB 03	5'CATCCCCCTG3'	37
42	OPC 19	5'GTTGCCAGCC3'	37
43	OPB 13	5'TTCCCCCGCT3'	37

Table 3.5. List of ISSR primers used for screening DNA samples from nutmeg accessions

Sl. No.	Primer name	Primer sequence	Annealing temperature (^{0}C)
1	ISSR 01	5' CACCGCACGCACGCACG3'	51
2	UBC 893	5' AGCAGCAGCAGCGT53'	41
3	UBC 820	5' GTGTGTGTGTGTGTGTC3'	47
4	UBC 814	5' CTCTCTCTCTCTCTCTA3'	45
5	$(TC)_7C$	5' TCTCTCTCTCTCTCC3'	41
6	$(CT)_7AC$	5' CTCTCTCTCTCTCTAC3'	47
7	ISSR 22	5' GACAGACAGACAGC3'	39
8	ISSR 23	5' ACACACACACACACT3'	39
9	ISSR 25	5' ACACACACACACACG3'	41
10	ISSR 26	5' CTCCTCCTCGC3'	33
11	UBC 808	5' AGAGAGAGAGAGAGAGC3'	47
12	UBC 809	5' AGAGAGAGAGAGAGAGG3'	47
13	UBC810	5' GAGAGAGAGAGAGAGAT3'	45
14	UBC812	5' GAGAGAGAGAGAGAGAA3'	47
15	UBC816	5' CACACACACACACACAT3'	45
16	UBC856	5' ACACACACACACACACYA3'	49
17	UBC857	5' ACACACACACACACYG3'	45
18	UBC864	5' ATGATGATGATGATGATG3'	43

The reaction mixture was given a short spin and was subjected to polymerase chain reaction.

The PCR tubes were placed in the thermal cycler firmly and the following programme with optimized annealing temperature for each primer was advocated.

Step 1: Initial denaturation : 93^0 C for 5 minutes
Step 2: Denaturation : 93^0 C for 30 seconds
Step 3: Primer annealing : (temperature set according to primer)
Step 4: Primer extension : 72^0 C for 1 minute
Step 5: Final extension : 72^0 C for 10 minutes
Step 6: 4^0 C to hold the samples infinitely

Steps 2, 3 and 4 were set to run for 40 cycles.

The same PCR reaction mixture and condition were used for both RAPD and ISSR markers. Amplification products were resolved specific on a 1.2 % agarose gel for RAPD markers and 1.5 % agarose gel for ISSR markers stained with ethidium bromide and electrophoresed at 70 volts for 2.5 hours. The gels were photographed and visualized in a gel documenter system (Alpha Imager, USA).

The bands were scored visually for their presence (1) or absence (0) with each primer. The characteristic matrix based on the scores was used for constructing dendrogram using NTSys pc software version 2.1 (Rohlf, 1992).

3.3 INTER CLUSTER ASSOCIATION OF QUALITATIVE CLUSTER WITH OTHER CLUSTER AGGLOMERATIONS

Inter cluster association of two cluster agglomerations was worked out by finding the per cent distribution of the members of a specific cluster over the clusters of the other cluster agglomerations.

Mean, standard deviation and coefficient of variation were worked out for all the different quantitative clusters to assess the quantum of variability exhibited by the formation characters in each cluster.

The inter cluster association formula proposed by Latha (2010) was modified to accommodate within variability of each of the quantitative cluster.

The perceived morphological dimensions of the members of the eleven qualitative and ten quantitative cluster agglomerations were calculated using the formula,

$$\text{Perceived morphological characteristics} = \frac{\sum_{i=1}^{n} Pi\ Wi\ Xi}{\sum_{i=1}^{n} Pi\ Wi}$$

Where, Pi = Per cent accessions falling in quantitative cluster i

Xi = Corresponding character mean based on the members falling in quantitative cluster i

Wi = Inverse of the standard deviation of the corresponding characters based on the members falling in quantitative cluster i

n = Total numbers of quantitative clusters

3.4 KEY FOR IDENTIFICATION OF ELITE NUTMEG TREE

The data base was generated selecting the best performed clusters from the perceived morphological dimension analysis. The key characters were selected based on the statistical analysis and commercial importance. Using these key quantitative characters, the statistical key was developed logically from the data base, which can serve as a tool for identifying an ideal nutmeg tree.

The step wise methodology adopted in the study is depicted in the following flow chart

FLOW CHART OF METHODOLOGY

Fifty select nutmeg accessions
(42 females + 4 monoecious + 4 males)

⇩

Morphological characterization
(51 qualitative characters, clustering and 38 quantitative characters, clustering)

⇩

Biochemical characterization
(Volatile oil, oleoresin & fixed oil in kernel and mace, clustering)

⇩

Selection of distinct accessions
(17 No's)

⇩

Pericarp analysis
(Ranking based on content of constituents)

⇩

Chemoprofiling of volatile oil: GC MS
(Grading based on percentage of compounds)

⇩

Isozyme analysis
(Peroxidase and polyphenol oxidase, clustering)

⇩

Molecular characterization

⇩

RAPD and ISSR clustering

⇩

Combined RAPD, ISSR clustering

⇩

Inter cluster association of qualitative clusters with other clusters

⇩

Key for identification of elite nutmeg tree

Results

4. RESULTS

The present study entitled "Characterization and evaluation of nutmeg (*Myristica fragrans* Houtt.) accessions" was carried out in the Department of Plantation Crops and Spices, College of Horticulture, Kerala Agricultural University, Thrissur, during 2012-2015.

The extensive nutmeg germplasm available in the farmers fields in Chalakudy region of Central Kerala was found ideal for carrying out the research work with the conceived objectives. The nutmeg plantations in and around Chalakudy were thoroughly surveyed and two plantations where maximum variability was observed, and all the trees well maintained, were selected for the study.

An outstanding advantage of the above said plantations was that both the farmers were progressive with an experimental outlook. Thus, the present investigation was taken up with the select 50 nutmeg accessions, belonging to same age of fifteen years.

As a preliminary step, general features of the accessions, including their source plants were gathered from the farmers. Another unique characteristic of these plantations was that they represented all most all nutmeg growing tracts of Kerala and the tree growth was good, as they enjoyed the most suited agro-climatic factors of Chalakudy river basin.

Among the select fifty accessions, forty two were females, four monoecious and four males. All the accessions were subjected to morphological, biochemical and molecular characterization and the results are presented one by one in the fore going sections.

4.1 MORPHOLOGICAL CHARACTERIZATION

Morphological characterization is well known for its applicability to derive economic and breeding gains from germplasm collections and families of related accessions. Both qualitative and quantitative observations were used for evaluating the nutmeg accessions morphologically.

4.1.1 Descriptor for qualitative evaluation

Morphological descriptors are tools for selecting appropriate accessions for breeding programmes. Due to non availability of an accepted descriptor for nutmeg, descriptors of other perennial crops published by Protection of Plant Varieties and Farmers Rights Authority, India and National Bureau of Plant Genetic Resources, New Delhi were referenced (PPVFRA, 2012 and BI, 2012). Based on the qualitative characters recorded in the study, a descriptor was developed for nutmeg. The descriptor and descriptor states are presented in Table 4.1.

4.1.2 Qualitative evaluation

Wide variability was present among the accessions, for 47 out of 51 qualitative characters observed. Characters *viz*., the leaf margin, fruit pubescence, grooves on nut and nature of fruit dehiscence were non variable characters among the accessions. Similarly, variability with respect to colour of filament and colour of anther were confined only to male and monoecious accessions. Hence, these characters were not included for further analysis.

4.1.2.1 Sex form and tree characters

Sex form and tree characters *viz*., stem colour, branching pattern, canopy shape, foliage density and colour of flushes were observed among the accessions evaluated (Table 4.2). Per cent of accessions distributed into each expression was calculated (Table 4.2a). Among the accessions evaluated, majority of the accessions

were dioecious (92%) and remaining were monoecious (8%). Expressions of sex form is given in Plate 4.1.

Table 4.1. Descriptor and descriptor states for qualitative evaluation of nutmeg accessions

Sl. No.	Character	Expression	Score
A – Qualitative characters			
90	Sex form	Dioecious Monoecious Others (specify)	1 2 9
91	Stem colour	Grey Dark grey Grey black Others (specify)	1 2 3 9
92	Branching pattern	Erect Spreading Drooping Others (specify)	1 2 3 9
93	Canopy shape	Conical Pyramidal Oblong Globular Others (specify)	1 2 3 4 9
94	Foliage density	Sparse Intermediate Abundant	1 3 5
95	Flushing pattern	Early Mid Late Others (specify)	1 2 3 9
96	Colour of flushes	Light green Greenish yellow Yellowish green Purple-green Others (specify)	1 2 3 4 9
97	Shape of mature leaf	Elliptic Oblong Ovate Obovate Lanceolate Others (specify)	1 2 3 4 5 9
98	Colour of mature leaf	Light green Green Dark green	1 2 3

Contd….

Table 4.1. Continued….

99	Leaf margin	Wavy Even Others (specify)	1 2 9
100	Shape of leaf apex	Acute Acuminate Obtuse Others (specify)	1 2 3 9
101	Season of flowering	Early (July) Mid (Late July- early August) Late (August) Others (specify)	1 2 3 9
102	Nature of flowering	Seasonal with three peak Round the year	1 2
103	Inflorescence type	Axillary raceme Umbellate cyme Others (specify)	1 2 9
104	Number of flowers per cluster	Single One-two One-three One-four Five and more Others (specify)	0 1 2 3 4 9
105	Frequency of flower clusters	Nil Sparse Intermediate Profuse Others (specify)	0 1 3 5 9
106	Nature of fruit bearing	Solitary Cluster Others (specify)	1 2 9
107	Periodicity of fruit bearing	Seasonal Round the year	1 2
108	Number of fruits per cluster	Single One –two One-three One-four Five and more Others (specify)	0 1 2 3 4 9
109	Frequency of fruit clusters	Nil Sparse Intermediate Profuse Others (specify)	0 1 3 5 9

Contd…

Table 4.1. Continued…

110	Density of fruit bearing	Sparse Intermediate Profuse	1 2 3
111	Nature of fruit dehiscence	Dehiscent Indehiscent Others (specify)	1 2 9
112	Bearing season	Very Early (March- April) Early (May-early June) Mid(Late June-August) Late (After August) Round the year	1 2 3 4 5
113	Colour of pedicel	Green Pale green Dark green Others (specify)	1 2 3 9
114	Colour of perianth	Creamy Creamy white Creamy yellow Greenish creamy Others (specify)	1 2 3 4 9
115	Shape of perianth	Bell shape Cylindrical Others (specify)	1 2 9
116	Colour of filament	Creamy white White Light yellow cream Others (specify)	1 2 3 9
117	Colour of anther	Pale yellow Yellow Others (specify)	1 2 9
118	Colour of pistil	Light green Greenish yellow Light yellow green Others (specify)	1 2 3 9
119	Colour of immature fruit	Pale green Green Green- pale yellow Others (specify)	1 2 3 9
120	Colour of mature fruit	Light yellow Yellow Yellow-light green Others (specify)	1 2 3 9
121	Shape of fruit	Round Oval Ovoid Pyriform	1 2 3 4

Table 4.1 Continued….

		Oblong Others (specify)	5 9
122	Shape of fruit base	Round Pointed Others (specify)	1 2 9
123	Shape of fruit apex	Acute Obtuse Round Others (specify)	1 2 3 9
124	Fruit pubescence	Absent Present	0 1
125	Number of seeds per fruit	No seed/Rudimentary One seed Two seeds Others (specify)	0 1 2 9
126	Number of splits in split fruits	Full Partial Others (specify)	1 2 9
127	Number of splits in split fruits	Two splits Three splits Four splits Others (specify)	1 2 3 9
128	Shape of mace	Round Oval Oblong Others (specify)	1 2 3 9
129	Colour of mace (fresh)	Deep red Red Orange-red Yellow Others (specify)	1 2 3 4 9
130	Colour of mace (dry)	Red Scarlet red Orange-red Yellow Others (specify)	1 2 3 4 9
131	Nature of mace	Entire Slightly dissected Intermediate Highly dissected Others (specify)	1 2 3 4 9
132	Beakness of mace	Absent Present	0 1

133	Attachment of mace to nut	Loose Compact	1 2
134	Shape of nut	Round Oval Oblong Others (specify)	1 2 3 9
135	Colour of nut (fresh)	Brown Dark brown Greyish brown Black Others (specify)	1 2 3 4 9
136	Colour of nut (dry)	Light brown Brown Dark brown Black Others (specify)	1 2 3 4 9
137	Grooves on nut	Absent Present	0 1
138	Nature of groove on nut	Shallow Pronounced Others (specify)	1 2 9
139	Colour of kernel (dry)	Grey Dark grey Light brown (coriander colour) Others (specify)	1 2 3 9
140	Nature of groove on kernel	No grooves Shallow Pronounced Others (specify)	0 1 2 9

Table 4. 2. Sex form and tree characters of nutmeg accessions

Sl. No.	Accessions	Sex form	Stem colour	Branching pattern	Canopy shape	Foliage density	Flushing pattern	Colour of flushes
1	Acc.1	Dioecious	Grey black	Spreading	Globular	Abundant	Mid	Yellowish green
2	Acc.2	Dioecious	Grey	Spreading	Pyramidal	Intermediate	Early	Greenish yellow
3	Acc.3	Dioecious	Dark grey	Erect	Pyramidal	Sparse	Mid	Yellowish green
4	Acc.4	Dioecious	Grey	Spreading	Pyramidal	Abundant	Mid	Light green
5	Acc.5	Dioecious	Grey	Spreading	Pyramidal	Abundant	Mid	Greenish yellow
6	Acc.6	Dioecious	Grey	Spreading	Oblong	Sparse	Mid	Yellowish green
7	Acc.7	Dioecious	Grey	Spreading	Pyramidal	Sparse	Early	Yellowish green
8	Acc.8	Dioecious	Grey	Spreading	Pyramidal	Abundant	Mid	Greenish yellow
9	Acc.9	Dioecious	Grey black	Spreading	Pyramidal	Abundant	Mid	Yellowish green
10	Acc.10	Dioecious	Grey	Spreading	Conical	Abundant	Early	Yellowish green
11	Acc.11	Dioecious	Dark grey	Spreading	Pyramidal	Abundant	Mid	Greenish yellow
12	Acc.12	Dioecious	Dark grey	Spreading	Pyramidal	Abundant	Mid	Light green
13	Acc.13	Dioecious	Dark grey	Spreading	Pyramidal	Abundant	Mid	Greenish yellow

Table 4.2. Continued…..

14	Acc.14	Dioecious	Grey	Spreading	Oblong	Intermediate	Mid	Yellowish green
15	Acc.15	Dioecious	Grey	Spreading	Pyramidal	Intermediate	Mid	Greenish yellow
16	Acc.16	Dioecious	Grey black	Spreading	Pyramidal	Abundant	Mid	Greenish yellow
17	Acc.17	Dioecious	Grey	Spreading	Oblong	Abundant	Mid	Yellowish green
18	Acc.18	Dioecious	Grey	Spreading	Pyramidal	Abundant	Early	Yellowish green
19	Acc.19	Dioecious	Grey	Spreading	Pyramidal	Sparse	Mid	Yellowish green
20	Acc.20	Dioecious	Grey	Spreading	Oblong	Intermediate	Late	Light green
21	Acc.21	Dioecious	Grey	Spreading	Pyramidal	Abundant	Late	Yellowish green
22	Acc.22	Dioecious	Grey	Spreading	Pyramidal	Abundant	Mid	Greenish yellow
23	Acc.23	Dioecious	Grey	Spreading	Conical	Abundant	Mid	Yellowish green
24	Acc.24	Dioecious	Dark grey	Spreading	Conical	Intermediate	Mid	Yellowish green
25	Acc.25	Dioecious	Grey	Spreading	Pyramidal	Intermediate	Mid	Greenish yellow
26	Acc.26	Dioecious	Dark grey	Spreading	Pyramidal	Intermediate	Mid	Yellowish green
27	Acc.27	Dioecious	Dark grey	Spreading	Pyramidal	Abundant	Mid	Greenish yellow
28	Acc.28	Dioecious	Grey	Spreading	Pyramidal	Abundant	Mid	Yellowish green

29	Acc.29	Dioecious	Grey	Spreading	Pyramidal	Intermediate	Mid	Yellowish green
30	Acc.30	Dioecious	Grey	Spreading	Pyramidal	Abundant	Mid	Yellowish green
31	Acc.31	Dioecious	Grey	Spreading	Pyramidal	Abundant	Mid	Greenish yellow
32	Acc.32	Dioecious	Grey	Spreading	Pyramidal	Abundant	Mid	Yellowish green
33	Acc.33	Dioecious	Grey black	Spreading	Pyramidal	Intermediate	Mid	Greenish yellow
34	Acc.34	Dioecious	Grey	Spreading	Globular	Intermediate	Mid	Greenish yellow
35	Acc.35	Dioecious	Dark grey	Spreading	Globular	Abundant	Mid	Yellowish green
36	Acc.36	Dioecious	Dark grey	Spreading	Pyramidal	Abundant	Mid	Yellowish green
37	Acc.37	Dioecious	Grey	Spreading	Pyramidal	Abundant	Early	Yellowish green
38	Acc.38	Dioecious	Grey	Spreading	Pyramidal	Abundant	Mid	Yellowish green
39	Acc.39	Dioecious	Grey	Spreading	Pyramidal	Abundant	Early	Greenish yellow
40	Acc.40	Dioecious	Grey	Spreading	Pyramidal	Abundant	Early	Yellowish green
41	Acc.41	Dioecious	Dark grey	Spreading	Globular	Abundant	Mid	Greenish yellow
42	Acc.42	Dioecious	Grey	Spreading	Globular	Abundant	Mid	Yellowish green
43	Acc.(H)1	Monoecious	Grey	Spreading	Globular	Abundant	Mid	Yellowish green

44	Acc.(H)2	Monoecious	Grey	Spreading	Pyramidal	Abundant	Mid	Greenish yellow
45	Acc.(H)3	Monoecious	Grey	Spreading	Pyramidal	Abundant	Mid	Greenish yellow
46	Acc.(H)4	Monoecious	Grey	Erect	Oblong	Abundant	Mid	Greenish yellow
47	Acc.(M)1	Dioceous	Grey	Erect	Oblong	Abundant	Mid	Greenish yellow
48	Acc.(M)2	Dioceous	Grey	Spreading	Globular	Intermediate	Mid	Greenish yellow
49	Acc.(M)3	Dioceous	Grey	Spreading	Globular	Intermediate	Mid	Greenish yellow
50	Acc.(M)4	Dioceous	Dark grey	Erect	Oblong	Abundant	Mid	Greenish yellow

Table 4.2a. Distribution of sex form and tree characters among nutmeg accessions

Sl. No.	Character	Expression	Frequency (%)
1	Sex form	Dioecious Monoecious	92.00 8.00
2	Stem colour	Grey Dark grey Grey black	70.00 8.00 22.00
3	Branching pattern	Erect Spreading	8.00 92.00
4	Canopy shape	Conical Pyramidal Oblong Globular	6.00 64.00 14.00 16.00
5	Foliage density	Sparse Intermediate Abundant	8.00 24.00 68.00
6	Flushing pattern	Early Mid Late	14.00 82.00 4.00
7	Colour of flushes	Light green Greenish yellow Yellowish green	6.00 44.00 50.00

Monoecious

Gynoecious

Androecious

Plate 4.1. Expressions of sex form

Spreading

Erect

Plate 4.2. Expressions of branching pattern

Three types of stem colour were observed in the accessions evaluated *viz*., grey, dark grey and grey black, of which majority of the accessions were having grey stem colour (70%), followed by grey black (22%) or dark grey (8%).

Branching pattern among the accessions evaluated showed predominance of spreading types (92%) over erect types (8%). Expressions of branching pattern is presented in Plate 4.2.

Four types of canopy shapes *viz*., conical, pyramidal, oblong and globular were observed among the accessions evaluated (Plate 4.3). Pyramidal canopy shape (64%) was common, followed by globular (16%) and oblong (14%). A few accessions *viz*., Acc. 10, Acc. 23 and Acc. 24 exhibited conical canopy shape (6%).

Sparse, intermediate and abundant types of foliage density were observed in the evaluated accessions (Plate 4.4). Majority of the accessions had abundant foliage density (68%), followed by intermediate foliage density (24%) and sparse foliage density (8%).

Variation was also observed among the accessions evaluated for flushing pattern; with the largest number mid flushing (82%), followed by early flushing (14%) and late flushing (4%) noticed in Acc. 20 and Acc. 21.

Fifty per cent of the accessions had flushes of yellowish green colour followed by greenish yellow (44%) and light green colour (6%).

4.1.2.2 Leaf characters

Leaf characters are one of the most easily observable qualitative traits representing the genetic make up of the tree. The fifth leaf from the bud at the tip of the branch was regarded as the mature leaf for evaluating the leaf characters (Table 4.3 and Table 4.3a). The mature leaf shape observed in the accessions had a

Conical

Pyramidal

Oblong

Globular

Plate 4.3. Expressions of canopy shape

Abundant

Intermediate

Sparse

Plate 4.4. Expressions of foliage density

Table 4.3. Leaf characters of nutmeg accessions

Sl. No.	Accessions	Shape of mature leaf	Colour of mature leaf	Shape of leaf apex
1	Acc.1	Elliptic	Green	Acuminate
2	Acc.2	Lanceolate	Green	Acuminate
3	Acc.3	Elliptic	Green	Acute
4	Acc.4	Elliptic	Green	Acute
5	Acc.5	Elliptic	Green	Acute
6	Acc.6	Elliptic	Green	Acute
7	Acc.7	Elliptic	Green	Acute
8	Acc.8	Elliptic	Green	Acute
9	Acc.9	Elliptic	Dark green	Acuminate
10	Acc.10	Elliptic	Light green	Acute
11	Acc.11	Obovate	Green	Acuminate
12	Acc.12	Elliptic	Green	Acute
13	Acc.13	Elliptic	Green	Acuminate
14	Acc.14	Elliptic	Green	Acuminate
15	Acc.15	Elliptic	Green	Acuminate
16	Acc.16	Lanceolate	Green	Acuminate
17	Acc.17	Lanceolate	Green	Acuminate
18	Acc.18	Obovate	Green	Obtuse
19	Acc.19	Elliptic	Green	Acuminate
20	Acc.20	Elliptic	Green	Acuminate
21	Acc.21	Elliptic	Green	Acuminate
22	Acc.22	Elliptic	Green	Acuminate
23	Acc.23	Elliptic	Green	Acute
24	Acc.24	Ovate	Green	Acute
25	Acc.25	Elliptic	Green	Acute
26	Acc.26	Elliptic	Green	Acuminate
27	Acc.27	Elliptic	Dark green	Acute
28	Acc.28	Elliptic	Green	Acuminate
29	Acc.29	Elliptic	Green	Acute
30	Acc.30	Oblong	Green	Acute
31	Acc.31	Elliptic	Green	Acuminate

Contd…..

Table 4.3. Continued…

32	Acc.32	Elliptic	Green	Acuminate
33	Acc.33	Elliptic	Light green	Acuminate
34	Acc.34	Elliptic	Green	Acuminate
35	Acc.35	Elliptic	Dark green	Acute
36	Acc.36	Obovate	Dark green	Acute
37	Acc.37	Elliptic	Light green	Acuminate
38	Acc.38	Elliptic	Green	Acute
39	Acc.39	Elliptic	Green	Acuminate
40	Acc.40	Elliptic	Green	Acuminate
41	Acc.41	Lanceolate	Green	Acuminate
42	Acc.42	Elliptic	Green	Acuminate
43	Acc.(H) 1	Elliptic	Green	Acute
44	Acc.(H) 2	Elliptic	Green	Acuminate
45	Acc.(H) 3	Elliptic	Green	Acute
46	Acc.(H) 4	Elliptic	Green	Acute
47	Acc.(M) 1	Elliptic	Green	Acute
48	Acc.(M) 2	Elliptic	Green	Acute
49	Acc.(M) 3	Elliptic	Green	Acute
50	Acc.(M) 4	Elliptic	Green	Acute

Table 4.3a. Distribution of leaf characters among nutmeg accessions

Sl. No.	Character	Expression	Frequency (%)
1	Shape of mature leaf	Elliptic Oblong Ovate Obovate Lanceolate	82.00 2.00 2.00 6.00 8.00
2	Colour of mature leaf	Light green Green Dark green	6.00 86.00 8.00
3	Shape of leaf apex	Acute Acuminate Obtuse	48.00 50.00 2.00

Obovate **Elliptic** **Oblong** **Lanceolate** **Ovate**

Plate 4.5. Expressions of shape of mature leaf

Light green **Green** **Dark green**

Plate 4.6. Expressions of colour of mature leaf

predominance of elliptic shape (82%), followed by lanceolate (8%), obovate (6%); ovate and oblong (2% each). Expressions of shape of mature leaf is given in Plate 4.5.

With regard to the mature leaf colour, 86 per cent were green, eight per cent dark green and six per cent light green (Plate 4.6).

With reference to the shape of leaf apex, 50 per cent of accessions had acuminate leaf apex and 48 per cent had acute leaf apex. Obtuse leaf apex (2%) was noticed only in Acc. 18.

4.1.2.3 Flowering and fruiting pattern

Flowering and fruiting pattern of the accessions evaluated are presented in Table 4.4 and Table 4.4a. In the case of nature of flowering, most of the accessions exhibited seasonal flowering (80%) with three peaks during the months of July-August, October- November and late January- February as against the year round flowering (20%). Accessions 8 and 22 were prominent females, bearing flowers round the year.

In the main flowering season, early flowering (July) was noticed in 12 per cent of the accessions, mid flowering (late July to early August) in 50 per cent of the accessions and late flowering (late August) in the rest (38%). Early flowering accessions were accession 1 and 18. Male accessions exhibited staggered flowering throughout the year.

Among the accessions, female accessions (84%) had axillary raceme type of inflorescence and the monoecious accessions (16%) had umbellate cyme inflorescence (Plate 4.7).

Number of flowers per cluster also varied among the accessions evaluated. Majority of the accessions were bearing one to two flowers in a cluster (50%),

Table 4.4. Flowering and fruiting pattern of nutmeg accessions

Sl. No.	Accessions	Season of flowering	Periodicity of flowering	Inflorescence type	Number of flowers per cluster	Frequency of flower clusters	Nature of fruit bearing	Periodicity of fruit bearing	Number of fruits per cluster	Frequency of fruit clusters	Density of fruit bearing	Bearing season
1	Acc.1	Early	Seasonal	Axillary raceme	One-three	Profuse	Cluster	Seasonal	One-three	Profuse	Intermediate	Very early
2	Acc.2	Mid	Seasonal	Axillary raceme	One-two	Sparse	Cluster	Seasonal	One-two	Sparse	Sparse	Mid
3	Acc.3	Mid	Seasonal	Axillary raceme	One-two	Intermediate	Cluster	Seasonal	One-two	Intermediate	Sparse	Mid
4	Acc.4	Mid	Seasonal	Axillary raceme	One-three	Intermediate	Cluster	Seasonal	One-two	Intermediate	Intermediate	Early
5	Acc.5	Late	Seasonal	Axillary raceme	One-two	Intermediate	Cluster	Seasonal	One-two	Intermediate	Intermediate	Mid
6	Acc.6	Mid	Seasonal	Axillary raceme	One-two	Intermediate	Cluster	Seasonal	One-two	Intermediate	Intermediate	Mid
7	Acc.7	Late	Seasonal	Axillary raceme	One-three	Sparse	Cluster	Seasonal	One-two	Sparse	Intermediate	Mid
8	Acc.8	Mid	Round the year	Axillary raceme	One-three	Profuse	Cluster	Round the year	One-three	Profuse	Profuse	Round the year
9	Acc.9	Mid	Seasonal	Axillary raceme	One-three	Profuse	Cluster	Seasonal	One-three	Profuse	Intermediate	Early
10	Acc.10	Mid	Seasonal	Axillary raceme	One-two	Intermediate	Cluster	Seasonal	One-two	Intermediate	Intermediate	Mid
11	Acc.11	Mid	Seasonal	Axillary raceme	One-two	Intermediate	Cluster	Seasonal	One-two	Intermediate	Intermediate	Early
12	Acc.12	Mid	Seasonal	Axillary raceme	One-two	Profuse	Cluster	Seasonal	One-two	Profuse	Intermediate	Mid
13	Acc.13	Late	Seasonal	Axillary raceme	One-three	Intermediate	Cluster	Seasonal	One-two	Intermediate	Intermediate	Mid
14	Acc.14	Mid	Seasonal	Axillary raceme	Five and more	Profuse	Cluster	Seasonal	One-four	Profuse	Profuse	Mid
15	Acc.15	Mid	Seasonal	Axillary raceme	One-two	Intermediate	Cluster	Seasonal	One-two	Intermediate	Intermediate	Mid
16	Acc.16	Late	Seasonal	Axillary	One-two	Intermediate	Cluster	Seasonal	One-two	Intermediate	Sparse	Mid

Contd…..

Table 4.4. Continued….

				raceme								
17	Acc.17	Late	Seasonal	Axillary raceme	One-two	Intermediate	Cluster	Seasonal	One-two	Intermediate	Intermediate	Mid
18	Acc.18	Early	Seasonal	Axillary raceme	One-three	Profuse	Cluster	Seasonal	One-two	Profuse	Intermediate	Very early
19	Acc.19	Mid	Seasonal	Axillary raceme	One-four	Profuse	Cluster	Seasonal	One-four	Profuse	Profuse	Early
20	Acc.20	Late	Seasonal	Axillary raceme	One-two	Sparse	Solitary	Seasonal	Single	Nil	Sparse	Late
21	Acc.21	Mid	Seasonal	Axillary raceme	One-three	Profuse	Cluster	Seasonal	One-two	Profuse	Profuse	Early
22	Acc.22	Mid	Round the year	Axillary raceme	One-three	Profuse	Cluster	Round the year	One-two	Profuse	Profuse	Round the year
23	Acc.23	Late	Seasonal	Axillary raceme	One-two	Profuse	Cluster	Seasonal	One-two	Profuse	Profuse	Mid
24	Acc.24	Mid	Seasonal	Axillary raceme	One-three	Intermediate	Cluster	Seasonal	One-two	Intermediate	Intermediate	Mid
25	Acc.25	Mid	Seasonal	Axillary raceme	One-two	Profuse	Cluster	Seasonal	One-two	Profuse	Intermediate	Mid
26	Acc.26	Mid	Seasonal	Axillary raceme	One-two	Intermediate	Cluster	Seasonal	One-two	Intermediate	Profuse	Mid
27	Acc.27	Late	Seasonal	Axillary raceme	One-three	Profuse	Cluster	Seasonal	One-two	Profuse	Profuse	Mid
28	Acc.28	Late	Seasonal	Axillary raceme	One-two	Sparse	Cluster	Seasonal	One-two	Sparse	Sparse	Late
29	Acc.29	Late	Seasonal	Axillary raceme	One-two	Intermediate	Cluster	Seasonal	One-two	Intermediate	Intermediate	Mid
30	Acc.30	Mid	Seasonal	Axillary raceme	One-two	Profuse	Cluster	Seasonal	One-two	Profuse	Intermediate	Mid
31	Acc.31	Mid	Seasonal	Axillary raceme	One-two	Sparse	Solitary	Seasonal	Single	Nil	Sparse	Late
32	Acc.32	Late	Seasonal	Axillary raceme	One-two	Sparse	Solitary	Seasonal	Single	Nil	Sparse	Mid
33	Acc.33	Late	Seasonal	Axillary raceme	One-two	Sparse	Solitary	Seasonal	Single	Nil	Sparse	Mid
34	Acc.34	Mid	Seasonal	Axillary raceme	One-two	Profuse	Cluster	Seasonal	One-two	Profuse	Intermediate	Early

Contd…

Table 4.4. Continued…..

35	Acc.35	Late	Seasonal	Axillary raceme	One-two	Intermediate	Cluster	Seasonal	One-two	Intermediate	Sparse	Early
36	Acc.36	Late	Seasonal	Axillary raceme	One-three	Profuse	Cluster	Seasonal	One-three	Profuse	Sparse	Mid
37	Acc.37	Mid	Seasonal	Axillary raceme	One-three	Intermediate	Cluster	Seasonal	One-three	Intermediate	Intermediate	Mid
38	Acc.38	Late	Seasonal	Axillary raceme	One-two	Profuse	Cluster	Seasonal	One-two	Profuse	Intermediate	Mid
39	Acc.39	Mid	Seasonal	Axillary raceme	One-two	Intermediate	Cluster	Seasonal	One-two	Intermediate	Intermediate	Mid
40	Acc.40	Late	Seasonal	Axillary raceme	One-three	Intermediate	Cluster	Seasonal	One-three	Intermediate	Profuse	Mid
41	Acc.41	Mid	Seasonal	Axillary raceme	One-two	Profuse	Cluster	Seasonal	One-two	Profuse	Intermediate	Mid
42	Acc.42	Mid	Seasonal	Axillary raceme	One-three	Intermediate	Cluster	Seasonal	One-three	Intermediate	Intermediate	Mid
43	Acc.(H)1	Mid	Round the year	Umbellate cyme	Five and more	Intermediate	Cluster	Round the year	One-two	Intermediate	Sparse	Mid
44	Acc.(H)2	Late	Round the year	Umbellate cyme	One-three	Sparse	Cluster	Seasonal	One-two	Sparse	Sparse	Mid
45	Acc.(H)3	Late	Round the year	Umbellate cyme	One-four	Sparse	Solitary	Seasonal	Single	Nil	Sparse	Mid
46	Acc.(H)4	Late	Round the year	Umbellate cyme	One-four	Intermediate	Cluster	Seasonal	One-two	Intermediate	Sparse	Mid
47	Acc.(M)1	Early	Round the year	Umbellate cyme	Five and more	Profuse	NA	NA	NA	NA	NA	NA
48	Acc.(M)2	Early	Round the year	Umbellate cyme	Five and more	Profuse	NA	NA	NA	NA	NA	NA
49	Acc.(M)3	Early	Round the year	Umbellate cyme	Five and more	Profuse	NA	NA	NA	NA	NA	NA
50	Acc.(M)4	Early	Round the year	Umbellate cyme	Five and more	Profuse	NA	NA	NA	NA	NA	NA

Table 4.4a. Distribution of flowering and fruiting pattern among nutmeg accessions

Sl. No.	Character	Expression	Frequency (%)
1	Season of flowering	Early (July) Mid (Late July- early August) Late (August)	12.00 50.00 38.00
2	Nature of flowering	Seasonal with three peaks Round the year	80.00 20.00
3	Inflorescence type	Axillary raceme Umbellate cyme	84.00 16.00
4	Number of flowers per cluster	One-two One-three One-four Five and more	50.00 32.00 6.00 12.00
5	Frequency of flower clusters	Sparse Intermediate Profuse	18.00 40.00 42.00
6	Nature of fruit bearing	Solitary Cluster	10.86 89.13
7	Periodicity of fruit bearing	Seasonal Round the year	93.47 6.52
8	Number of fruits per cluster	Single One –two One-three One-four	10.86 69.56 15.21 4.34
9	Frequency of fruit clusters	Nil Sparse Intermediate Profuse	10.86 8.69 43.47 36.95
10	Density of fruit bearing	Sparse Intermediate Profuse	30.43 50.00 19.55
11	Bearing season	Very Early (March- April) Early (May-early June) Mid(Late June-August) Late (After August) Round the year	4.34 15.21 69.56 6.52 4.34

Axillary raceme **Umbellate cyme**

Plate 4.7. Expressions of inflorescence type

Single flower

Two flowers/cluster

Three flowers/cluster

Four flowers/cluster

Plate 4.8. Expressions of number of ♀ flowers per cluster

followed by one to three (32%), one to four (6%) and five and more flowers in a cluster (12%). Expressions of number of flowers per cluster is presented in Plate 4.8.

Frequency of flower clusters was profuse in forty two per cent accessions, intermediate in forty per cent accessions and sparse in eighteen per cent accessions.

With regard to the nature of fruit bearing, majority of the evaluated accessions had cluster bearing habit (89.13 %). Solitary bearing habit was noticed in 10.86 per cent accessions.

The periodicity of fruit bearing of different accessions showed that majority were seasonal (93.47%). Round the year fruit bearing (6.52%) was observed in the accessions Acc. 8, Acc. 22 and Acc. (H) 1.

Wide variation was observed in the accessions evaluated for the number of fruits per cluster (Plate 4.9). Majority of the accessions were bearing one to two fruits in a cluster (69.56%), followed by the accessions bearing one to three fruits in a cluster (15.21%) in contrast with 10.86 per cent of the accessions as solitary fruit bearing types. Accessions 14 and 19 were bearing one to four fruits in a cluster (4.34%).

The frequencies of fruit clusters were aligned as intermediate (43.47%), profuse (36.95%), no clusters (10.86%) and sparse (8.69%).

Different fruit bearing densities were observed in the evaluated accessions like intermediate bearing (50%), sparse bearing (30.43%) and profuse bearing (19.55%).

An observation on season of bearing revealed that, majority were peak bearing (June- August, 69.56%), very few were early bearing (15.21%), late bearing (6.52%), very early (4.34%) as also round the year bearing (4.34%).

4.1.2.4 Floral characters

The floral characters of various accessions are presented in Table 4.5 and Table 4.5a. Characters *viz*., colour of filament and colour of anther were observed only in monoecious and male accessions.

Three colours of pedicel were observed among the accessions. Majority were green (84%), followed by dark green (10%) and pale green (6%).

Majority of the accessions had creamy perianth (74%), followed by accessions with creamy white (14%), creamy yellow and greenish creamy (6% each) perianth.

In the case of shape of perianth, 98 per cent had bell shaped perianth. One male accession (Acc. (M) 3) possessed cylindrical perianth (Plate 4.10).

Two filament colours were observed in the monoecious and male accessions *viz*., white (75%) and creamy white (25%). Similarly two anther colours were observed *viz*., pale yellow (87.50%) and yellow (12.50%).

Pistil colours of the female accessions could be streamlined as light green (93.47%), greenish yellow (4.34%) and light yellow green (2.17%).

4.1.2.5 Fruit characters

Data on fruit characters of the evaluated accessions are presented in Table 4.6 and Table 4.6a. Immature fruit colour showed good variability among the evaluated accessions. Majority of the accessions had pale green fruits (69.56%) followed by green-pale yellow (17.39%) and green (13.04%). Similarly, mature fruit colour observed was yellow (91.30%), yellow-light green (4.76%) and light yellow (4.34%).

Table 4.5. Floral characters of nutmeg accessions

Sl. No.	Accessions	Colour of pedicel	Colour of perianth	Shape of perianth	Colour of filament	Colour of anther	Colour of pistil
1	Acc.1	Dark green	Creamy	Bell shape	NA	NA	Light green
2	Acc.2	Green	Creamy	Bell shape	NA	NA	Greenish yellow
3	Acc.3	Green	Creamy	Bell shape	NA	NA	Light green
4	Acc.4	Green	Creamy	Bell shape	NA	NA	Light green
5	Acc.5	Dark green	Greenish creamy	Bell shape	NA	NA	Light green
6	Acc.6	Light green	Creamy white	Bell shape	NA	NA	Greenish yellow
7	Acc.7	Green	Creamy	Bell shape	NA	NA	Light green
8	Acc.8	Green	Creamy	Bell shape	NA	NA	Light green
9	Acc.9	Dark green	Greenish creamy	Bell shape	NA	NA	Light green
10	Acc.10	Green	Creamy	Bell shape	NA	NA	Light green
11	Acc.11	Dark green	Creamy	Bell shape	NA	NA	Light green
12	Acc.12	Green	Creamy	Bell shape	NA	NA	Light green
13	Acc.13	Green	Creamy	Bell shape	NA	NA	Light green
14	Acc.14	Green	Creamy	Bell shape	NA	NA	Light green
15	Acc.15	Green	Creamy	Bell shape	NA	NA	Light green
16	Acc.16	Green	Creamy	Bell shape	NA	NA	Light green
17	Acc.17	Green	Creamy	Bell shape	NA	NA	Light green
18	Acc.18	Green	Creamy	Bell shape	NA	NA	Light green
19	Acc.19	Green	Creamy	Bell shape	NA	NA	Light green
20	Acc.20	Green	Creamy	Bell shape	NA	NA	Light green
21	Acc.21	Green	Creamy	Bell shape	NA	NA	Light green
22	Acc.22	Green	Creamy white	Bell shape	NA	NA	Light green
23	Acc.23	Green	Creamy	Bell shape	NA	NA	Light green
24	Acc.24	Green	Creamy	Bell shape	NA	NA	Light green
25	Acc.25	Green	Creamy	Bell shape	NA	NA	Light green
26	Acc.26	Green	Creamy	Bell shape	NA	NA	Light green
27	Acc.27	Dark green	Greenish creamy	Bell shape	NA	NA	Light green
28	Acc.28	Green	Creamy	Bell shape	NA	NA	Light green
29	Acc.29	Green	Creamy	Bell shape	NA	NA	Light green
30	Acc.30	Green	Creamy	Bell shape	NA	NA	Light green

Table 4.5. Continued….

31	Acc.31	Green	Creamy	Bell shape	NA	NA	Light green
32	Acc.32	Green	Creamy	Bell shape	NA	NA	Light green
33	Acc.33	Green	Creamy	Bell shape	NA	NA	Light green
34	Acc.34	Green	Creamy	Bell shape	NA	NA	Light green
35	Acc.35	Green	Creamy	Bell shape	NA	NA	Light green
36	Acc.36	Green	Creamy	Bell shape	NA	NA	Light green
37	Acc.37	Light green	Creamy	Bell shape	NA	NA	Light green
38	Acc.38	Green	Creamy	Bell shape	NA	NA	Light green
39	Acc.39	Green	Creamy white	Bell shape	NA	NA	Light green
40	Acc.40	Green	Creamy	Bell shape	NA	NA	Light green
41	Acc.41	Green	Creamy white	Bell shape	NA	NA	Light green
42	Acc.42	Green	Creamy	Bell shape	NA	NA	Light green
43	Acc.(H)1	Green	Creamy yellow	Bell shape	White	Pale yellow	Light green
44	Acc.(H)2	Green	Creamy	Bell shape	White	Pale yellow	Light green
45	Acc.(H)3	Green	Creamy	Bell shape	White	Pale yellow	Light green
46	Acc.(H)4	Light green	Creamy yellow	Bell shape	Creamy white	Pale yellow	Light yellow green
47	Acc(M)1	Green	Creamy white	Bell/ Cylindrical	Creamy white	Yellow	NA
48	Acc(M)2	Green	Creamy yellow	Bell/ Cylindrical	White	Pale yellow	NA
49	Acc(M)3	Green	Creamy white	Cylindrical	White	Pale yellow	NA
50	Acc(M)4	Green	Creamy white	Bell/ Cylindrical	White	Pale yellow	NA

Table 4.5a. Distribution of floral characters among nutmeg accessions

Sl. No.	Character	Expression	Frequency (%)
1	Colour of pedicel	Green Light green Dark green	84.00 6.00 10.00
2	Colour of perianth	Creamy Creamy white Creamy yellow Greenish creamy	74.00 14.00 6.00 6.00
3	Shape of perianth	Bell shape Cylindrical	98.00 2.00
4	Colour of filament	Creamy white White	25.00 75.00
5	Colour of anther	Pale yellow Yellow	87.50 12.50
6	Colour of pistil	Light green Greenish yellow Light yellow green	93.47 4.34 2.17

Table 4.6. Variability for fruit characters in nutmeg accessions

Sl. No.	Accessions	Colour of immature fruit	Colour of mature fruit	Shape of fruit	Shape of fruit base	Shape of fruit apex	Number of seeds per fruit	Number of splits in split fruits	No. of splits in split fruits
1	Acc.1	Pale green	Yellow	Ovoid	Round	Obtuse	One seed	Full splitting	Two splits
2	Acc.2	Green	Yellow	Ovoid	Pointed	Obtuse	One seed	Full splitting	Two splits
3	Acc.3	Pale green	Yellow	Round	Round	Round	One seed	Full splitting	Two splits
4	Acc.4	Pale green	Yellow	Ovoid	Round	Obtuse	One seed	Full splitting	Two splits
5	Acc.5	Green	Yellow-light green	Ovoid	Pointed	Round	One seed	Partial	Two splits
6	Acc.6	Pale green	Light yellow	Ovoid	Round	Round	One seed	Full splitting	Two splits
7	Acc.7	Green-pale yellow	Yellow	Pyriform	Pointed	Obtuse	One seed	Full splitting	Two splits
8	Acc.8	Pale green	Yellow	Oval	Pointed	Acute	One seed	Full splitting	Two splits
9	Acc.9	Green	Yellow	Ovoid	Round	Round	One seed	Full splitting	Two splits
10	Acc.10	Pale green	Yellow	Oval	Pointed	Acute	One seed	Full splitting	Two splits
11	Acc.11	Green-pale yellow	Yellow	Round	Round	Round	One seed	Full splitting	Two splits
12	Acc.12	Pale green	Yellow	Oval	Pointed	Acute	One seed	Full splitting	Two splits
13	Acc.13	Pale green	Yellow	Round	Round	Round	One seed	Full splitting	Two splits
14	Acc.14	Pale green	Yellow	Round	Round	Round	One seed	Full splitting	Two splits
15	Acc.15	Pale green	Yellow	Round	Round	Round	One seed	Full splitting	Two splits
16	Acc.16	Green	Yellow	Round	Round	Round	One seed	Full splitting	Two splits
17	Acc.17	Pale green	Yellow-light green	Round	Round	Round	One seed	Full splitting	Two splits

Table 4.6. Continued….

18	Acc.18	Pale green	Yellow	Round	Round	Round	One seed	Full splitting	Two splits
19	Acc.19	Green	Yellow	Pyriform	Pointed	Acute	One seed	Full splitting	Two splits
20	Acc.20	Pale green	Yellow	Ovoid	Round	Round	One seed	Full splitting	Two splits
21	Acc.21	Pale green	Yellow	Round	Round	Round	One seed	Full splitting	Two splits
22	Acc.22	Green	Yellow	Ovoid	Pointed	Round	One seed	Full splitting	Two splits
23	Acc.23	Pale green	Yellow	Ovoid	Pointed	Obtuse	One seed	Full splitting	Two splits
24	Acc.24	Pale green	Yellow	Ovoid	Pointed	Obtuse	One seed	Full splitting	Two splits
25	Acc.25	Green-pale yellow	Yellow	Round	Round	Round	One seed	Full splitting	Two splits
26	Acc.26	Pale green	Yellow	Ovoid	Pointed	Obtuse	One seed	Full splitting	Two splits
27	Acc.27	Pale green	Yellow	Ovoid	Pointed	Obtuse	One seed	Full splitting	Two splits
28	Acc.28	Pale green	Yellow	Ovoid	Pointed	Obtuse	One seed	Full splitting	Two splits
29	Acc.29	Pale green	Yellow	Ovoid	Pointed	Obtuse	One seed	Full splitting	Two splits
30	Acc.30	Pale green	Yellow	Ovoid	Pointed	Round	One seed	Full splitting	Two splits
31	Acc.31	Pale green	Yellow	Round	Round	Round	One seed	Full splitting	Two splits
32	Acc.32	Green-pale yellow	Yellow	Round	Round	Round	One seed	Full splitting	Two splits
33	Acc.33	Green-pale yellow	Yellow	Ovoid	Pointed	Obtuse	One seed	Full splitting	Two splits
34	Acc.34	Pale green	Yellow	Round	Round	Round	One seed	Full splitting	Two splits
35	Acc.35	Pale green	Yellow	Round	Round	Round	One seed	Full splitting	Two splits
36	Acc.36	Pale green	Yellow	Round	Round	Round	One seed	Full splitting	Two splits
37	Acc.37	Pale green	Yellow	Round	Round	Round	One seed	Full splitting	Two splits
38	Acc.38	Pale green	Yellow	Ovoid	Pointed	Round	One seed	Full splitting	Two splits

39	Acc.39	Pale green	Yellow	Round	Round	Round	One seed	Full splitting	Two splits
40	Acc.40	Green-pale yellow	Yellow	Round	Round	Round	One seed	Full splitting	Two splits
41	Acc.41	Pale green	Yellow	Ovoid	Pointed	Round	One seed	Full splitting	Two splits
42	Acc.42	Green-pale yellow	Yellow	Round	Round	Round	One seed	Full splitting	Two splits
43	Acc.(H)1	Green-pale yellow	Yellow	Ovoid	Pointed	Round	Two seeds	Full splitting	Three splits
44	Acc.(H)2	Pale green	Yellow	Round	Round	Round	One seed	Full splitting	Two splits
45	Acc.(H)3	Pale green	Yellow	Round	Round	Round	One seed	Full splitting	Two splits
46	Acc.(H)4	Pale green	Light yellow	Round	Round	Round	Two seeds	Full splitting	Four splits

Table 4.6a. Distribution of fruit characters among nutmeg accessions

Sl. No.	Character	Expression	Frequency (%)
1	Colour of immature fruit	Pale green Green Green- pale yellow	69.56 13.04 17.39
2	Colour of mature fruit	Light yellow Yellow Yellow-light green	4.34 91.30 4.76
3	Shape of fruit	Round Oval Ovoid Pyriform	47.82 6.52 41.30 4.34
4	Shape of fruit base	Round Pointed	58.69 41.30
5	Shape of fruit apex	Acute Obtuse Round	8.69 23.91 67.39
6	Number of seeds per fruit	One seed Two seeds	95.65 4.34
7	Number of splits in split fruits	Full Partial	97.82 2.17
8	Number of splits in split fruit	Two splits Three splits Four splits	95.65 2.17 2.17

Single fruit

Two fruits/cluster

Three fruits/cluster

Four fruits/cluster

Plate 4.9. Expressions of number of fruits per cluster

Bell shape **Cylindrical**

Plate 4.10. Expressions of shape of perianth

Round **Ovoid**

Pyriform **Oval**

Plate 4.11. Expressions of shape of fruit

Four fruit shapes were observed; round, oval, ovoid and pyriform (Plate 4.11). Forty seven per cent of the accessions had round fruits, followed by ovoid fruits (41.30%) and oval fruits (6.52%). Acc. 7 and Acc. 19 had pyriform fruits (4.34%).

Two types of fruit base were observed among the accessions evaluated *viz*., round (58.69%) and pointed (41.30%). As regards shape of fruit apex, 67.39 per cent had round fruit apex followed by obtuse (23.91%) and acute (8.69%) fruit apex shapes.

Number of seeds per fruit was one in majority of the accessions (95.65%). Monoecious accessions, Acc. (H) 1 and Acc. (H) 4 contained two seeds per fruit (Plate. 4.12).

Two types of fruit splitting could be observed in the accessions. The per cent of accessions exhibiting full fruit splitting was 97.82 per cent. Acc. 5 alone was observed to have partial fruit splitting habit (Plate 4.13).

In majority of the accessions, ripened fruits split into two splits (95.65%). In Acc. (H) 1 and Acc. (H) 4, fruits split into three splits and four splits respectively (Plate 4.12).

4.1.2.6 Mace and nut characters

Mace and nut characters of all the accessions evaluated are presented in Table 4.7 and Table 4.7a. Three mace shapes were observed among the evaluated accessions, with majority having oval (60.86%) shape followed by accessions with round (21.73%) and oblong (17.39%) mace shapes.

In majority of the accessions, fresh mace colour was red (78.26%), and a few had deep red mace colour (17.39%). In Acc. 13 and Acc. 21, fresh mace was orange-red in colour (Plate 4.14). Upon drying, mace colour changed to scarlet red (91.30%), red or orange red (4.34% each).

Nature of mace was classified into four types (Plate 4.15); majority had slightly dissected mace (56.52%) followed by intermediate (34.78%) and very few had highly dissected mace (4.34%). Entire mace could be observed in Acc. 14 and Acc. 38 only.

Beakness of mace was observed only in the accession 39 and the remaining accessions were devoid of mace beakness (Plate 4.16).

Attachment of mace to nut was compact in majority of the accessions (93.47%); very few had loose attachment of mace to nut (6.52%).

Three nut shapes were observed among the accessions; a large number had oval nuts (60.86%) followed by round (21.73%) and oblong (17.39%) shaped nuts.

With respect to fresh nut colour, shining black was more common (58.69%) followed by dark brown (34.78%) and brown (6.52%). On drying, the nut colour changed to dark brown (86.95%), brown (8.69%) or light brown (4.34%). Plate 4.17 displays the expressions of colour of fresh nut.

Nuts were observed to have shallow grooves (56.52%) or pronounced grooves on the surface of nut (43.47%). Expressions of grooves on nut is shown in Plate 4.18.

Kernel colour also varied among the accessions. In majority of the trees, it was grey (56.52%) followed by dark grey (39.13%). In Acc. 36 and Acc. (H) 1, light brown kernel was observed.

The kernels also had grooves of various nature (Plate 4.19). It was shallow in most of the accessions (54.34%) followed by accessions having no grooves (28.26%) and pronounced grooves (17.39%).

The above results on various qualitative characters bring forth the plausibility of numerous undulating combinations of the qualitative characteristics. An exclusive grouping of the accessions based on disjoint subsets of the forty seven qualitative

Table 4.7. Variability in mace and nut characters in nutmeg accessions

Sl. No.	Accessions	Shape of mace	Colour of mace		Nature of mace	Beakness of mace	Attachment of mace to nut	Shape of nut	Colour of nut		Nature of groove on nut	Kernel colour (dry)	Nature of groove on kernel
			Fresh	Dry					Fresh	Dry			
1	Acc.1	Oblong	Red	Red	Slightly dissected	Absent	Compact	Oblong	Shining black	Dark brown	Pronounced	Dark grey	Pronounced
2	Acc.2	Oblong	Deep red	Scarlet red	Slightly dissected	Absent	Compact	Oblong	Dark brown	Dark brown	Pronounced	Grey	No grooves
3	Acc.3	Oval	Red	Scarlet red	Slightly dissected	Absent	Compact	Oval	Dark brown	Dark brown	Shallow	Dark grey	Shallow
4	Acc.4	Round	Red	Scarlet red	Slightly dissected	Absent	Compact	Round	Shining black	Dark brown	Shallow	Grey	Shallow
5	Acc.5	Round	Red	Scarlet red	Slightly dissected	Absent	Compact	Round	Dark brown	Dark brown	Pronounced	Dark grey	Shallow
6	Acc.6	Oval	Red	Scarlet red	Slightly dissected	Absent	Compact	Oval	Dark brown	Dark brown	Pronounced	Dark grey	Pronounced
7	Acc.7	Oval	Red	Scarlet red	Intermediate	Absent	Compact	Oval	Dark brown	Dark brown	Pronounced	Dark grey	Pronounced
8	Acc.8	Oblong	Red	Scarlet red	Slightly dissected	Absent	Compact	Oblong	Shining black	Dark brown	Shallow	Dark grey	Shallow
9	Acc.9	Oval	Red	Scarlet red	Intermediate	Absent	Compact	Oval	Shining black	Dark brown	Shallow	Grey	No grooves
10	Acc.10	Oval	Red	Red	Slightly dissected	Absent	Compact	Oval	Shining black	Dark brown	Shallow	Grey	Shallow
11	Acc.11	Round	Red	Scarlet red	Intermediate	Absent	Compact	Round	Shining black	Dark brown	Pronounced	Grey	No grooves
12	Acc.12	Oval	Red	Scarlet red	Intermediate	Absent	Compact	Oval	Shining black	Dark brown	Pronounced	Dark grey	Shallow
13	Acc.13	Round	Orange red	Orange red	Intermediate	Absent	Compact	Round	Dark brown	Dark brown	Pronounced	Grey	No grooves
14	Acc.14	Oval	Deep red	Scarlet red	Entire	Absent	Compact	Oval	Shining black	Dark brown	Pronounced	Grey	No grooves
15	Acc.15	Oblong	Red	Scarlet red	Intermediate	Absent	Compact	Oblong	Shining black	Dark brown	Shallow	Dark grey	Shallow
16	Acc.16	Oval	Red	Scarlet red	Slightly dissected	Absent	Compact	Oval	Shining	Dark	Shallow	Grey	Shallow

Contd….

Table 4.7. Continued….

									black	brown			
17	Acc.17	Oval	Deep red	Scarlet red	Slightly dissected	Absent	Compact	Oval	Brown	Dark brown	Pronounced	Grey	Shallow
18	Acc.18	Oval	Red	Scarlet red	Slightly dissected	Absent	Compact	Oval	Shining black	Dark brown	Shallow	Dark grey	Shallow
19	Acc.19	Oblong	Red	Scarlet red	Slightly dissected	Absent	Compact	Oblong	Shining black	Dark brown	Shallow	Dark grey	Pronounced
20	Acc.20	Oval	Red	Scarlet red	Intermediate	Absent	Compact	Oval	Shining black	Dark brown	Shallow	Grey	Shallow
21	Acc.21	Round	Orange red	Orange red	Intermediate	Absent	Compact	Round	Dark brown	Brown	Shallow	Grey	No grooves
22	Acc.22	Oval	Red	Scarlet red	Slightly dissected	Absent	Compact	Oval	Shining black	Dark brown	Shallow	Grey	Shallow
23	Acc.23	Oval	Red	Scarlet red	Intermediate	Absent	Compact	Oval	Shining black	Dark brown	Shallow	Grey	No grooves
24	Acc.24	Oval	Red	Scarlet red	Slightly dissected	Absent	Compact	Oval	Shining black	Dark brown	Shallow	Grey	Shallow
25	Acc.25	Oval	Red	Scarlet red	Slightly dissected	Absent	Compact	Oval	Dark brown	Dark brown	Pronounced	Grey	No grooves
26	Acc.26	Oval	Red	Scarlet red	Intermediate	Absent	Compact	Oval	Shining black	Dark brown	Shallow	Dark grey	Pronounced
27	Acc.27	Oval	Red	Scarlet red	Slightly dissected	Absent	Loose	Oval	Shining black	Dark brown	Shallow	Grey	No grooves
28	Acc.28	Oval	Red	Scarlet red	Intermediate	Absent	Compact	Oval	Shining black	Brown	Shallow	Grey	Shallow
29	Acc.29	Round	Deep red	Scarlet red	Slightly dissected	Absent	Compact	Round	Dark brown	Dark brown	Pronounced	Dark grey	Shallow
30	Acc.30	Oval	Red	Scarlet red	Intermediate	Absent	Compact	Oval	Brown	Light brown	Shallow	Grey	Pronounced
31	Acc.31	Oblong	Red	Scarlet red	Intermediate	Absent	Compact	Oblong	Shining black	Dark brown	Shallow	Grey	Shallow
32	Acc.32	Oblong	Red	Scarlet red	Intermediate	Absent	Loose	Oblong	Dark brown	Dark brown	Shallow	Dark grey	Pronounced
33	Acc.33	Oblong	Red	Scarlet red	Highly dissected	Absent	Compact	Oblong	Brown	Light brown	Pronounced	Grey	Shallow
34	Acc.34	Oval	Deep red	Scarlet red	Intermediate	Absent	Compact	Oval	Shining black	Dark brown	Pronounced	Grey	Shallow

Contd….

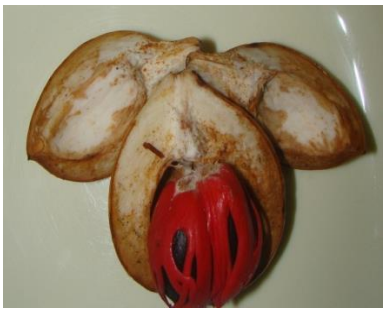
Table 4.7. Continued….

35	Acc.35	Oval	Red	Scarlet red	Intermediate	Absent	Compact	Oval	Dark brown	Dark brown	Pronounced	Grey	No grooves
36	Acc.36	Round	Red	Scarlet red	Slightly dissected	Absent	Loose	Round	Dark brown	Dark brown	Pronounced	Light brown	Shallow
37	Acc.37	Oval	Red	Scarlet red	Slightly dissected	Absent	Compact	Oval	Shining black	Dark brown	Shallow	Dark grey	Shallow
38	Acc.38	Oval	Deep red	Scarlet red	Entire	Absent	Compact	Oval	Shining black	Dark brown	Pronounced	Grey	No grooves
39	Acc.39	Round	Deep red	Scarlet red	Slightly dissected	Present	Compact	Round	Dark brown	Dark brown	Pronounced	Grey	Shallow
40	Acc.40	Round	Red	Scarlet red	Slightly dissected	Absent	Compact	Round	Shining black	Dark brown	Shallow	Dark grey	No grooves
41	Acc.41	Round	Red	Scarlet red	Slightly dissected	Absent	Compact	Round	Dark brown	Dark brown	Shallow	Dark grey	Pronounced
42	Acc.42	Oval	Deep red	Scarlet red	Slightly dissected	Absent	Compact	Oval	Shining black	Dark brown	Shallow	Dark grey	Shallow
43	Acc.(H)1	Oval	Red	Scarlet red	Slightly dissected	Absent	Compact	Oval	Shining black	Dark brown	Pronounced	Light brown	Shallow
44	Acc.(H)2	Oval	Red	Scarlet red	Slightly dissected	Absent	Compact	Oval	Shining black	Dark brown	Shallow	Grey	Shallow
45	Acc.(H)3	Oval	Red	Scarlet red	Slightly dissected	Absent	Compact	Oval	Dark brown	Brown	Pronounced	Grey	Shallow
46	Acc.(H)4	Oval	Red	Scarlet red	Highly dissected	Absent	Compact	Oval	Dark brown	Brown	Shallow	Dark grey	No grooves

Table 4.7a. Distribution of mace and nut characters among nutmeg accessions

Sl. No.	Character	Expression	Frequency (%)
1	Shape of mace	Round Oval Oblong	21.73 60.86 17.39
2	Mace colour (fresh)	Deep red Red Orange-red	17.39 78.26 4.34
3	Mace colour (dry)	Red Scarlet red Orange-red	4.34 91.30 4.34
4	Nature of mace	Entire Slightly dissected Intermediate Highly dissected	4.34 56.52 34.78 4.34
5	Beakness of mace	Absent Present	97.82 2.17
6	Attachment of mace to nut	Loose Compact	6.52 93.47
7	Shape of nut	Round Oval Oblong	21.73 60.86 17.39
8	Nut colour (fresh)	Brown Dark brown Shining black	6.52 34.78 58.69
9	Nut colour (dry)	Light brown Brown Dark brown	4.34 8.69 86.95
10	Nature of groove on nut	Shallow Pronounced	56.52 43.47
11	Kernel colour (dry)	Grey Dark grey Light brown (coriander colour)	56.52 39.13 4.34
12	Nature of groove on kernel	No grooves Shallow Pronounced	28.26 54.34 17.39

Two splits



Three splits

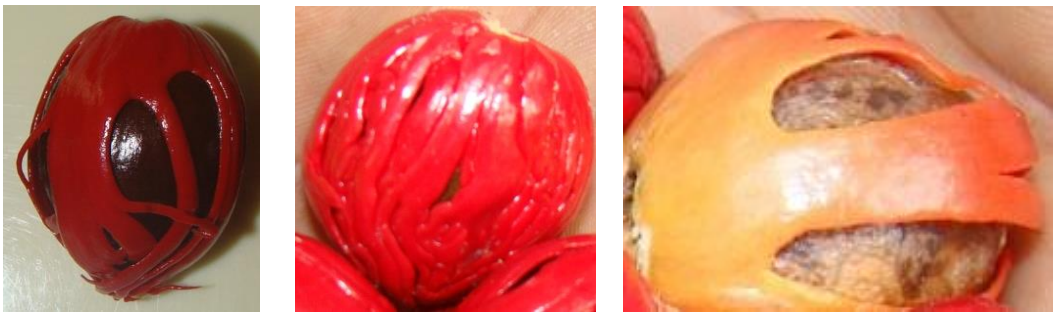
Four splits

Plate 4.12. Expressions of number of splits & number of seeds per fruit

Full splitting

Partial splitting

Plate 4.13. Expressions of nature of fruit splitting



Deep red **Red** **Orange-red**

Plate 4.14. Expressions of colour of fresh mace

Entire **Slightly dissected** **Intermediate** **Highly dissected**

Plate 4.15. Expressions of nature of mace

Beaked mace **No beak**

Plate 5.16. Expressions of beakness of mace

Black **Dark brown** **Brown** **Greyish brown**

Plate 4.17 Expressions of colour of fresh nut

Pronounced **Shallow**

Plate 4.18. Expressions of grooves on nut

Deep **Shallow** **No grooves**

Plate 4.19. Expressions of grooves on kernel

characters is a near impossibility, a fruitless exercise if attempted. Since the data on qualitative characters can be measured only utmost with an ordinary scale, it is most apt that grouping be attempted based on similarities worked out with the vector of characters at disposal. Agglomerative hierarchical clustering based on the Jaccard's similarity coefficient, using unweighted pair group method with arithmetic mean (UPGMA) is the most apt method that can be co-opted to evolve groups of accessions at any default level of similarity admissible. The results ensuing out of the

above mentioned classification of accessions will bolt the first step of the ladder towards characterization of accessions.

4.1.3 Clustering based on qualitative characters

Forty seven qualitative characters were considered and dendrogram was formulated.

All the forty six accessions including female and monoecious were used in formulating dendrogram. Accessions could be grouped into 11 clusters at 66 per cent similarity level (Fig. 4.1). The 11 clusters obtained and accessions constituting each cluster are presented in Table 4.8.

Cluster IV was the largest one having 20 accessions. In cluster VII, there were seven members. Cluster V and VI contained four accessions each. Cluster III included three accessions. Accession 2 and 33 were included in cluster IX. Cluster II included Acc. 9 and Acc. 27. Cluster I, VIII, X and XI included single accessions each namely Acc. 1, Acc. 36, Acc. (H) 1 and Acc. (H) 4 respectively. These accessions were distinct in their qualitative characters when compared to other female and monoecious accessions.

The above exercise has paved the way to align each accession with the rest of seemingly nearby accessions in a systematic way as the rider at default similarity is pulled back to fold down the multiribbed umbrella of dendrogram.

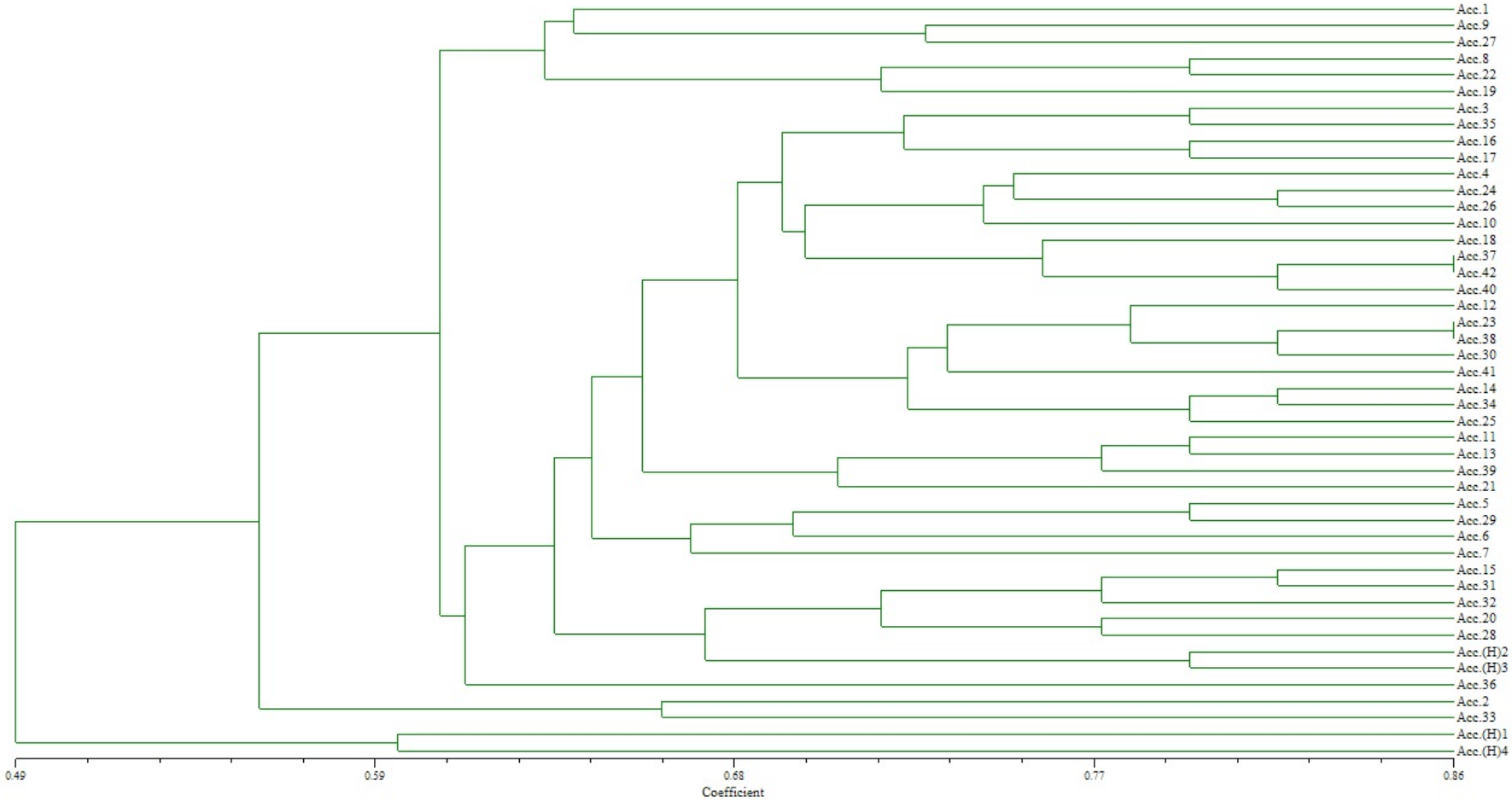


Fig. 4. 1. UPGMA dendrogram of qualitative characteristics of nutmeg accessions

Table 4.8. Clustering based on qualitative characters in nutmeg accessions

Cluster number	Number of accessions	Cluster members
I	1	Acc.1
II	2	Acc.9, Acc.27
III	3	Acc.8, Acc.22, Acc.19
IV	20	Acc.3, Acc.35, Acc.16, Acc.17, Acc.4, Acc.24, Acc.26, Acc.10, Acc.18, Acc.37, Acc.42, Acc.40, Acc.12, Acc.23, Acc.38, Acc.30, Acc.41, Acc.14, Acc.34, Acc.25
V	4	Acc.11, Acc.13, Acc.39, Acc.21
VI	4	Acc.5, Acc.29, Acc.6, Acc.7
VII	7	Acc.15, Acc.31, Acc.32, Acc.20, Acc.28, Acc.(H)2, Acc.(H)3
VIII	1	Acc.36
IX	2	Acc.2, Acc.33
X	1	Acc.(H)1
XI	1	Acc.(H)4

Standing at this step where the strings of the folded umbrella are tightened, the next step is to explore each constellation of accessions henceforth christened as cluster though more precise measurements, especially made in the interval scale. To assess the extent of variability, observations made on thirty eight quantitative characters were analysed initially as a one way classification. Out of the thirty eight quantitative characters, ten were growth characters and observations could be recorded on the four male accessions in addition to the already accounted forty six accessions. Thus, the data on growth parameters were analysed including the observations on male accessions so that a comprehensive assessment of the variability of the growth parameters could be made, in relation to sex manifestation. The rest of the sex linked parameters were assessed for their variability based on the available observations from forty six accessions.

4.1.4 Quantitative evaluation

All the accessions were evaluated based on various quantitative parameters and the results are presented below.

4.1.4.1 Growth parameters

Data on growth parameters of fifty accessions of nutmeg evaluated in the study are presented in Table 4.9. The accessions varied significantly with regard to tree height. Maximum tree height was recorded in Acc. 21 (12.35 m) which was followed by Acc. 42 (11.02 m), Acc. (H) 4 (10.85 m), Acc. (M) 1 (10.71 m), Acc. 16 (10.54 m) and Acc. 10 (10.31 m). Minimum tree height was recorded in Acc. 20 (3.20 m). Other accessions were intermediate with respect to tree height.

There was significant difference in tree girth among the accessions. Maximum tree girth was recorded in Acc. 12 (63.51 cm). The girth of Acc. (H) 4 (53.34 cm), Acc. 21 (58.02 cm) and Acc. 42 (56.12 cm) were on par with Acc. 12. Minimum tree girth was recorded in Acc. 25 (20.33 cm).

Table 4.9. Performance of nutmeg accessions for growth parameters

Accessions	Tree height (m)	Tree girth (cm)	Canopy spread (m)		No. of orthotrops/tree	Intermodal length (cm)	Leaf length (cm)	Leaf breadth (cm)	Leaf area (cm^2)	Chlorophyll content
			E-W	N-S						
Acc. 1	6.90 (2.81)	37.71 (6.21)	6.03 (2.65)	5.50 (2.54)	6.00(2.61)	30.48 (5.54)	11.60 (3.54)	4.08(2.25)	34.49(5.95)	57.50(7.64)
Acc. 2	6.45 (2.72)	47.24 (6.94)	4.30 (2.30)	4.75 (2.39)	1.50(1.57)	21.50(4.74)	11.85(3.58)	3.83(2.19)	33.95(5.91)	56.20(7.56)
Acc. 3	6.37 (2.71)	37.97 (6.24)	5.40 (2.52)	5.45(2.54)	1.50(1.57)	26.30(5.21)	10.18(3.34)	3.61(2.14)	24.89(5.08)	60.95(7.86)
Acc. 4	7.30 (2.88)	38.09 (6.25)	5.30 (2.51)	5.25(2.50)	7.00(2.82)	25.51(5.14)	9.02(3.16)	4.26(2.29)	23.90(4.99)	59.90(7.79)
Acc. 5	7.79 (2.96)	49.01 (7.07)	5.13 (2.47)	5.79(2.60)	9.00(3.15)	26.43(5.23)	11.72(3.56)	5.00(2.45)	37.09(6.16)	57.40(7.64)
Acc. 6	6.80 (2.79)	36.81 (6.14)	5.08 (2.46)	5.31(2.51)	8.00(2.99)	26.44(5.23)	12.17(3.63)	4.76(2.40)	43.08(6.64)	51.90(7.27)
Acc. 7	6.20 (2.68)	38.34 (6.27)	4.43 (2.33)	4.72(2.39)	19.00(4.47)	30.18(5.57)	9.82(3.29)	3.04(2.01)	20.29(4.61)	56.00(7.54)
(Acc. 8	8.23 (3.03)	44.63 (6.75)	6.10 (2.66)	5.42(2.53)	1.00(1.41)	20.10(4.59)	9.95(3.30)	4.13(2.26)	29.74(5.54)	60.90(7.86)
Acc. 9	8.67 (3.10)	46.99 (6.92)	6.55 (2.74)	6.09(2.66)	5.50(2.54)	30.44(5.60)	12.02(3.60)	4.75(2.39)	38.49(6.28)	67.50(8.27)
Acc. 10	10.31 (3.36)	50.27 (7.16)	5.02 (2.45)	5.63(2.57)	2.00(1.73)	20.53(4.63)	12.16(3.62)	4.27(2.29)	31.26(5.67)	49.80(7.12)
Acc. 11	8.15 (3.02)	40.64 (6.45)	5.09 (2.46)	5.61(2.57)	1.00(1.41)	32.06(5.74)	10.25(3.35)	3.85(2.20)	26.01(5.19)	57.65(7.65)
Acc. 12	8.30 (3.05)	63.51 (8.03)	6.69 (2.77)	7.22(2.86)	1.00(1.41)	32.31(5.77)	11.16(3.48)	3.59(2.14)	28.39(5.42)	58.25(7.69)
Acc. 13	5.82 (2.61)	46.35 (6.85)	5.20 (2.47)	4.65(3.37)	5.50(2.54)	21.59(4.75)	10.01(3.31)	3.90(2.21)	29.71(5.53)	51.50(7.24)
Acc. 14	5.60 (2.56)	38.30 (6.26)	6.20 (2.68)	6.49 (2.73)	18.50(4.41)	28.28(5.41)	11.04(3.47)	4.16(2.27)	34.85(5.98)	57.45(7.64)
Acc. 15	8.10 (3.01)	44.57 (6.74)	6.30 (2.69)	6.55 (2.74)	28.50(5.43)	21.59(4.74)	11.20(3.49)	4.07(2.25)	34.08(5.91)	50.30(7.15)
Acc. 16	10.54 (3.98)	53.35 (7.37)	5.89 (2.62)	5.42 (2.53)	25.50(5.14)	24.25(5.02)	11.94(3.59)	3.91(2.21)	29.44(5.51)	58.90(7.73)
Acc. 17	8.27 (3.04)	48.00 (7.00)	5.30 (2.50)	4.75 (2.39)	16.50(4.17)	30.48(5.60)	9.11(3.18)	3.93(2.22)	24.31(5.03)	49.05(7.70)
Acc. 18	9.07 (3.17)	52.83 (7.33)	6.45(2.72)	7.20 (2.86)	6.50(2.72)	23.70(4.96)	11.21(3.49)	4.58(2.36)	38.04(6.24)	54.60(7.45)
Acc. 19	5.80 (2.60)	29.20 (5.49)	3.81 (2.19)	3.76 (2.18)	1.501.57)	22.13(4.80)	9.52(3.24)	3.83(2.19)	25.64(5.16)	58.05(7.68)
Acc. 20	3.20 (2.05)	34.29 (5.94)	3.49 (2.12)	3.30 (2.07)	1.00(1.41)	18.73(4.43)	11.53(3.54)	3.23(2.05)	34.88(5.98)	50.65(7.18)
Acc. 21	12.35 (3.65)	58.02 (7.68)	8.85 (3.13)	8.55 (3.08)	15.50(4.06)	26.67(5.25)	11.60(3.54)	4.31(2.30)	38.48(6.28)	53.95(7.41)
Acc. 22	6.80 (2.79)	38.57 (6.29)	5.92 (2.62)	5.75 (2.59)	2.00(1.70)	31.61(5.69)	11.40(3.51)	4.50(2.34)	35.98(6.06)	61.25(7.88)
Acc. 23	9.39 (3.22)	41.91 (6.55)	4.19 (2.27)	3.68 (2.16)	6.50(2.72)	30.62(5.62)	11.44(3.52)	4.55(2.35)	37.50(6.20)	63.95(8.05)
Acc. 24	8.87 (3.14)	48.26 (7.01)	4.80 (2.40)	4.40 (2.32)	3.50(2.09)	33.02(5.83)	10.97(3.45)	4.70(3.38)	38.36(6.25)	61.80(7.92)
Acc. 25	8.00 (3.00)	20.33 (4.61)	7.20 (2.86)	7.03 (2.83)	1.00(1.41)	33.43(5.86)	11.16(3.48)	4.10(2.25)	30.98(5.65	59.95(7.80)
Acc. 26	6.64 (2.76)	44.44 (6.73)	5.12 (2.47)	5.10 (2.47)	2.50(1.82)	25.60(5.09)	11.38(3.51)	4.36(2.31)	38.11(6.25)	61.60(7.91)
Acc. 27	6.26 (2.69)	53.34 (7.37)	5.59 (2.56)	5.21 (2.49)	13.00(3.73)	23.03(4.90)	11.10(3.47)	4.93(2.43)	35.71(6.05)	68.60(8.34)
Acc. 28	7.14 (2.85)	33.01 (5.83)	3.11 (2.02)	3.13 (2.03)	2.50(1.86)	25.00(5.09)	11.74(3.57)	4.41(2.32)	32.89(5.82)	50.20(7.15)
Acc. 29	7.37 (2.89)	31.38 (5.67)	4.55 (2.35)	4.40 (2.32)	3.50(2.09)	33.86(5.90)	10.10(3.33)	4.31(2.30)	32.20(5.75	59.90(7.80)

Contd…

Table 4.9. Continued….

Acc. 30	9.01 (3.16)	45.71 (6.83)	5.90 (2.62)	6.62 (2.76)	10.50(3.39)	27.64(5.34)	11.79(3.57)	4.12(2.26)	39.27(6.34)	52.75(7.33)
Acc. 31	6.60 (2.75)	45.84 (6.84)	5.30 (2.50)	5.20 (2.48)	6.50(2.72)	29.21(5.48)	10.43(3.38)	3.77(2.18)	30.60(5.62)	57.35(7.63)
Acc. 32	9.03 (3.16)	43.67 (6.68)	5.09 (2.46)	5.89 (2.62)	9.50(3.21)	30.32(5.59	12.86(3.72)	4.68(2.38)	42.15(6.56)	56.30(7.57)
Acc. 33	7.53 (2.91)	46.14 (6.86)	5.85 (2.61)	5.20 (2.48)	1.50(1.57)	20.98(4.67)	11.23(3.49)	4.73(2.39)	38.49(6.28)	52.15(7.28)
Acc. 34	8.94 (3.15)	55.84 (7.53)	7.39 (2.89)	7.58 (2.93)	3.50(2.11)	24.21(5.02)	12.61(3.68)	4.30(2.30)	36.48(6.10)	59.80(7.79)
Acc. 35	7.18 (2.86)	55.86 (7.54)	6.48 (2.37)	6.20 (2.68)	33.00(5.82)	27.52(5.33)	11.30(3.50)	4.71(2.39)	37.86(6.23)	67.30(8.26)
Acc. 36	7.21 (2.87)	50.83 (7.19)	8.21 (3.03)	7.19 (2.86)	8.50(3.08)	27.44(5.33)	10.43(3.38)	3.80(2.19)	35.14(6.01)	67.25(8.26)
Acc. 37	8.41 (3.06)	53.84 (7.40)	5.30 (2.51)	5.52 (2.55)	3.00(2.00)	23.87(4.97)	13.26(3.77)	3.87(2.20)	39.00(6.32)	47.00(6.92)
Acc. 38	7.80 (2.96)	48.24 (7.01)	5.61(2.57)	5.58 (2.56)	6.00(2.63)	22.03(4.79)	9.63(3.26)	3.85(2.20)	29.76(5.54)	55.15(7.49)
Acc. 39	9.21 (3.19)	53.35 (7.37)	7.29 (2.88)	7.11 (2.84)	6.50(2.73)	27.73(5.35)	11.74(3.57)	5.36 (2.52)	47.18(6.93)	62.65(7.97)
Acc. 40	8.15 (3.02)	39.36 (6.35)	6.03 (2.65)	5.91 (2.62)	1.00 (1.41)	36.39 (6.10)	11.74 (3.57)	4.45 (2.33)	36.83 (6.14)	58.25 (9.69)
Acc. 41	6.94 (2.81)	52.70 (7.32)	8.15 (3.02)	8.85 (3.13)	12.50 (3.64)	22.80 (4.87)	11.53 (3.54)	3.63 (2.15)	30.21 (5.58)	59.30 (7.76)
Acc. 42	11.02 (3.46)	56.12 (7.55)	9.02 (3.16)	7.61 (2.93)	1.00 (1.41)	20.41 (4.62)	11.57 (3.54)	4.26 (2.29)	36.46 (6.12)	58.55 (7.71)
Acc. (H)1	6.05 (2.65)	34.28 (5.94)	6.53 (2.74)	6.21 (2.68)	1.00 (1.41)	29.08 (5.48)	10.45 (3.38)	4.18 (2.27)	34.13 (5.92)	62.50 (7.96
Acc. (H)2	8.25 (3.04)	45.72 (6.83)	5.63 (2.57)	6.21 (2.68)	6.50 (2.73)	28.28 (5.41)	9.90 (3.30)	3.44 (2.10)	25.51 (5.14)	62.35 (7.95)
Acc. (H)3	5.71 (2.59)	25.91 (5.18)	4.15 (2.27)	4.29 (2.30)	2.50 (1.86)	26.45 (5.23)	9.63(3.26)	3.63 (2.15)	24.70 (5.06)	60.90 (7.86)
Acc. (H)4	10.85 (3.44)	58.46 (7.71)	7.89 (2.98)	8.31 (3.05)	1.00 (1.41)	20.04 (4.58)	12.62(3.69)	5.38 (2.52)	54.63 (7.45)	64.85 (8.11)
Acc. (M)1	10.70 (3.42)	47.00 (6.92)	4.90 (2.42)	5.90 (2.62)	4.00(2.23)	25.02(5.10)	10.57(3.40)	4.52(2.34)	27.80 (5.35)	55.15 (7.49)
Acc. (M)2	7.12(2.85)	36.82 (6.15)	5.20(2.49)	6.20(2.68)	1.00(1.41)	23.88(4.98)	10.15(3.33)	3.68(2.16)	25.06 (5.09)	48.10 (7.00)
Acc. (M)3	7.14(2.85)	43.18(6.64)	6.10(2.66)	5.50(2.55)	6.50(2.73)	28.75(5.45)	9.61(3.25)	4.12(2.26)	32.58 (5.79)	58.85 (7.73)
Acc. (M) 4	8.67(3.11)	53.34 (7.37)	8.90(3.14)	9.30(3.20)	2.00(1.73)	41.48(6.51)	10.42(3.38)	3.65(2.15)	27.71 (5.35)	49.10 (7.07)
C.D (0.05)	0.72(0.12)	5.01(0.38)	1.00(0.19)	0.77(0.15)	3.19(0.54)	7.77(0.73)	0.92(0.13)	0.47(0.10)	7.05 (0.58)	6.41 (0.42)
C.V (%)	4.53(2.05)	5.54(2.81)	8.51(3.61)	6.54(2.82)	23.06(10.52)	14.40(6.91)	4.17(1.93)	5.64(2.26)	10.45 (4.99)	5.52 (2.73)

***Figures in parenthesis are square root transformed values**

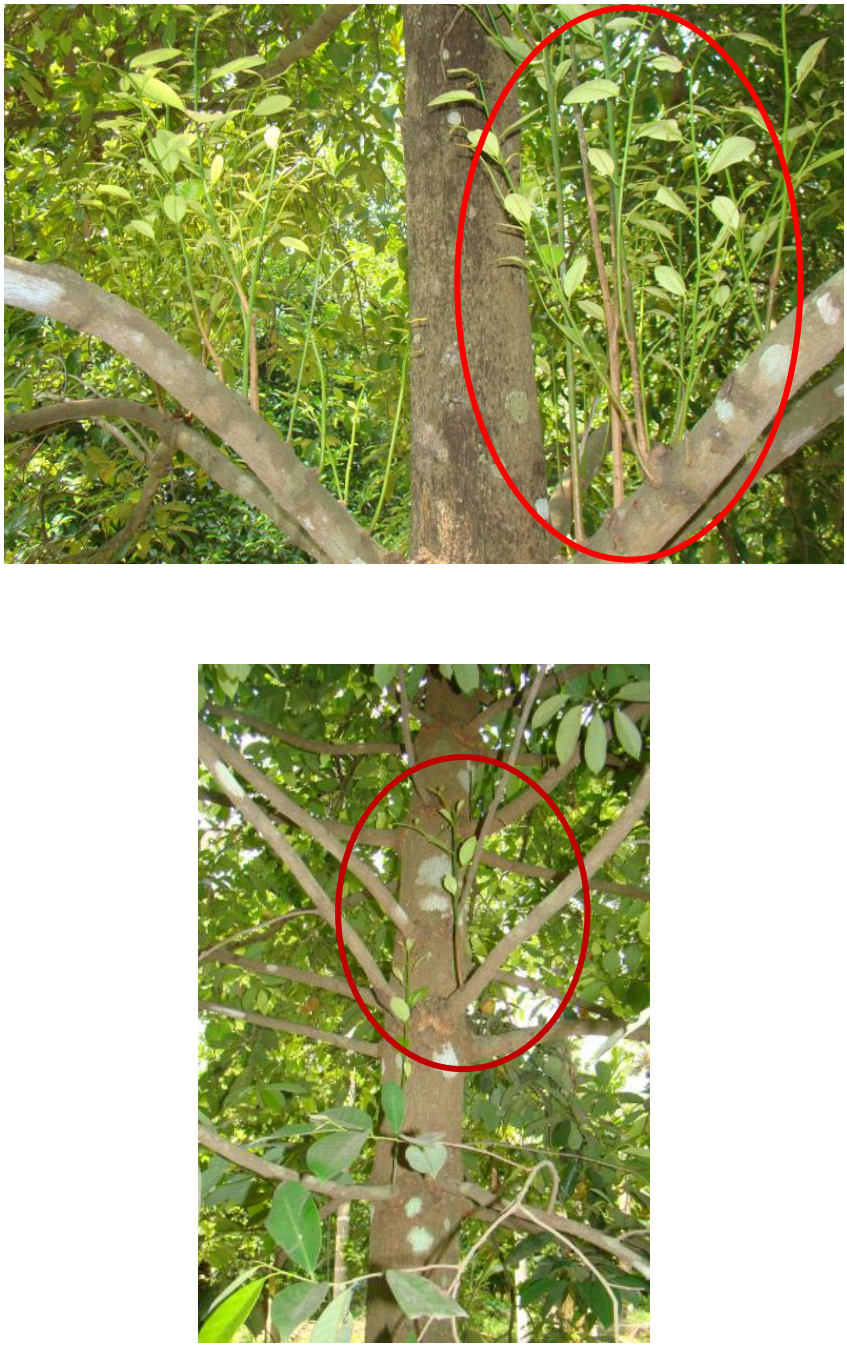


Plate 4.20. Variation in number of orthotrops

Accessions differed significantly for canopy spread in both E-W and N-S directions. Maximum E-W spread was recorded in Acc. 42 (9.02 m) and minimum E-W tree spread in Acc.28 (3.11 m). Maximum N-S tree spread was registered in Acc (M) 4 (9.30 m). Similar trends were observed in minimum canopy spread in both E-W and N-S directions.

The number of orthotrops per tree varied from 1.00 to 28.50 (Plate 4.20). Significantly highest number of orthotrops was recorded in Acc.15 (28.50), which was closely followed by Acc.16 (25.50).

Internodal length of accessions was recorded and it was significantly high in Acc. (M) 4 (41.48 cm) followed by Acc.40 (36.39 cm). The minimum internodal length was recorded in Acc. 20 (18.73 cm).

Leaf length was in the range, 9.02 cm (Acc. 4) to 13.26 cm (Acc. 37), while the leaf breadth significantly varied from 3.04 cm (Acc. 7) to 5.38 cm (Acc. (H) 4). The accessions Acc. 32, Acc. (H) 4 and Acc. 34 registered long leaves. Similarly, the accessions Acc. 39 and Acc. 5 possessed wider leaves.

Significantly highest leaf area was recorded in Acc. (H) 4 (54.63 cm^2). The leaf area of Acc. 39 (47.18 cm^2) was on par with that of Acc. (H) 4. The lowest leaf area was recorded in Acc. 7 (20.29 cm^2).

Maximum chlorophyll content was observed in the Acc. 27 (68.60) followed by Acc. 9, Acc. 5 and Acc. 36, which were on par with each other, whereas minimum chlorophyll content was recorded in Acc. 37 (47.00).

4.1.4.2 Flower characters and fruit set

Data pertaining to the floral characters and fruit set are given in Table 4.10. Results of the analysis of variance revealed that all the floral characters showed significant variation across the nutmeg accessions under study.

Table 4.10. Variability for flower characters and fruit set in nutmeg accessions

Accessions	No. of flowers/10cm^2	Length of flower (mm)	Breadth of flower (mm)	Length of tepal (mm)	Breadth of tepal (mm)	Length of pistil (mm)	Breadth of pistil (mm)	Fruit set percentage
Acc. 1	5.30 (2.50)	8.31(3.05)	5.82(2.61)	7.44(2.90)	5.07(2.46)	5.79(2.60)	3.02(2.00)	30.37(5.60)
Acc. 2	3.75(2.17)	7.89(2.98)	4.89(2.42)	7.35(2.88)	4.08(2.25)	5.14(2.47)	2.39(1.84)	14.82(3.97)
Acc. 3	3.50(2.11)	8.34(3.05)	4.98(2.44)	7.63(2.93)	4.95(2.44)	5.67(2.58)	2.87(1.96)	28.05(5.39)
Acc. 4	4.50(2.34)	8.63(3.10)	5.35(2.52)	8.08(3.01)	4.43(2.33)	5.94(2.63)	2.80(1.95)	32.02(5.74)
Acc. 5	5.50(2.54)	9.33(3.21)	5.64(2.57)	8.09(3.01)	4.76(2.40)	4.97(2.44)	2.55(1.88)	19.50(4.52)
Acc. 6	5.25(2.49)	8.60(3.10)	6.16(2.67)	7.83(2.97)	4.55(2.35)	5.87(2.62)	3.22(2.05)	10.20(3.34)
Acc. 7	5.00(2.44)	10.07(3.32)	4.78(2.40)	9.33(3.21)	4.47(2.33)	6.41(2.72)	2.67(1.91)	15.09(4.01)
Acc. 8	4.25(2.29)	8.08(3.01)	5.53(2.55)	7.05(2.83)	5.32(2.51)	5.33(2.51)	2.68(1.91)	44.15(6.72)
Acc. 9	4.25(2.29)	8.72(3.11)	5.34(2.51)	7.59(2.93)	4.37(2.31)	5.53(2.55)	3.05(2.01)	40.70(6.45)
Acc. 10	3.75(2.17)	8.80(3.13)	4.15(2.27)	6.83(2.79)	3.38(2.09)	5.89(2.62)	2.74(1.93)	11.62(3.53)
Acc. 11	5.25(2.49)	8.12(3.02)	5.30(2.51)	7.38(2.89)	4.97(2.44)	5.51(2.55)	2.81(1.95)	26.32(5.22)
Acc. 12	5.50(2.54)	8.78(3.12)	6.24(2.69)	7.83(2.97)	5.38(2.52)	5.97(2.64)	3.26(2.06)	11.62(3.55)
Acc. 13	5.25(2.49)	8.60(3.09)	5.93(2.63)	7.29(2.88)	5.09(2.46)	5.22(2.49)	2.58(1.89)	15.46(4.05)
Acc. 14	6.75(2.77)	9.01(3.16)	5.80(2.60)	8.22(3.03)	5.42(2.53)	5.88(2.62)	2.78(1.94)	38.17(6.25)
Acc. 15	5.25(2.49)	9.35(3.21)	5.89(2.62)	8.23(3.03)	4.93(2.43)	5.67(2.58)	2.95(1.98)	26.32(5.22)
Acc. 16	5.75(2.59)	8.33(3.05)	5.14(2.47)	7.25(2.87)	4.37(2.31)	4.74(2.39)	2.34(1.82)	16.78(4.20)
Acc. 17	3.50(2.11)	8.76(3.12)	5.22(2.49)	7.40(2.90)	4.52(2.35)	5.52(2.55)	2.63(1.90)	35.66(6.05)
Acc. 18	4.50(2.34)	10.98(3.46)	5.84(2.61)	9.46(3.23)	5.28(2.50)	6.84(2.80)	2.99(1.99)	28.05(5.39)
Acc. 19	5.75(2.59)	7.39(2.89)	5.59(2.56)	7.08(2.84)	4.91(2.43)	5.14(2.47)	2.73(1.93)	36.73(6.14)
Acc. 20	3.75(2.17)	7.30(2.88)	5.04(2.45)	7.16(2.85)	4.09(2.25)	4.28(2.29)	2.17(1.78)	6.55(2.74)
Acc. 21	7.00(2.82)	7.28(2.87)	4.56(2.35)	6.26(2.69)	4.11(2.61	4.82(2.41)	2.23(1.79)	29.57(5.52)
Acc. 22	5.25(2.49)	10.07(3.32)	5.52(2.55)	8.75(3.12)	4.89(2.42)	6.23(2.69)	2.76(1.93)	36.80(6.14)
Acc. 23	4.50(2.34)	10.07(3.32)	5.85(2.61)	8.76(3.12)	4.59(2.36)	5.62(2.57)	2.73(1.93)	32.46(5.770
Acc. 24	5.75(2.59)	8.88(3.14)	5.36(2.52)	7.71(2.95)	5.02(2.45)	5.26(2.50)	2.73(1.93)	18.82(4.45)
Acc. 25	5.25(2.49)	8.98(3.15)	5.29(2.50)	7.22(2.86)	5.76(2.60)	4.92(2.43)	2.76(1.93)	12.68(3.69)
Acc. 26	5.25(2.49)	8.65(3.10)	5.27(2.50)	7.85(2.97)	4.89(2.42)	5.86(2.61)	2.72(1.92)	33.82(5.90)
Acc. 27	3.25(2.06)	9.06(3.17)	5.90(2.62)	8.28(3.04)	5.83(2.61)	5.97(2.64)	2.98(1.99)	34.62(5.96)
Acc. 28	4.75(2.39)	7.46(2.90)	5.45(2.54)	7.05(2.83)	5.11(2.47)	4.08(2.25)	2.16(1.77)	6.45(2.72)
Acc. 29	5.00(2.44)	7.48(2.91)	5.21(2.49)	6.67(2.76)	3.92(2.21	5.13(2.47)	2.63(1.90)	23.10(4.90)
Acc. 30	3.50(2.12)	7.35(2.89)	5.19(2.48)	6.17(2.67)	5.53(2.55	4.30(2.30)	2.38(1.83)	35.05(6.000

Contd….

Table 4.10. Continued….

Acc. 31	4.50(2.34)	9.95(3.31)	5.25(2.50)	8.40(3.06)	4.79(2.40)	4.26(2.29)	2.60(1.89)	6.50(2.72)
Acc. 32	2.50(1.87)	8.30(3.05)	5.48(2.54)	7.89(2.98)	5.45(2.53)	6.42(2.72)	2.71(1.92)	23.40(4.93)
Acc. 33	5.50(2.54)	9.61(3.25)	5.31(2.51)	8.35(3.05)	4.54(2.35)	5.32(2.51)	2.69(1.92)	14.92(3.99)
Acc. 34	6.00(2.64)	7.85(2.97)	5.80(2.60)	7.70(2.95)	4.73(2.39)	5.59(2.56)	2.68(1.91)	26.87(5.28)
Acc. 35	6.50(2.73)	8.54(3.090	5.45(2.54)	7.93(2.98)	5.158(2.48)	5.83(2.61)	2.63(1.90)	10.30(3.36)
Acc. 36	4.50(2.34)	8.51(3.08)	5.31(2.53)	8.23(3.03)	4.44(2.32	5.69(2.58)	2.98(1.99)	29.62(5.53)
Acc. 37	5.25(2.49)	9.53(3.25)	5.05(2.46)	8.04(3.00)	5.18(2.48)	5.60(2.56)	2.91(1.97)	17.10(4.25)
Acc. 38	5.50(2.54)	8.24(3.04)	6.00(2.64)	7.96(2.99)	5.45(2.54)	5.68(2.58)	2.73(1.93)	21.37(4.73)
Acc. 39	4.75(2.39)	7.93(2.98)	5.17(2.48)	6.64(2.76)	5.70(2.58)	5.55(2.56)	2.36(1.83)	20.51(4.63)
Acc. 40	3.75(2.17)	7.54(2.92)	4.81(2.40)	6.64(2.76)	4.54(2.35)	4.51(2.34)	2.33(1.82)	29.62(5.53)
Acc. 41	5.50(2.54)	9.32(3.21)	5.91(2.62)	8.21(3.03)	5.80(2.60)	6.17(2.67)	3.14(2.03)	34.90(5.99)
Acc. 42	4.25(2.29)	8.08(3.01)	3.92(2.21)	6.67(2.76)	5.45(2.53)	5.70(2.58)	2.48(1.86)	19.83(4.56)
Acc.(H)1	10.25(3.34)	9.00(3.16)	4.44(2.33)	10.01(3.31)	4.41(2.32)	7.09(2.84)	2.91(1.97)	20.82(4.67)
Acc. (H)2	5.75(2.59)	8.44(3.07)	5.43(2.53)	8.32(3.05)	5.52(2.55)	4.68(2.38)	2.37(1.83)	6.15(2.67)
Acc. (H)3	8.25(3.03)	9.69(3.27)	6.42(2.72)	9.00(3.16)	5.66(2.58)	5.95(2.63)	2.88(1.97)	8.36(3.06)
Acc. (H)4	6.75(2.78)	11.25(3.50)	7.67(2.94)	9.48(3.23)	6.49(2.73)	8.71(3.11)	4.03(2.24)	14.25(3.90)
Acc. (M)1	8.50 (3.08)	8.26(3.04)	6.73(2.78)	8.08(3.01)	6.01(2.64)	NA	NA	NA
Acc. (M)2	14.00(3.87)	8.47(3.07)	4.76(2.39)	8.35(3.05)	4.54(2.35)	NA	NA	NA
Acc. (M)3	8.00(2.99)	10.55(3.39)	5.79(2.60)	8.95(3.15)	5.67(2.58)	NA	NA	NA
Acc.(M) 4	10.75(3.41)	8.50(3.08)	4.81(2.41)	8.10(3.01)	4.54(2.35)	NA	NA	NA
C.D (0.05)	1.69(0.31)	0.85(0.13)	0.74(0.15)	0.75(0.12)	0.78(0.16)	0.72(0.13)	0.27(0.07)	2.45(0.29)
C.V (%)	15.24(6.02)	4.81(2.13)	6.78(2.87)	4.72(2.08)	7.84(3.32)	6.41(2.61)	5.05(1.86)	5.29(3.09)

***Figures in parenthesis are square root transformed values**

Number of flowers per 10 cm^2 was significantly higher in male accessions (Acc. (M) 1 and Acc. (M) 4) and that of Acc. (H) 1 was on par with the predecessors. Number of flowers per 10 cm^2 in female accessions varied from 2.50 (Acc. 32) to 7.00 (Acc. 21).

Significant difference was noticed in the length as well as breadth of flower. Maximum length of flower was recorded in Acc. (H) 4 (11.25 mm) and minimum was in Acc. 21 (7.28 mm). Breadth of flower also recorded maximum value in Acc. (H) 4 (7.67 mm).

Accessions differed significantly for length and breadth of tepal. Maximum length of tepal was recorded in Acc. (H) 1 (10.01 mm), whereas minimum was recorded in Acc. 30 (6.17 mm). Breadth of tepal significantly varied from 3.38 mm (Acc. 10) to 6.49 mm (Acc. (H) 4).

Length and breadth of pistil differed significantly among the accessions. Both length and breadth of pistil recorded maximum value in Acc. (H) 4 (8.71 and 4.03 mm, respectively). Minimum values for length and breadth of pistil were recorded in Acc. 28 (4.08 and 2.16 mm, respectively).

The statistical analysis revealed significant difference in the fruit set percentage, which was highest in Acc. 8 (44.15%). The lowest fruit set percentage was recorded in Acc. 28 (6.45%).

4.1.4.3 Fruit parameters

Data on the observations on fruit parameters are presented in Table 4.11. Analysis of variance for the fruit parameters revealed significant variations among accessions for all the parameters except shelling percentage.

The fruit weight was measured as the average fruit weight of twenty fruits. Maximum fruit weight was recorded in Acc. 24 (99.56 g) which was significantly

higher than that of all other accessions. The fruit weight of accessions such as Acc. 37 (93.47 g), Acc. 5 (90.45 g), Acc. 36 (87.97 g) and Acc. 9 (87.96 g) were on par with each other. Minimum fruit weight was recorded in Acc. 29 (39.33 g).

Fruit length significantly varied from 42.14 mm (Acc. 31) to 66.25 mm (Acc. 37) with corresponding values of 35.19 mm (Acc. 28) to 57.43 mm (Acc.24) for fruit breadth.

Significant difference was noticed among the accessions for pericarp thickness. Highest pericarp thickness was observed in Acc. 5 (15.70 mm). The pericarp thickness of Acc. 12 (14.96 mm), Acc. 6 (14.80 mm), Acc. 37 (14.47 mm), Acc. 24 (14.38 mm) and Acc. 41 (14.33 mm) were on par with that of Acc. 5. Lowest thickness of pericarp was recorded in Acc. 31 (8.26 mm).

Accessions differed significantly for fresh and dry mace weights. Fresh mace weight ranged from 0.91 g (Acc.33) to 5.27 g (Acc. 14). Accessions 14, 18, 17, 41 and 42 recorded significantly higher fresh mace weights. The dry mace weight varied from 0.46 g (Acc. 30) to 2.61 g (Acc.18).

There were significant differences among the accessions for fresh and dry nut weight. Fresh nut weight recorded maximum value in Acc. 24 (13.67 g). The fresh nut weight of Acc. 41 (13.60 g), Acc. 7 (13.39 g), Acc. 6 (13.14 g), Acc. 18 (12.38 g) and Acc. 27 (12.16 g) were on par with highest recorded fresh nut weight. The dry nut weight recorded maximum value in Acc. 41 (11.01 g) and that of Acc. 18 (10.52 g) and Acc. 27 (10.17 g) were on par with the above. Minimum fresh and dry nut weights were observed in Acc. (H) 4 (4.41 g and 3.56 g, respectively).

Shell thickness varied significantly from 0.80 mm (Acc. 23) to 1.42 mm (Acc. 1). The lowest value was recorded in Acc. 23 (0.80 mm), which was on par with Acc. 28 (0.87 mm) and Acc.19 (0.88 mm).

Table 4.11. Variability for fruit characters in nutmeg accessions

Accessions	Fruit weight (g)	Fruit length (mm)	Fruit breadth (mm)	Thickness of pericarp (mm)	Fresh mace weight (g)	Dry mace weight (g)	Fresh nut weight (g)	Dry nut weight (g)	Shell thickness (mm)	Kernel weight (g)
Acc. 1	71.91 **(**8.53)	62.63(7.97)	50.90 (7.20)	13.65 (3.82)	2.43 (1.85)	1.48 (1.57)	11.78 (3.57)	9.69 (3.26)	1.42(1.55)	6.63(2.76)
Acc. 2	51.18(7.22)	65.30(8.14)	43.98 (6.70)	9.96(3.31)	2.23 (1.79)	1.26 (1.50)	9.26 (3.20)	7.30 (2.88)	1.09(1.44)	4.85(2.42)
Acc. 3	71.13 (8.49)	59.81(7.79)	51.03(7.21)	12.91 (3.73)	2.06 (1.74)	1.07 (1.44)	11.04 (3.47)	8.47 (3.07)	0.97(1.40)	6.61(2.75)
Acc. 4	58.67 (7.72)	57.64(7.65)	46.42 (6.88)	11.44 (3.52)	2.76(1.94)	1.59 (1.61)	8.65 (3.10)	6.47 (2.73)	1.13(1.46)	4.51(2.34)
Acc. 5	90.45 (9.56)	62.74(7.98)	56.25(7.56)	15.70 (4.08)	2.94 (1.98)	1.30 (1.51)	10.22 (3.34)	7.03 (2.83)	1.07(1.44)	5.39(2.52)
Acc. 6	75.50 (8.74)	56.24(7.56)	54.06 (7.42)	14.80 (3.97)	2.86 (1.96)	1.23 (1.49)	13.14 (3.72)	8.06 (3.01)	1.05(1.43)	5.80 (2.60)
Acc. 7	68.17 (8.31)	62.06(7.94)	48.96 (7.06)	9.81(3.28)	1.96 (1.72)	0.55 (1.24)	13.29 (3.78)	7.46 (2.90)	0.95(1.39)	4.79(2.40)
Acc. 8	54.90 (7.47)	62.51(7.97)	44.20 (6.72)	10.68 (3.41)	1.53 (1.59)	0.87 (1.36)	11.14 (3.48)	8.28 (3.04)	0.94(1.39)	6.61(2.75)
Acc. 9	87.96 (9.43)	63.30(8.01)	56.19 (7.56)	14.08(3.88)	2.52 (1.87)	1.44 (1.56)	11.44 (3.52)	8.37 (3.06)	1.00(1.41)	6.89(2.81)
Acc. 10	56.49 (7.58)	61.16(7.88)	46.05(6.85)	12.23 (3.63)	2.12 (1.76)	1.20 (1.48)	9.61 (3.25)	7.35 (2.89)	1.02(1.42)	5.78(2.60)
Acc. 11	58.63 (7.72)	53.23(7.36)	48.80 (7.05)	10.75 (3.42)	2.71 (1.92)	1.56(1.60)	11.83 (3.58)	8.81 (3.13)	1.01(1.42)	7.02(2.83)
Acc. 12	73.15 (8.61)	61.98(7.93)	51.55 (7.25)	14.96 (3.99)	1.47 (1.57)	0.68 (1.29)	8.06 (3.01)	5.82 (2.61)	1.07(1.44)	4.71(2.39)
Acc. 13	55.91 (7.54)	52.92(7.34)	47.47 (6.96)	10.77 (3.43)	2.13 (1.76)	0.97 (1.40)	8.94 (3.15)	7.40 (2.89)	1.08(1.44)	5.54(2.55)
Acc. 14	70.76 (8.47)	57.93(7.67)	51.40 (7.23)	11.74 (3.56)	5.27 (2.49)	2.14 (1.76)	10.11 (3.33)	5.77(2.60)	1.06(1.43)	4.22(2.28)
Acc. 15	77.22 (8.84)	60.07(7.81)	53.26 (7.36)	13.12 (3.75)	2.18 (1.78)	1.10 (1.52)	11.31 (3.51)	7.54 (2.92)	1.15(1.46)	5.28(2.50)
Acc. 16	54.67 (7.45)	52.20(7.29)	52.20 (7.29)	12.07 (3.61)	1.07 (1.44)	0.51 (1.22)	7.58 (2.92)	5.78 (2.60)	0.98(1.40)	4.04(2.24)
Acc. 17	73.52 (8.63)	60.55(7.84)	52.71 (7.32)	12.83 (3.71)	3.77 (2.18)	1.41 (1.55)	11.46 (3.53)	7.05 (2.83)	0.96(1.40)	4.41(2.32)
Acc. 18	79.84(8.99)	57.84(7.67)	55.28 (7.50)	13.74 (3.83)	3.91 (2.21)	2.61 (1.90)	12.38 (3.65)	10.52(2.39)	1.14(1.46)	7.68(2.94)
Acc. 19	55.98(7.54)	63.44(8.02)	44.40 (6.73)	10.43 (3.38)	1.47 (2.57)	0.89 (1.37)	10.66 (3.41)	7.96 (2.99)	0.88(1.37)	6.18(2.68)
Acc. 20	46.94(6.92)	46.11(6.86)	35.63(6.050)	9.15 (3.18)	1.20 (2.48)	0.70 (1.30)	6.92 (2.81)	5.18 (2.48)	0.90(1.37)	3.59(2.14)

Acc. 21	58.87(7.73)	49.67(7.11)	48.54(7.03)	13.26 (3.77)	1.26 (1.50)	0.77 (1.33)	8.00 (3.00)	6.91 (2.81)	1.00(1.41)	4.87(2.42)
Acc. 22	65.19(8.12)	58.98(7.74)	50.00 (7.14)	11.95 (3.60)	1.66 (1.63)	0.98(1.40)	12.36 (3.65)	8.55(3.09)	1.02(1.42)	6.88(2.80)
Acc. 23	63.04(8.00)	58.26(7.69)	49.13 (7.08)	12.10(3.61)	1.89 (1.70)	0.71 (1.30)	8.80 (3.13)	6.69 (2.75)	0.80(1.34)	4.34(2.31)
Acc. 24	99.5610.02)	64.69(8.10)	57.43 (7.64)	14.38 (3.92)	2.15 (1.77)	0.93(1.39)	13.67 (3.83)	7.43 (2.90)	1.12(1.45)	5.65(2.57)
Acc. 25	61.46 (7.90)	53.94(7.41)	50.02 (7.14)	11.09 (3.47)	2.57 (1.89)	1.16(1.47)	9.21 (3.19)	6.23 (2.69)	1.14(1.46)	4.08(2.24)
Acc. 26	54.48 (7.44)	58.14(7.69)	44.64 (6.75)	10.24 (3.35)	1.47 (1.57)	0.68 (1.29)	10.34 (3.36)	7.60(2.93)	1.03(1.42)	5.42(2.53)
Acc. 27	81.41 (9.07)	60.22(7.82)	54.09 (7.42)	13.31 (3.78)	1.89 (1.70)	1.16 (1.47)	12.16 (3.62)	10.17(3.34)	0.90(1.38)	7.89(2.98)
Acc. 28	40.44 (6.43)	49.24(7.08)	35.19 (6.01)	8.42 (3.06)	1.63 (1.62)	0.98 (1.40)	6.20 (2.68)	5.49 (2.56)	0.87(1.36)	4.13(2.26)
Acc. 29	39.33 (6.34)	53.88(7.40)	40.92 (6.47)	8.99 (3.16)	1.53 (1.59)	0.62 (1.27)	7.78 (2.96)	6.27 (2.69)	1.01(1.41)	3.39(2.09)
Acc. 30	54.58 (7.45)	57.33(7.63)	45.80 (6.84)	10.59 (3.40)	1.07(1.44)	0.46(1.20)	9.95 (3.30)	6.48 (2.73)	1.09(1.44)	5.07(2.46)
Acc. 31	40.79 (6.46)	42.14(6.56)	37.35 (6.19)	8.26 (3.04)	1.82 (1.68)	1.08 (1.44)	7.21(2.86)	6.13 (2.67)	0.89(1.37)	4.20(2.28)
Acc. 32	51.48 (7.24)	55.77(7.53)	45.75 (6.83)	11.27 (3.50)	1.67 (1.63)	0.84(1.35)	9.52(3.24)	6.09(2.66)	0.90(1.38)	4.93(2.43)
Acc. 33	69.17 (8.37)	61.94(7.93)	50.49 (7.17)	11.93(3.59)	0.91 (1.38)	0.58(1.25)	8.76 (3.12)	5.32 (2.51)	1.10(1.45)	3.31(2.07)
Acc. 34	66.24 (8.20)	54.22(7.43)	51.58 (7.25)	12.94 (3.73)	1.65 (1.62)	1.11(1.45)	9.27 (3.20)	6.52 (2.73)	0.96(1.40)	4.56(2.35)
Acc. 35	51.62 (7.23)	55.81(7.53)	46.68 (6.90)	10.57(3.40)	2.28 (1.81)	1.35 (1.53)	9.51 (3.24)	7.98(2.99)	1.11(1.45)	5.81(2.61)
Acc. 36	87.97 (9.43)	60.42(7.83)	56.35 (7.57)	13.55 (3.81)	2.71 (1.92)	1.06(1.43)	10.66(3.41)	6.76 (2.78)	1.15(1.46)	4.93(2.43)
Acc. 37	93.47 (9.79)	66.25(8.20)	56.56 (7.58)	14.47 (3.93)	3.28 (2.07)	1.32(1.52)	11.73 (3.56)	6.65 (2.76)	1.14(1.46)	4.51(2.34)
Acc. 38	47.77 (6.98)	55.03(7.48)	43.47 (6.66)	10.13 (3.33)	2.94 (1.98)	1.31 (1.52)	6.51 (2.74)	4.17 (2.27)	1.04(1.42)	4.92(2.40)
Acc. 39	55.97 (7.54)	51.92(7.27)	49.94 (7.13)	11.70 (3.56)	2.76(1.94)	1.40 (1.55)	7.09 (2.84)	4.60 (2.36)	1.16(1.47)	3.15(2.03)
Acc. 40	57.29 (7.63)	49.80(7.12)	48.10 (7.00)	11.04 (3.47)	2.24(1.80)	0.77 (1.33)	11.18 (3.49)	6.41 (2.72)	1.12(1.45)	4.91(2.43)
Acc. 41	72.80 (8.59)	63.11(8.00)	51.34 (7.23)	14.33 (3.89)	3.72 (2.17)	2.03(1.72)	13.60 (3.82)	11.01(3.46)	1.10(1.45)	8.04(3.00)
Acc. 42	74.18 (8.66)	58.67(7.72)	52.80 (7.33)	13.54 (3.81)	3.10 (2.02)	1.84 (1.68)	11.45 (3.52)	8.86 (3.14)	1.02(1.42)	6.68(2.77)
Acc.(H)1	69.41 (8.38)	61.22(7.88)	50.25 (7.15)	12.06 (3.61)	2.80 (1.95)	1.07 (1.44)	11.47(3.53)	7.30 (2.88)	1.07(1.44)	5.42(2.53)

Table 4.11. Continued…..

Acc. (H)2	49.33 (7.09)	56.19(7.56)	44.50 (6.74)	10.02(3.32)	1.80(1.6)	0.77(1.33)	9.12 (3.18)	6.05 (2.65)	1.09(1.44)	4.36(2.31)
Acc. (H)3	69.40 (8.37)	61.20(7.86)	50.24 (7.14)	12.05 (3.60)	2.78 (1.94)	1.06(1.43)	11.46 (3.53)	7.29(2.87)	1.06 (1.43)	5.41(2.52)
Acc. (H)4	51.54 (9.24)	49.56(7.11)	45.09 (6.78)	12.15 (3.62)	1.17 (1.47)	0.56(1.25)	4.41 (2.37)	3.56 (2.13)	0.90(1.37)	2.65(1.91)
C.D (0.05)	4.85 (0.30)	2.53(0.16)	2.15 (0.15)	1.50(0.20)	0.62 (0.13)	0.30 (0.09)	1.76(0.20)	0.98 (0.17)	0.12(0.04)	1.10(0.23)
C.V (%)	3.73 (1.87)	2.18 (1.06)	2.18 (1.07)	6.21 (2.76)	13.83(3.85)	13.47(3.11)	8.74 (3.75)	6.93 (3.12)	5.77(1.46)	10.49(4.60

***Figures in parenthesis are square root transformed values**

Table 4.11. Continued…..

Accessions	Fruit volume (cm^3)	Nut volume (cm^3)	Mace volume (cm^3)	Kernel volume (cm^3)	Nut length (mm)	Nut breadth (mm)	Ratio of nut to mace	Shelling percentage	No. of fruits/m^2
Acc. 1	59.25(7.76)	10.35(3.36)	1.86 (1.69)	5.53(2.55)	25.14(5.11)	34.71(5.97)	4.86(2.42)	68.55 (8.34)	19.00 (4.47)
Acc. 2	50.79(7.19)	9.16(3.18)	2.64 (1.90)	5.64(2.57)	34.46(5.95)	22.38(4.83)	4.15 (2.27)	66.87 (8.23)	7.25(2.87)
Acc. 3	67.86(8.29)	11.86(3.58)	2.71 (1.92)	6.63(2.75)	32.15(5.75)	25.66(5.16)	5.37 (2.52)	78.15(8.89)	14.75(3.96)
Acc. 4	48.25(7.01)	7.65 (2.93)	2.10(1.76)	4.55(2.35)	32.97(5.82)	22.35(4.83)	3.13 (2.03)	69.79(8.41)	17.25(4.26)
Acc. 5	79.75(8.98)	9.95 (3.30)	2.25 (1.80)	6.70(2.77)	31.01(5.65)	24.91(5.09)	3.47 (2.11)	76.74 (8.81)	15.75(4.09)
Acc. 6	67.50(8.27)	10.02(3.32)	3.56(2.13)	6.07(2.65)	30.51(5.61)	25.40(5.13)	4.56 (2.35)	72.16 (8.55)	10.00(3.31)
Acc. 7	58.04(7.68)	12.87(3.72)	2.55 (1.88)	8.23(3.03)	36.63(6.13)	25.79(5.17)	6.77 (2.78)	64.21 (8.07)	13.00(3.73)
Acc. 8	45.75(6.83)	9.80 (3.28)	1.71 (1.64)	6.00(2.64)	36.26(6.10)	24.07(5.00)	7.24 (2.87)	79.77(8.98)	25.75(5.16)
Acc. 9	68.95(8.36)	10.50(3.39)	2.27 (1.81)	7.83(2.97)	33.90(5.90)	25.97(5.19)	4.54 (2.35)	82.76 (9.14)	18.75(4.42)
Acc. 10	56.79(7.60)	9.44 (3.22)	3.44 (2.10	5.18(2.48)	33.07(5.83)	23.77(4.97)	4.52 (2.35)	78.59 (8.92)	10.00(3.31)
Acc. 11	49.50(7.10)	10.80(3.43)	2.10 (1.76)	7.45(2.90)	31.59(5.70)	26.71(5.26)	4.35(2.31)	79.76 (8.98)	10.50(3.39)
Acc. 12	70.35(8.44)	8.56 (3.09)	2.04(1.74)	4.87(2.41)	29.29(5.50)	23.98(4.99)	5.46 (2.54)	81.03 (9.05)	16.50(4.12)
Acc. 13	52.75(7.33)	8.50(3.08)	3.10 (2.02)	6.55(2.74)	29.80(5.55)	24.53(5.05)	4.22 (2.28)	74.86(8.71)	15.50(4.04)

Contd….

Table 4.11. Continued….

Acc. 14	65.22(8.13)	10.29(3.36)	4.73(2.39)	6.70(2.77)	33.43(5.86)	24.00(5.00)	2.05(1.74)	64.49 (8.09)	20.75(4.66)
Acc. 15	65.53(8.15)	10.73(3.42)	2.85(1.96)	6.18(2.68)	32.68(5.80)	25.18(5.11)	5.19 (2.49)	70.06 (8.42)	11.50(3.51)
Acc. 16	48.01(7.00)	5.22 (2.49)	1.00(1.41)	3.75(2.17)	25.76(5.17)	20.46(4.63)	6.68 (2.77)	70.00(8.40)	10.75(3.42)
Acc. 17	66.50(8.21)	10.15(3.33)	3.70(2.16)	5.32(2.51)	32.61(5.79)	25.34(5.13)	3.07 (2.01)	62.55(7.97)	17.00(4.23)
Acc. 18	65.58(8.16)	10.96(3.45)	3.38(2.09)	6.44(2.72)	34.91(5.98)	26.85(5.27)	3.16 (2.04)	72.98 (8.60)	14.00(3.85)
Acc. 19	50.00(7.13)	10.45(3.38)	2.19(1.78)	5.50(2.55)	36.41(6.11)	23.11(4.91)	7.24 (2.87)	77.79(8.87)	21.25(4.71)
Acc. 20	38.60(6.28)	6.12 (2.66)	1.00(1.41)	3.60(2.14)	25.37(5.13)	16.88(4.22)	5.75 (2.59)	69.45 (8.39)	4.00(2.23)
Acc. 21	51.04(7.21)	7.81 (2.96)	1.21(1.48)	4.71(2.38)	27.74(5.36)	23.84(4.98)	6.39 (2.71)	70.65 (8.46)	25.00(5.09)
Acc. 22	53.25(7.36)	12.00 (3.60)	1.85(1.68)	6.50(2.73)	32.89(5.82)	26.23(5.21)	7.42 (2.90)	80.60(9.03)	31.50(5.69)
Acc. 23	51.90(7.26)	9.05 (3.17)	2.40 (1.84)	6.15(2.67)	29.41(5.51)	23.38(4.93)	4.65 (2.37)	72.11 (8.53)	30.50(5.61)
Acc. 24	89.36(9.30)	13.21 (3.77)	3.71 (2.17)	6.50(2.73)	36.41(6.11)	26.64(5.25)	6.35 (3.71)	75.99 (8.77)	15.00(3.96)
Acc. 25	57.91(7.67)	7.43(2.90)	3.506 (2.12)	5.62(2.57)	29.61(5.53)	23.55(4.95)	3.57 (2.13)	65.55 (8.15)	11.50(3.53)
Acc. 26	59.28(7.76)	9.92 (3.30)	2.50 (1.87)	6.42(2.72)	33.85(5.90)	22.64(4.86)	7.05 (2.83)	71.18 (8.49)	20.00(4.58)
Acc. 27	76.25(8.78)	11.89 (3.58)	2.47(1.86)	7.36(2.89)	32.61(5.79)	25.28(5.12)	6.41 (2.72)	77.66 (8.86)	23.00(4.88)
Acc. 28	39.13(6.33)	6.13 (2.67)	1.00(1.41)	4.01(2.23)	26.03(5.19)	17.90(4.34)	4.40 (2.32)	66.64 (8.21)	5.00(2.44)
Acc. 29	38.69(6.29)	7.27 (2.87)	1.94 (1.71)	2.50(1.86)	28.21(5.40)	21.04(4.69)	5.06 (2.46)	56.55 (7.51)	19.504.48)
Acc. 30	56.66(7.58)	9.88 (3.30)	1.83(1.68)	6.00(2.64)	33.59(5.88)	22.95(4.89)	9.27 (3.20)	78.66 (8.91)	16.25(4.15)
Acc. 31	35.25(6.01)	7.42 (2.90)	1.00(1.41)	5.02(2.45)	22.16(4.81)	19.55(4.53)	3.94 (2.22)	68.49(8.33)	4.75(2.38)
Acc. 32	51.90 7.26)	9.00 (3.16)	2.75 (1.93)	6.00(2.64)	33.96(5.91)	22.73(4.87)	5.70 (2.58)	81.31(9.05)	9.75(3.27
Acc. 33	69.25 8.37)	9.50 (3.23)	2.00 (1.73)	5.70(2.58)	32.01(5.74)	23.38(4.93)	9.68 (3.26)	62.34 (7.95)	8.25(3.00)
Acc. 34	53.61 7.39)	9.61 (3.25)	1.94 (1.71)	5.98(2.64)	29.68(5.53)	24.89(5.08)	5.66 (2.58)	72.34 (8.49)	17.25(4.26)
Acc. 35	45.31**(**6.79)	9.28 (3.20)	2.05 (1.74)	6.27(2.69)	30.83(5.64)	24.44(5.04)	4.16 (2.27)	72.97 (8.59)	7.75(2.95)
Acc. 36	79.00(8.94)	9.61(3.25)	3.03 (2.00)	5.93(2.63)	30.91(5.64)	25.35(5.13)	3.93 (2.22)	72.92 (8.59)	20.75(4.65)

Acc. 37	80.50(9.01)	11.30 (3.50)	4.05 (2.24)	5.70(2.58)	34.29(5.94)	25.33(5.13)	3.57 (2.13)	67.74 (8.29)	12.50(3.67)
Acc. 38	21.94(4.22)	7.60(2.93)	4.00 (2.23	4.57(2.36)	27.37(5.32)	20.73(4.66)	2.20 (1.79)	71.59 (8.51)	14.00(3.87)
Acc. 39	53.56(7.38)	6.28 (2.69)	2.27 (1.81)	4.90(2.42)	27.70(5.35)	22.26(4.82)	2.56 (1.88)	68.35 (8.31)	14.25(3.89)
Acc. 40	40.97(6.47)	10.20(3.34)	3.00 (1.99)	5.31(2.51)	30.93(5.65)	25.81(5.17)	4.98(2.44)	77.63 (8.84)	24.50(5.04)
Acc. 41	57.09(7.61)	12.63 (3.69)	3.49 (2.11)	6.99(2.82	34.48(5.95)	27.53(5.34)	3.65(2.15)	73.12 (8.60)	16.25 (4.12)
Acc. 42	69.00(8.36)	11.19(3.49)	3.75 (2.18)	6.32(2.70)	30.68(5.62)	25.67(5.16)	3.69 (2.16)	75.48(8.73)	12.50(3.66)
Acc.(H)1	66.75(8.22)	11.15(3.48)	3.00 (1.98)	6.46(2.73	33.91(5.90)	25.32(5.13)	4.09 (2.22)	74.52 (8.68)	6.00(2.64)
Acc. (H)2	57.00(7.61)	8.43(3.06)	2.70(1.91)	4.72(2.38	30.38(5.60)	22.58(4.85)	5.02(2.45)	73.43(8.60)	2.75(1.92)
Acc. (H)3	66.74(8.22)	11.14(3.48)	2.98 (1.96)	6.45(2.73)	33.90(5.90)	25.31(5.12)	4.08 (2.24)	74.51(8.68)	4.49(2.32)
Acc. (H)4	46.66(6.90)	4.00(2.23)	1.00 (1.41)	2.00(1.73	24.58(5.05)	17.23(4.27)	3.76 (2.18)	74.28 (8.67)	8.25(3.02)
C.D (0.05)	11.55(1.09)	1.18 (0.18)	0.88(0.22)	1.31(0.25)	2.03 (0.17)	1.19 (0.12)	0.91 (0.18)	NS	5.50(0.68)
C.V (%)	9.96(7.15)	6.18 (2.80)	17.32 6.00)	11.35(4.87)	3.22 (1.55)	2.45 (1.21)	9.12 (3.78)	12.00 (6.04)	18.59(8.79)

***Figures in parenthesis are square root transformed values** ***NS- Non significant**

Kernel weight differed significantly among the accessions. Plate 4.21 depicts the variation in kernel weight. Highest kernel weight was recorded in Acc. 41 (8.04 g) and that of Acc. 27 (7.89 g), Acc. 18 (7.68 g) and Acc. 11 (7.02 g) were on par with that of the aforesaid accessions. The lowest kernel weight was observed in Acc. (H) 4 (2.65 g).

Significant differences were noticed among the accessions for volume of fruit, nut, mace and kernel. Fruit volume ranged from 21.94 cm^3 (Acc. 38) to 89.36 cm^3 (Acc. 24). Highest fruit volume was recorded in Acc. 24 (89.36 cm^3) and that of Acc. 37 (80.50 cm^3) and Acc. 36 (79.00 cm^3) were on par with that of this accession. Highest nut volume was recorded in Acc. 24 (13.21 cm^3). The nut volume of Acc.22 (12.00 cm^3) and Acc. 42 (11.19 cm^3) were on par with that of Acc. 24. The lowest nut volume was recorded by Acc. (H) 4 (4.00 cm^3). Mace volume varied form 1.00 cm^3 (Acc. 16) to 4.73 cm^3 (Acc. 14). The mace volume of Acc. 14, Acc.37 and Acc. 38 were on par with each other. Maximum kernel volume was recorded in Acc. 7 (8.23 cm^3) followed by Acc. 9 (7.83 cm^3), Acc. 11 (7.45 cm^3) and Acc. 27 (7.36 cm^3), which were on par with that of Acc. 7.

Nut length as also breadth differed significantly among the accessions. Longest nut was observed in Acc. 7 (36.63 mm) followed by Acc. 19 (36.41 mm), Acc. 24 (36.41 mm) and Acc. 8 (36.26 mm). Accession 31 possessed shortest nut (22.16 mm). Maximum nut breadth was recorded in Acc. 1 (34.71 mm), which was significantly higher than that of other accessions. The nut breadth of accessions, 11 (26.11 mm), 24 (26.64 mm) and 22 (26.23 mm) were on par. Minimum nut breadth was recorded in Acc. 20 (16.88 mm).

Significant difference was noticed for ratio of nut to mace. Maximum nut to mace ratio was recorded in Acc. 33 (9.68), which was on par with Acc. 30 (9.27). Accession 14 registered minimum nut to mace ratio (2.05).

Maximum shelling percentage was recorded in Acc. 9 (82.76), which was followed by Acc. 12, Acc. 32 and Acc. 22. The lowest shelling percentage was noticed in Acc. 29 (55.55).

The statistical analysis revealed significant difference in the number of fruits per m^2. Maximum number of fruits per m^2 was recorded in Acc. 22 (31.50) and that of Acc. 23 (30.50), Acc. 8 (25.75) and Acc. 21 (25.00) were on par with that of Acc. 22. Minimum number of fruits per m^2 was recorded in Acc. 20 (4.00).

4.1.5 Yield and number of fruits per tree

All the yield parameters were recorded over two consecutive years to bring into picture the true variability of the accessions as regards yield. Year wise data for 2013 and 2014, and their pooled mean values are presented in Table 4.12.

The accessions showed significant differences in both the years with respect to the average number of fruits per tree. During the year 2013, the number of fruits per tree varied from 27 (Acc.31) to 4420 (Acc. 9). The number of fruits per tree of Acc. 8 (3835), Acc. 22 (3690), Acc. 21 (3325), Acc. 18 (3252) and Acc. 12 (3015) were significantly higher with more than 3000 fruits per tree and were on par with that of Acc. 9. Besides, Acc. 1, Acc. 38, Acc. 25, Acc. 34, Acc. 23, Acc. 27, Acc. 36 and Acc. 14 yielded more than 2000 fruits per tree.

During 2014, Accession 9 (4775) and 8 (4455) recorded consistently higher number of fruits per tree and were significantly superior over other accessions as regards number of fruits. Acc.1 and Acc. 22 yielded more than 3000 fruits per tree. Accessions such as Acc. 14, Acc. 12, Acc. 21 and Acc. 34 produced more than 2000 fruits per tree.

The pooled means of the number of fruits per tree for two consecutive years varied from 29 (Acc. 31) to 4597 (Acc. 9). Accessions 9 (4597), 8 (4145), 22 (3507) and 1 (3283) were noticed as bearing prominently higher number of fruits per tree,

Table 4.12. Yield and number of fruits per tree in nutmeg accessions

Accessions	No. of fruits (tree/year)			Pooled yield (kg/tree/year)				
	2013	2014	Pooled mean	Fresh mace	Dry mace	Fresh nut	Dry nut	Kernel
Acc.1	2817.50	3750.00	3283.75	8.01 (2.82)	4.89 (2.20)	38.67 (6.21)	31.74 (5.63)	21.77 (4.66)
Acc.2	177.50	115.00	146.25	0.32 (0.57)	0.18 (0.42)	1.35 (1.16)	1.05 (1.02)	0.71 (0.84)
Acc.3	1462.50	1457.50	1460.00	3.01 (1.73)	1.57 (1.25)	16.14 (4.01)	12.41 (3.51)	9.66 (3.10)
Acc.4	870.00	310.00	590.00	1.63 (1.27)	0.93 (0.96)	5.10 (2.25)	3.80 (1.95)	2.65 (1.63)
Acc.5	1205.00	1525.00	1365.00	4.02 (2.00)	1.78 (1.33)	13.81 (3.71)	9.57 (3.09)	7.35 (2.70)
Acc.6	1272.50	1060.00	1166.25	3.33 (1.82)	1.42 (1.19)	14.97 (3.86)	9.45 (3.06)	6.76 (2.59)
Acc.7	650.00	680.00	665.00	1.30 (1.14)	0.37 (0.60)	8.83 (2.97)	4.96 (2.22)	3.18 (1.78)
Acc.8	3835.00	4455.00	4145.00	6.37 (2.52)	3.60 (1.89)	46.19 (6.79)	34.33 (5.85)	27.39 (5.23)
Acc.9	4420.00	4775.00	4597.50	11.60 (3.40)	6.64 (2.57)	52.62 (7.25)	38.49 (6.23)	31.70 (5.63)
Acc.10	782.50	610.00	696.25	1.47 (1.21)	0.83 (0.91)	6.70 (2.58)	5.12 (2.26)	4.03 (2.00)
Acc.11	1202.50	1070.00	1136.25	3.09 (1.75)	1.77 (1.33)	13.50 (3.66)	9.96 (3.15)	7.95 (2.88)
Acc.12	3015.00	2525.00	2770.00	4.09 (2.02)	1.88 (1.37)	22.35 (4.72)	16.12 (4.01)	13.07 (3.61)
Acc.13	610.00	567.50	588.75	1.25 (1.11)	0.57 (0.75)	5.26 (2.29)	4.35 (2.08)	3.26 (1.80)
Acc.14	2050.00	2580.00	2315.00	12.17 (3.46)	4.94 (2.20)	23.42 (4.83)	13.36 (3.65)	9.76 (3.11)
Acc.15	422.50	725.00	573.75	1.25 (1.11)	0.63 (0.79)	6.50 (2.54)	4.32 (2.07)	3.02 (1.73)
Acc.16	1372.50	1175.00	1273.75	1.36 (1.17)	0.64 (0.80)	9.65 (3.10)	7.36 (2.71)	5.14 (2.26)
Acc.17	825.00	840.00	832.50	3.16 (1.77)	1.17 (1.08)	9.54 (3.08)	5.87 (2.42)	3.68 (1.91)
Acc.18	3252.50	1175.00	2213.75	8.65 (2.94)	5.78 (2.40)	27.40 (5.23)	23.29 (4.82)	17.00 (4.12)
Acc.19	675.00	1015.00	845.00	1.24 (1.11)	0.75 (0.86)	9.00 (2.99)	6.74 (2.59)	5.22 (2.28)
Acc.20	38.50	42.50	40.50	0.04 (0.22)	0.02 (0.16)	0.28 (0.52)	0.20 (0.45)	0.14 (0.38)
Acc.21	3325.00	2280.00	2802.50	3.52 (1.87)	2.16 (1.46)	22.52 (4.73)	19.43 (4.40)	13.65 (3.69)
Acc.22	3690.00	3325.00	3507.50	5.82 (2.41)	3.44 (1.85)	43.21 (6.57)	29.94 (5.47)	24.18 (4.91)
Acc.23	2287.50	1525.00	1906.25	3.62 (1.89)	1.35 (1.16)	16.84 (4.09)	8.98 (2.99)	6.36 (2.52)
Acc.24	577.00	565.00	571.00	1.22	0.53	7.79	4.25	3.23

Contd…

Table 4.12. Continued…

				(1.10)	(0.72)	(2.79)	(2.05)	(1.79)
Acc.25	2415.00	1975.00	2195.00	5.64 (2.37)	2.56 (1.60)	20.22 (4.49)	13.68 (3.69)	8.96 (2.99)
Acc.26	937.00	855.00	896.00	1.31 (1.14)	0.61 (0.78)	9.25 (3.03)	6.80 (2.60)	4.85 (2.20)
Acc.27	2277.50	1825.00	2051.25	3.89 (1.97)	2.38 (1.54)	24.96 (4.99)	20.87 (4.56)	16.17 (4.02)
Acc.28	46.00	47.50	46.75	0.07 (0.27)	0.04 (0.21)	0.29 (0.53)	0.25 (0.50)	0.19 (0.43)
Acc.29	1257.50	1127.50	1192.50	1.83 (1.35)	0.74 (0.86)	9.27 (3.04)	7.50 (2.72)	4.02 (2.00)
Acc.30	2030.00	1885.00	1957.50	2.09 (1.44)	0.89 (0.94)	19.41 (4.40)	12.665 (3.557)	9.94 (3.15)
Acc.31	27.00	32.50	29.75	0.05 (0.23)	0.03 (0.17)	0.21 (0.46)	0.18 (0.42)	0.12 (0.355)
Acc.32	420.00	345.00	382.50	0.64 (0.80)	0.32 (0.56)	3.62 (1.90)	2.32 (1.528)	1.899 (1.372)
Acc.33	242.50	255.00	248.75	0.22 (0.47)	0.14 (0.38)	2.18 (1.47)	1.32 (1.15)	0.82 (0.90)
Acc.34	2350.00	2055.00	2202.50	3.62 (1.90)	2.44 (1.56)	20.37 (4.51)	14.31 (3.77)	10.09 (3.16)
Acc.35	602.50	435.00	518.75	1.18 (1.08)	0.70 (0.83)	4.93 (2.21)	4.12 (2.02)	3.03 (1.73)
Acc.36	2086.00	1068.50	1577.25	4.29 (2.06)	1.67 (1.29)	16.78 (4.09)	10.69 (3.26)	7.79 (2.78)
Acc.37	935.00	885.00	910.00	2.98 (1.72)	1.21 (1.09)	10.66 (3.26)	6.05 (2.45)	4.10 (2.02)
Acc.38	2610.00	1805.00	2207.50	6.50 (2.55)	2.89 (1.70)	14.36 (3.79)	9.21 (3.03)	10.89 (3.24)
Acc.39	870.00	660.00	765.00	2.12 (1.45)	1.07 (1.03)	5.42 (2.32)	3.51 (1.87)	2.39 (1.54)
Acc.40	1462.50	1105.00	1283.75	2.87 (1.69)	0.99 (0.99)	14.33 (3.78)	8.26 (2.86)	6.29 (2.50)
Acc.41	1438.50	1667.50	1553.00	5.75 (2.39)	3.17 (1.77)	21.19 (4.57)	17.20 (4.12)	12.50 (3.51)
Acc.42	687.50	725.00	706.25	2.19 (1.48)	1.30 (1.14)	8.08 (2.84)	6.26 (2.50)	4.714 (2.169)
Acc. (H) 1	215.00	175.00	195.00	0.54 (0.73)	0.20 (0.45)	2.24 (1.49)	1.42 (1.19)	1.05 (1.02)
Acc. (H) 2	77.50	52.50	65.00	0.11 (0.34)	0.04 (0.22)	0.58 (0.76)	0.38 (0.62)	0.28 (0.53)
Acc. (H) 3	89.50	67.50	78.50	0.22 (0.46)	0.08 (0.29)	0.89 (0.94)	0.57 (0.75)	0.42 (0.65)
Acc. (H) 4	400.00	305.00	352.50	0.41 (0.64)	0.19 (0.44)	1.55 (1.24)	1.25 (1.12)	0.93 (0.96)
CD (0.05)	616.69	566.47	406.97	1.46 (0.28)	0.75 (0.19)	3.20 (0.38)	2.70 (0.37)	2.65 (8.57)
CV (%)	21.84	22.51	22.16	23.91 (8.53)	24.19 (8.98)	11.59 (5.71)	14.06 (6.64)	17.68 (0.41)

***Figures in parenthesis are square root transformed values**

whereas, accessions 21, 12, 14, 18, 34, 38 and 27 could be categorised as better yielders.

Pooled mean of fresh and dry mace yield per tree per year differed significantly among the accessions. The fresh mace yield per tree varied from 0.05 kg (Acc. 31) to 12.17 kg (Acc. 14). Fresh mace yield of Acc. 9 (11.60 kg/tree) was on par with that of accession 14 (12.17 kg/tree). Similarly, dry mace yield varied from 0.03 kg per tree (Acc. 31) to 6.64 kg per tree (Acc. 9). Dry mace yield was significantly higher in Acc. 9 (6.64 kg/tree) followed by Acc. 18 (5.78 kg/tree), Acc. 14 (4.94 kg/tree) and Acc. 1 (4.89 kg/tree).

In case of nut yield, fresh as well as dry nut yield per tree per year differed significantly among the accessions. The fresh nut yield varied from 0.21 kg per tree (Acc. 31) to 52.62 kg per tree (Acc. 9). Fresh nut yield was significantly higher in Acc. 9 (52.62 kg/tree) followed by Acc. 8 (46.19 kg/tree), Acc. 22 (43.21 kg/tree) and Acc. 1 (38.67 kg/tree). Similarly, dry nut yield varied from 0.18 kg per tree (Acc. 31) to 38.49 kg per tree (Acc. 9). Significantly highest dry nut yield was recorded in Acc. 9 (38.49 kg/tree) followed by Acc. 8 (34.44 kg/tree), Acc. 1 (31.74 kg/tree) and Acc. 22 (29.94 kg/tree).

Kernel yield varied significantly among the accessions, it varied from 0.12 kg per tree (Acc. 31) to 31.70 kg per tree (Acc. 9). Highest kernel yield was recorded in Acc. 9 (31.70 kg/tree) followed by Acc. 8 (27.39 kg/tree) and Acc. 22 (24.18 kg/tree).

The results presented on the various quantitative parameters again highlight the quantum of variability of the inherent quantitative characteristics. Though the easily readable traits of a tree are its qualitative characteristics, it is difficult to probe into the likely quantitative characteristics through the qualitative traits. Since the same extent of oblique dimensionality as in the case of qualitative characteristics exist for quantitative characteristics, the grouping of accessions based on a

multivariate distance scale of measurement was conceived of. Mahalanobis D^2 was found to be the most apt distance scale and this distance was further utilised for formation of groups on a hierarchal way. The results of the quantitative cluster analysis are given below.

4.1.6 Clustering based on quantitative characters

Among the forty seven characters studied, only 26 economically important and significant characters were used for assessment of genetic divergence among the genotypes, using Mahalanobis D^2 statistic.

All the forty six accessions were grouped into ten clusters (Table 4.13). Cluster I included the highest number of accessions. Cluster II, III and IV were having six accessions each. Cluster VI had five accessions. Cluster VII included four accessions and cluster IX was having three accessions. Cluster V, VIII and X had two accessions each with identical quantitative characters.

The intra and inter cluster distances are presented in Table 4.14. Among the ten different clusters, the maximum inter cluster distance was observed between clusters V and III (4362.74), followed by cluster VIII and cluster III (3064.26) and cluster X and cluster III (3049.42). The minimum inter cluster distance was observed between clusters II and I (963.86).

At this stage, it is desirable that the orientation of a set of quantitative characters with in the vector space generated from the qualitative clusters for each quantitative character is visualised.

The herculean task was taken up in two stages. Initially the inter cluster association as detailed in section 3.3 was worked out. The results are presented as follows.

Table 4.13. Cluster members of D^2 analysis of nutmeg accessions

Cluster number	Number of accessions	Cluster members
I	10	Acc.3, Acc.5, Acc.6, Acc.7, Acc.11, Acc.15, Acc.34, Acc.36, Acc.(H)1, Acc.(H)3
II	6	Acc.12, Acc.16, Acc.32, Acc.33, Acc.39, Acc.(H)4
III	6	Acc.13, Acc.20, Acc.28, Acc.31, Acc.35, Acc.(H)2
IV	6	Acc.8, Acc.19, Acc.21, Acc.22, Acc.26, Acc.30
V	2	Acc.9, Acc.18
VI	5	Acc.2, Acc.4, Acc.25, Acc.29, Acc.38
VII	4	Acc.10, Acc.24, Acc.37, Acc.42
VIII	2	Acc. 14, Acc.41
IX	3	Acc.17, Acc.23, Acc.40
X	2	Acc.1, Acc.27

Table 4.14. Inter and intra cluster D^2 values of nutmeg accessions

	I	II	III	IV	V	VI	VII	VIII	IX	X
I	580.62									
II	963.86	691.50								
III	1424.87	1223.06	719.77							
IV	1451.01	1647.50	2909.41	640.26						
V	1854.89	2817.92	4362.74	1484.91	828.56					
VI	1084.21	1207.38	1255.63	1570.83	2507.40	780.87				
VII	987.74	1195.31	2184.66	1920.77	1814.22	1725.21	725.38			
VIII	1409.55	2569.00	3064.26	1656.08	1511.38	1818.87	2398.65	1180.11		
IX	1202.47	1161.46	2185.15	1393.12	2358.25	1458.98	1391.48	2311.30	816.95	
X	1196.71	1994.38	3049.42	1646.07	1677.38	2277.62	1661.85	1654.76	1489.24	1429.98

4.1.7 Inter cluster association of qualitative and quantitative clusters

It is already mentioned that the cluster analysis based on qualitative characters resulted in 11 clusters and that based on quantitative characters resulted in 10 clusters. The extent of linkage between the qualitative and quantitative clustering patterns is presented in Table 4.15.

All the accessions of qualitative clusters I, III, X and XI were distributed in quantitative clusters X, IV, I and II respectively.

The accessions in qualitative cluster II were found to be equally distributed in the quantitative clusters V and X. Similarly, the accessions in qualitative cluster IX were also found to be equally distributed in quantitative clusters II and VI.

In case of qualitative cluster IV, a wide range of distribution of the accessions over almost all the quantitative clusters was noticed with 20 per cent falling in cluster VII; fifteen per cent each falling in cluster VI and IX; ten per cent each in clusters I, II, IV and VIII; five per cent in each clusters III and V.

The accessions in qualitative cluster V were found equally distributed through the quantitative clusters I, II III and IV. Seventy five per cent of accessions of the qualitative cluster VI were found to be falling in quantitative cluster I and the remaining 25 per cent were found falling in cluster VI.

In the case of qualitative cluster VII, 57.14 per cent of the accessions fell in quantitative cluster III followed by 28.57 per cent of accessions falling in quantitative cluster I; while the remaining 14.28 per cent of the accessions fell in quantitative cluster II.

Now the second stage of the task as mentioned above has been taken up as follows. Initially the summary statistics of all the quantitative characters of the members *per se* of qualitative clusters were worked out. The results are presented below.

Table 4.15. Inter cluster association of qualitative and quantitative clusters

Qualitative cluster	Number of accessions	Per cent of accessions falling in different quantitative clusters									
		I	II	III	IV	V	VI	VII	VIII	IX	X
I	**1**	*	*	*	*	*	*	*	*	*	100
II	**2**	*	*	*	*	50	*	*	*	*	50
III	**3**	*	*	*	100	*	*	*	*	*	*
IV	**20**	10	10	5	10	5	15	20	10	15	*
V	**4**	25	25	25	25	*	*	*	*	*	*
VI	**4**	75	*	*	*	*	25	*	*	*	*
VII	**7**	28.57	14.28	57.14	*	*	*	*	*	*	*
VIII	**1**	100	*	*	*	*	*	*	*	*	*
IX	**2**	*	50	*	*	*	50	*	*	*	*
X	**1**	100	*	*	*	*	*	*	*	*	*
XI	**1**	*	100	*	*	*	*	*	*	*	*

4.1.8 Summary statistics of quantitative clusters

The mean, standard deviation (SD) and coefficient of variation (CV) computed for all the 26 quantitative characters of each of the quantitative clusters are furnished in Table 4.16. When the coefficient of variability among the cluster members for each of the quantitative clusters were assessed, it was observed that number of fruits per tree, number of fruits per m^2, fruit set percentage and number of flowers per 10 cm^2 contributed to maximum variability in cluster I.

In cluster II, number of fruits per tree, ratio of nut to mace, fruit set percentage, fresh and dry weight of mace as well as volume of nut, mace and kernel were the most variable characters. With regard to cluster III, the most variable

Table 4.16. Summary statistics of quantitative characters in different quantitative clusters

Characters	Cluster I			Cluster II			Cluster III			Cluster IV			Cluster V		
	Mean	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)
Plant height (m)	7.13	1.07	15.07	9.24	1.27	13.81	6.37	1.73	27.31	8.14	2.36	29.06	8.87	0.28	3.26
Plant girth (cm)	41.42	8.80	21.26	53.08	7.40	13.95	43.52	8.55	3.49	43.43	9.44	21.75	49.91	4.12	8.27
Canopy spread E-W-(m)	5.77	1.30	22.62	6.45	1.03	16.07	4.87	1.30	26.71	5.95	1.65	27.74	6.50	0.07	1.08
Canopy spread N-S-(m)	5.87	1.03	17.64	6.53	1.21	18.64	4.78	1.34	28.20	5.87	1.61	27.43	6.65	0.78	11.81
Leaf area (cm^2)	31.59	7.17	22.69	40.05	10.19	25.44	31.91	4.30	13.49	34.54	5.56	16.12	38.27	0.31	0.83
No. of flowers /10 cm^2	5.88	1.95	33.28	5.13	1.43	28.06	5.08	0.97	19.08	5.17	1.21	23.43	4.38	0.17	4.04
Fruit set percentage	21.12	7.66	36.28	16.92	4.33	25.62	8.57	3.72	43.42	36.02	4.78	13.29	34.37	8.94	26.02
No. of fruits per m^2	12.40	4.98	40.22	11.29	3.37	29.91	6.63	4.64	70.18	23.29	5.30	22.79	16.38	3.35	20.51
Fruit weight (g)	73.41	9.75	13.28	59.33	9.41	15.87	47.51	6.09	12.83	57.34	4.18	7.30	83.90	5.74	6.84
Fruit length (mm)	59.13	3.34	5.66	55.57	5.33	9.60	50.40	5.60	11.11	58.35	4.89	8.39	60.57	3.86	6.37
Fruit breadth (mm)	52.08	2.77	5.33	49.17	3.01	6.13	41.14	5.69	13.84	46.26	2.43	5.26	55.74	0.64	1.15
Thickness of pericarp (mm)	12.77	1.74	13.64	12.35	1.31	10.66	9.54	1.08	11.39	11.20	1.18	10.54	13.91	0.24	1.72
Fresh mace weight (g)	2.47	0.45	18.59	1.51	0.67	44.38	1.81	0.37	20.89	1.42	0.20	14.79	3.22	0.98	30.57
Dry mace weight (g)	1.12	0.25	22.62	0.76	0.33	43.54	0.98	0.23	23.81	0.78	0.18	24.08	2.03	0.83	41.10
Fresh nut weight (g)	11.37	1.22	10.75	7.58	1.76	23.35	7.99	1.37	17.18	10.41	1.44	13.86	11.91	0.65	5.51
Dry nut weight (g)	7.53	0.72	9.64	5.20	0.95	18.39	6.37	1.09	17.19	7.63	0.80	10.50	9.45	1.52	16.16
Shell thickness (mm)	1.05	0.07	6.97	1.02	0.11	10.78	0.99	0.11	12.00	0.99	0..07	7.41	1.07	0.09	9.25
Kernel weight (g)	5.53	0.77	14.05	3.80	0.91	24.03	4.61	0.87	18.98	5.84	0.83	14.35	7.29	0.55	7.56
Fruit volume (cm^3)	65.43	9.72	14.86	56.63	10.51	18.56	44.67	8.64	19.35	52.67	4.85	9.21	67.27	2.38	3.54

Nut volume (cm^3)	10.78	1.04	9.71	7.10	2.24	31.69	7.65	1.31	17.23	9.98	1.34	13.44	10.73	0.33	3.09
Mace volume (cm^3)	2.70	0.49	18.44	1.85	0.74	38.30	1.81	0.94	52.39	1.88	0.43	23.17	2.83	0.78	27.68
Kernel volume (cm^3)	6.61	0.72	10.91	4.54	1.46	32.38	5.03	1.18	23.57	5.86	0.66	11.33	7.14	0.98	13.77
Nut length (mm)	32.30	2.06	6.38	28.89	28.88	12.52	27.43	3.45	12.60	33.46	3.15	9.43	34.41	0.71	2.07
Nut breadth (mm)	25.45	0.52	2.06	21.68	2.48	11.46	20.99	3.33	15.87	23.81	1.30	5.48	26.41	0.62	2.38
Ratio of nut to mace	4.75	0.99	20.88	5.64	2.46	43.73	4.58	0.67	14.74	7.44	0.94	13.06	3.86	0.96	25.12
No. of fruits per tree	1095.55	758.87	69.26	1053.33	1045.85	99.28	233.58	289.16	123.79	2415.33	1402.17	58.05	3836.25	825.54	21.51

Contd…

Table 4.16. Continued…..

Characters	Cluster VI			Cluster VII			Cluster VIII			Cluster IX			Cluster X		
	Mean	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)
Plant height (m)	7.39	0.60	8.13	9.65	1.21	12.63	6.27	0.94	15.11	8.61	0.68	7.98	6.58	0.45	6.87
Plant girth (cm)	37.06	11.64	31.40	52.13	3.52	6.76	45.51	10.18	22.37	43.09	4.44	10.31	45.53	11.05	24.27
Canopy spread E-W-(m)	5.40	1.14	21.25	6.04	2.00	33.14	7.18	1.37	19.10	5.18	0.92	17.79	5.81	0.30	5.22
Canopy spread N-S-(m)	5.40	1.01	18.82	5.79	1.33	23.11	7.67	1.66	21.65	4.78	1.23	23.21	5.36	0.20	3.82
Leaf area (cm^2)	30.16	3.82	12.67	36.27	3.50	9.67	32.54	3.27	10.06	32.88	7.42	22.57	35.10	0.85	2.43
No. of flowers / 10 m^2	4.80	0.69	14.45	4.75	0.91	19.21	6.13	0.88	14.43	3.92	0.52	13.28	4.28	1.44	33.90
Fruit set percentage	20.80	7.63	36.71	16.85	3.65	21.70	36.54	2.30	6.30	32.58	3.01	9.25	32.50	3.00	9.24
No. of fruits per m^2	13.90	4.81	34.61	12.50	2.04	16.32	18.50	3.18	17.19	24.00	6.76	28.18	21.00	2.82	13.46
Fruit weight (g)	51.69	8.83	17.10	80.92	19.56	24.1	71.78	1.44	2.01	64.62	8.22	12.7	76.66	6.72	8.77

						7						2			
Fruit length (mm)	57.16	4.79	8.39	62.70	3.42	5.46	60.53	3.66	6.05	56.21	5.66	10.07	61.43	1.71	2.78
Fruit breadth (mm)	44.96	3.43	7.64	53.21	5.18	9.73	51.37	0.04	0.08	49.98	2.41	4.83	52.50	2.25	5.29
Thickness of pericarp (mm)	10.33	0.96	9.37	13.66	1.03	7.60	13.04	1.83	14.04	11.99	0.89	7.46	13.49	0.24	1.78
Fresh mace weight (g)	2.41	0.55	23.00	2.67	0.61	23.11	4.50	1.09	24.38	2.64	1.01	38.13	2.16	0.37	17.31
dry mace weight (g)	1.19	0.35	30.02	1.33	0.83	28.95	2.09	0.07	3.38	0.97	0.39	40.73	1.32	0.22	17.14
Fresh nut weight (g)	8.29	1.16	13.98	11.62	1.66	14.30	11.86	2.46	20.74	10.48	1.45	13.88	11.97	0.26	2.24
Dry nut weight (g)	6.09	1.15	18.91	7.58	0.92	12.21	8.39	3.70	44.16	6.05	1.21	20.07	9.93	0.33	3.41
Shell thickness (mm)	1.08	0.05	5.20	1.08	0.06	5.68	1.09	0.03	3.25	0.97	0.15	16.09	1.16	0.36	31.69
Kernel weight (g)	4.35	0.63	14.56	5.66	0.89	15.80	6.14	2.70	44.14	4.23	0.80	18.94	7.27	0.88	12.16
Fruit volume (cm^3)	43.52	13.89	31.91	73.92	14.13	19.12	61.16	5.75	9.41	53.13	12.80	24.09	67.75	12.02	17.74
Nut volume (cm^3)	7.83	0.76	9.78	11.29	1.54	13.64	11.46	1.65	14.43	9.80	0.65	6.63	11.12	1.08	9.79
Mace volume (cm^3)	2.84	0.89	31.47	3.74	0.24	6.66	4.11	0.87	21.33	3.04	0.64	21.26	2.17	0.43	19.83
Kernel volume (cm^3)	4.58	1.27	27.94	5.93	0.60	10.14	6.85	0.20	2.99	5.59	0.48	8.61	6.45	1.29	20.04
Nut length (mm)	30.53	3.06	10.03	33.62	2.39	7.12	33.96	0.74	2.18	30.99	1.60	5.18	28.88	5.28	18.28
Nut breadth (mm)	22.01	1.13	5.17	25.36	1.19	4.71	25.77	2.49	9.68	24.85	1.29	5.20	30.00	6.67	22.25
Ratio of nut to mace	3.63	1.07	29.54	4.54	1.28	28.38	2.86	1.13	39.55	4.24	1.02	24.11	5.64	1.10	19.55
No. of fruits per tree	1466.00	1032.98	70.46	745.50	151.69	20.34	1744.25	43.39	24.78	1525.00	733.25	48.08	2547.50	381.83	14.98

characters were number of fruits per tree, mace volume, number of fruits per m^2 and fruit set percentage.

In cluster IV, number of fruits per tree was the most variable character. In cluster V, fresh as well as dry weight of mace was the most variable character, while in cluster VI, number of fruits per tree, fruit and mace volume, dry weight of mace, number of fruits per m^2, fruit set percentage, tree girth and ratio of nut to mace were the most variable characters. As regards to cluster VII, canopy spread (E-W) was the most variable character.

In cluster VIII, the characters contributing to maximum variability were kernel weight, dry nut weight and ratio of nut to mace. With regard to cluster IX, number of fruits per tree and fresh and dry weight of mace were the most variable characters. Shell thickness and number of flowers per 10 cm^2 were the maximum variability contributing characters in cluster X.

The consistency of performance of each quantitative character among the accessions within a cluster varied over the different clusters. This result is a pointer towards further exploration of performance of each character taking into consideration the variability of each character cluster wise. Thus, the perception of morphological dimensions of quantitative characters for a set of qualitative characters was taken up as a final step towards the fulfilment of the task. The perceived morphological dimensions were worked out as detailed in section 3.3 and the results are presented below.

4.1.9 Perceived morphological dimensions

The inter cluster association of the members of qualitative cluster with that of the quantitative clusters was worked into. This quantum of association can be well utilised to build up the perceived morphological dimensions of the members of each qualitative cluster as detailed in the section and are presented in Table 4.17.

The perceived tree height recorded maximum value in cluster XI (10.85 m) followed by the tree height of cluster X (8.61 m) and cluster IV (8.17 m). Minimum perceived tree height was recorded in cluster VIII (6.27 m). The tree girth ranged between 39.79 cm (cluster VI) and 58.46 cm (cluster XI).

Canopy spread (E-W) recorded maximum value in cluster XI (7.89 m) and minimum value in cluster III (4.87 m), whereas canopy spread (N-S) was found maximum in cluster XI (8.31 m). Minimum value was in cluster III and cluster X (4.78 m). Highest perceived value for leaf area (cm^2) was recorded in cluster XI (54.63) and least was in cluster VI (31.05).

Number of flowers per 10 cm^2 varied from 3.92 (cluster X) to 6.75 (cluster XI). Fruit set percentage ranged from 8.57 (cluster III) to 33.65 (cluster II). Highest number of fruits per m^2 was recorded in cluster X (24.00) followed by the number of fruits per m^2 of cluster VIII and cluster II, whereas least was in cluster III (6.63).

Maximum fruit weight was recorded in cluster II (81.12 g) followed by the fruit weights of cluster I (73.41 g) and cluster VIII (71.78 g). Minimum fruit weight was recorded in cluster III (47.51 g). Longest fruit was found in cluster II (60.89 mm) and shortest fruit in cluster III (50.40 mm). Fruit breadth varied from 41.14 mm (cluster III) to 52.08 mm (cluster I). Pericarp had maximum thickness value of 13.74 mm in cluster II and the least thickness value of 9.54 mm in cluster III.

Fresh as well as dry weight of mace was found maximum in cluster VIII 4.50 g and 2.90 g respectively. Minimum fresh mace weight was in cluster III (1.81 g) and dry mace weight was in cluster V (0.93 g).

Highest fresh nut weight was recorded in cluster II (11.93 g) and least was in cluster III (7.99 g). Dry nut weight varied from 5.80 g (cluster IX) to 9.63 g (cluster II). Maximum shell thickness was noticed in cluster II (1.10 mm) and minimum in cluster XI (0.90 mm). Maximum kernel weight was found in cluster II (7.28 g)

Table 4.17. Perceived morphological dimensions of the different qualitative clusters

Character	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster X	Cluster XI
Plant height (m)	7.13	7.99	6.37	8.17	7.735	7.22	7.11	6.27	7.98	8.61	10.85
Plant girth (cm)	41.42	48.23	43.52	44.91	45.54	39.79	44.24	45.51	42.20	43.09	58.46
Canopy spread E-W-(m)	5.77	6.23	4.87	5.88	5.803	5.63	5.45	7.18	5.73	5.18	7.89
Canopy spread N-S-(m)	5.87	6.15	4.78	5.85	5.82	5.69	5.46	7.67	5.76	4.78	8.31
Leaf area (cm^2)	31.59	37.05	31.91	33.99	34.56	31.05	33.07	32.54	33.33	32.88	54.63
No. of flowers / 10 cm^2	5.88	4.34	5.08	4.81	5.38	5.47	5.38	6.13	4.90	3.92	6.75
Fruit set percentage	21.12	33.65	8.57	26.00	19.56	21.00	14.61	36.54	19.55	32.58	14.25
No. of fruits per m^2	12.40	18.15	6.63	16.27	12.54	12.95	9.53	18.50	13.06	24.00	8.25
Fruit weight (g)	73.41	81.12	47.51	67.42	61.34	65.31	59.12	71.78	54.14	64.62	51.54
Fruit length (mm)	59.13	60.89	50.40	58.52	56.12	58.39	54.50	60.53	56.64	56.21	49.56
Fruit breadth (mm)	52.08	47.85	41.14	50.13	48.01	49.42	46.52	51.37	46.31	49.98	45.09
Thickness of pericarp (mm)	12.77	13.74	9.54	12.25	11.71	11.86	11.20	13.04	10.97	11.99	12.15
Fresh mace weight (g)	2.47	2.81	1.81	2.69	1.88	2.44	2.01	4.50	2.12	2.64	1.17
Dry mace weight (g)	1.12	1.75	0.98	1.32	0.93	1.14	0.99	2.09	1.05	0.97	0.56
Fresh nut weight (g)	11.37	11.93	7.99	10.32	9.40	10.22	9.19	11.86	8.06	10.48	4.41
Dry nut weight (g)	7.53	9.63	6.37	7.13	6.62	6.99	6.62	8.39	5.80	6.05	3.56
Shell thickness (mm)	1.05	1.10	0.99	1.04	1.01	1.06	1.01	1.09	1.06	0.97	0.90
Kernel weight (g)	5.53	7.28	4.61	5.24	4.88	5.09	4.82	6.14	4.17	4.23	2.65
Fruit volume (cm^3)	65.43	67.45	44.67	57.91	56.49	57.26	54.3	61.16	47.72	53.13	46.66
Nut volume (cm^3)	10.78	10.87	7.65	9.73	8.92	9.68	8.74	11.46	7.59	9.80	4.00
Mace volume (cm^3)	2.70	2.57	1.81	2.981	1.86	2.75	2.15	4.11	2.52	3.04	1.00
Kernel volume (cm^3)	6.61	6.87	5.03	5.781	5.55	5.85	5.54	6.85	4.56	5.59	2.00
Nut length (mm)	32.30	32.28	27.43	32.02	30.46	31.64	29.43	33.96	30.00	30.99	24.58
Nut breadth (mm)	25.45	27.78	20.99	24.33	23.15	24.16	22.77	25.77	21.90	24.85	17.23
Ratio of nut to mace	4.75	6.63	4.58	4.227	5.389	4.332	4.81	2.86	4.27	4.24	3.76
No. of fruits per tree	1095.55	3341.93	233.58	1710.15	1105.73	1233.66	687.72	1744.25	1333.59	1525.00	400.00

followed by the kernel weights of cluster VIII (6.14 g) and cluster I (5.53 g). Minimum was in cluster IX (4.17 g).

Highest fruit volume was recorded in cluster II (67.45 cm^3) and lowest in cluster III (44.67 cm^3). Nut volume ranged from 7.65 cm^3 (cluster III) to 11.46 cm^3 (cluster VIII). Mace volume varied from 1.00 cm^3 (cluster XI) to 4.11 cm^3 (cluster VIII). Similarly, kernel volume ranged from 2.00 cm^3 (cluster XI) to 6.87 cm^3 (cluster II).

Longest nut was noticed in cluster VIII (33.96 mm) and shortest in cluster XI (24.58 mm). Widest nut was recorded in cluster II (27.78 mm) and thinnest in cluster XI (17.23 mm).

Ratio of nut to mace ranged from 2.86 (cluster VIII) to 6.63 (cluster II). Highest number of fruits per tree was noticed in cluster II (3341.93) followed by the number of fruits per tree of cluster VIII (1744.25), cluster IV (1710.15) and cluster X (1525.00). Lowest number of fruits per tree was in cluster XI (400).

The exploration of the data of the accessions typical but representative of the genus *Myristica* itself in terms of quality as also quantity has unveiled the characteristic of a nutmeg tree. The perceived morphological dimensions of the characters as enlisted above can well characterise a nutmeg tree. But further finer exploration into the genetic make up of the tree can be debugged through genetic variability studies. This will serve as a prelude for evaluation of nutmeg especially as an economically viable asset from plant kingdom.

4.1.10 Genetic variability studies

The genetic variability parameters including mean, range, standard deviation, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h^2), genetic advance (GA), genetic gain (GG), skewness and kurtosis for

26 quantitative characters studied in forty six accessions (forty two females and four monoecious) are presented in Table 4.18.

In the selected characters, PCV ranged between 9.39 to 83.90 per cent and GCV ranged between 9.14 and 81.05 per cent. Number of fruits per tree, number of fruits per m^2, fruit set percentage, dry and fresh weight of mace, mace volume and ratio of nut to mace exhibited higher values for PCV and GCV. Low PCV as well as GCV were recorded for fruit length and shell thickness recorded low GCV.

All the characters recorded higher values (>72.07%) for heritability. Heritability was highest for the characters namely fruit set percentage (98.64%) and fruit weight (97.19%).

Characters showed wide variation in genetic gain ranging between 16.68 per cent and 161.28 per cent. All the characters except shell thickness and fruit length exhibited high genetic gain (>20) whereas only moderate genetic gain was noticed in these two characters (10 to 20). Maximum genetic gain was exhibited for characters *viz.*, number of fruits per tree (161.28), fruit set percentage (93.36), number of fruits per m^2 (84.99) fresh (72.37) and dry (77.29) weight of mace, mace volume (62.97) as well as ratio of nut to mace (65.92).

Among the characters studied, number of flowers per 10 cm^2 and fresh and dry weight of mace exhibited highly positive skewed distribution and kurtosis. Ratio of nut to mace as well as number of fruits per tree also showed highly positive skewed distribution with meso kurtosis.

Moderately positive skewness and kurtosis were observed in shell thickness. Leaf area recorded moderately positive skewness with meso kurtotic distribution. Moderately negative skewness with meso kurtotic distribution was recorded in characters viz., fruit length, fruit breadth, nut volume, kernel volume as well as nut length. Among the characters studied, none showed highly negative skewed distribution.

Table 4.18. Descriptive statistics of nutmeg accessions

Sl. No.	Characters (unit)	Mean	Range	PCV (%)	GCV (%)	Heritability broad sense (%)	Genetic advance	Genetic gain (%)	Skewness	Kurtosis
1	Plant height (m)	7.79	3.20-12.35	21.75	21.22	95.17	3.33	42.64	0.24	0.73
2	Plant girth (cm)	44.77	20.33-63.51	20.83	20.06	92.69	17.81	39.78	-0.41	-0.09
3	Canopy spread (E-W) (m)	5.80	3.11-9.03	23.63	21.91	86.01	2.43	41.86	0.50	0.12
4	Canopy spread (N-S) (m)	5.77	3.13-8.85	23.14	22.07	90.96	2.50	43.36	0.30	0.02
5	Leaf area (cm^2)	33.94	20.29-54.63	20.41	17.72	75.37	10.75	31.68	0.53	0.88
6	No. of flowers/ $10cm^2$	5.11	2.50-10.25	28.16	23.91	72.07	2.14	41.81	1.36	3.96
7	Fruit set percentage	22.96	6.15-44.15	45.94	45.63	98.64	21.44	93.36	0.05	-1.09
8	No. of fruits/ m^2	14.66	2.75-31.50	48.54	44.75	85.00	12.46	84.99	0.44	-0.25
9	Fruit weight (g)	64.35	39.33-99.57	22.50	22.18	97.19	28.98	45.04	0.46	-0.28
10	Fruit length (mm)	57.55	42.15-66.25	9.39	9.14	94.60	10.54	18.31	-0.69	0.23
11	Fruit breadth (mm)	48.85	35.19-57.44	10.87	10.65	95.88	10.49	21.48	-0.68	0.39
12	Thickness of pericarp (mm)	11.94	8.26-15.70	15.75	14.45	84.12	3.26	27.30	0.16	-0.23
13	Fresh mace weight (g)	2.25	0.91-5.27	39.86	37.42	88.13	1.63	72.37	1.44	4.80
14	Dry mace weight (g)	1.12	0.46-2.62	41.05	39.24	91.41	0.86	77.29	1.32	2.72
15	Fresh nut weight (g)	10.01	4.42-13.67	21.98	20.19	84.33	3.82	38.19	-0.14	0.05
16	Dry nut weight (g)	7.07	3.56-11.01	22.99	21.97	91.33	3.06	43.26	0.27	0.14
17	Shell thickness (mm)	1.04	0.80-1.42	11.10	9.48	72.91	0.17	16.68	0.57	1.71
18	Kernel weight (g)	5.20	2.65-8.05	25.80	23.52	83.10	2.30	44.17	0.23	-0.49
19	Fruit volume (cm^3)	57.46	21.95-89.37	24.33	22.26	83.76	24.12	41.98	-0.32	1.74
20	Nut volume (cm^3)	9.49	4.00-13.21	21.72	20.81	91.82	3.90	41.08	-0.51	0.15
21	Mace volume (cm^3)	2.54	1.00-4.73	37.65	33.93	81.19	1.60	62.97	0.23	-0.71
22	Kernel volume (cm^3)	5.72	2.00-8.23	22.67	20.68	83.24	2.22	38.87	-0.64	0.80
23	Nut length (mm)	31.22	22.17-36.64	11.28	10.80	91.66	6.65	21.29	-0.54	-0.10
24	Nut breadth (mm)	23.99	16.88-34.72	12.36	12.13	96.31	5.89	24.53	0.23	3.33
25	Ratio of nut to mace	4.94	2.06-9.69	34.46	33.21	92.86	3.26	65.92	0.83	089
26	No. of fruits per tree	1398.09	27.00-4420.00	83.90	81.05	93.32	2254.87	161.28	0.90	0.02

*PCV & GCV (Sivsubramanian & Madhavamenon, 1973) - Low: less than 10%, Moderate: 10-20%, High: more than 20%

*H^2 (Johnson *et al*., 1995) - Low: less than 30%, Moderate: 30-60%, High: more than 60%

*GG (Johnson *et al*., 1995) - Low: less than 10%, Moderate: 10-20%, High: more than 20%

The characters economically valuable, either directly or indirectly were associated through genetic variability studies and the consistency of performance of each character as also inheritance was discussed. Next step in the evaluation study is the study of inter relationships of the different characters phenotypically and genotypically.

4.1.11 Association studies

The genotypic and phenotypic correlation coefficients were determined to obtain information on the relationship among twenty six growth and yield related characters of nutmeg accessions, and these are presented in Table 4.19 and Table 4.20, respectively.

4.1.11.1 Genotypic correlation coefficient analysis

Tree girth expressed a highly significant and positive correlation with tree height. Canopy spread in both E-W and N-S direction showed positive and significant correlation with tree height and tree girth. Canopy spread N-S had a strong positive correlation with canopy spread E-W. Leaf area had significant and positive correlation with tree height, tree girth as well as canopy spread in both E-W and N-S directions.

Number of flowers per 10 cm^2 had significant positive correlation with canopy spread in both E-W and N-S directions, whereas fruit set percentage showed significant positive correlation with canopy spread in E-W direction only. It expressed highly negative correlation with number of flowers per 10 cm^2. Number of fruits per m^2 showed high significant and positive correlation with fruit set percentage and negative correlation with number of flowers per 10 cm^2.

Fruit weight recorded positive and significant correlation with tree girth, canopy spread in both E-W and N-S directions, fruit set percentage and number of fruits per m^2.

Table 4.19. Genotypic correlation coefficients among growth, flower, yield and yield components in nutmeg accessions

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
2	0.57**																								
3	0.53**	0.54**																							
4	0.52**	0.55**	0.94**																						
5	0.39**	0.39**	0.36**	0.36**																					
6	-0.06	-0.09	0.22*	0.22*	0.03																				
7	0.08	0.03	0.24*	0.18	-0.001	-0.21*																			
8	0.14	0.06	0.20	0.08	0.06	-0.21*	0.84**																		
9	0.06	0.23*	0.26*	0.21*	0.11	0.09	0.26*	0.23*																	
10	-0.08	0.05	-0.03	-0.03	-0.19	0.04	0.34**	0.22*	0.67**																
11	0.27**	0.32**	0.44**	0.39**	0.16	0.13	0.31**	0.30**	0.92**	0.59**															
12	0.35**	0.45**	0.50**	0.49**	0.30**	0.12	0.24*	0.27**	0.90**	0.56**	0.92**														
13	-0.14	-0.03	0.19	0.22*	-0.10	0.17	0.20	0.002	0.45**	0.29**	0.47**	0.39**													
14	-0.03	0.11	0.28**	0.32**	-0.03	0.02	0.24*	-0.02	0.37**	0.22*	0.37**	0.37**	0.86**												
15	-0.19	-0.13	0.02	0.02	-0.24*	-0.07	0.41**	0.28**	0.70**	0.67**	0.65**	0.52**	0.44**	0.39**											
16	-	0.0	0.1	0.1	-	-	0.3	0.2	0.4	0.4	0.4	0.3	0.3	0.5	0.8										

Contd….

Table 4.19. Continued…

	0.16	1	5	4	0.26*	0.12	9**	1*	4**	7**	0**	8**	1**	3**	5*									
17	-0.02	0.03	0.35**	0.36**	0.03	0.21	-0.02	-0.05	0.39**	0.35**	0.45**	0.37**	0.42**	0.39**	0.37**	0.28**								
18	-0.12	0.09	0.15	0.17	-0.21*	-0.11	0.45**	0.27**	0.43**	0.46**	0.39**	0.41**	0.29**	0.51**	0.81**	0.95**	0.19							
19	0.05	0.20	0.19	0.18	0.13	0.09	0.12	0.09	0.95**	0.70**	0.86**	0.82**	0.34**	0.23*	0.65**	0.38**	0.32**	0.38**						
20	-0.22*	-0.06	-0.01	0.01	-0.25*	-0.004	0.40**	0.31**	0.68**	0.72**	0.59**	0.45**	0.39**	0.34**	0.99**	0.76**	0.24*	0.74**	0.63**					
21	-0.07	-0.06	0.12	0.14	-0.14	0.04	0.12	0.05	0.53**	0.51**	0.53**	0.37**	0.84**	0.58**	0.60**	0.25*	0.33**	0.26*	0.49**	0.55**				
22	-0.23*	-0.03	0.04	0.03	-0.2*	-0.07	0.37**	0.23*	0.59**	0.55**	0.56**	0.36**	0.40**	0.39**	0.84**	0.67**	0.15	0.72**	0.57**	0.89**	0.49**			
23	-0.13	-0.10	-0.12	-0.06	-0.25*	-0.04	0.44**	0.24*	0.47**	0.78**	0.41**	0.24*	0.28**	0.22*	0.76**	0.50**	0.08	0.51**	0.52**	0.78**	0.53**	0.68**		
24	-0.01	0.02	0.26*	0.21*	-0.15	0.05	0.41**	0.37**	0.65**	0.60**	0.68**	0.63**	0.42**	0.44**	0.83**	0.74**	0.67**	0.68**	0.58**	0.78**	0.45**	0.69**	0.42**	
25	0.02	-0.04	-0.16	-0.18	0.01	-0.17	0.16	0.23*	-0.08	0.12	-0.10	-0.14	-0.77**	-0.67**	0.10	0.08	-0.22*	0.07	0.04	0.17	-0.47**	0.09	0.23*	-0.007
26	0.25*	0.17	0.39**	0.34**	0.08	-0.12	0.62**	0.72**	0.28**	0.18	0.36**	0.39**	0.08	0.19	0.21*	0.28**	0.10	0.41**	0.06	0.17	-0.004	0.23*	0.09	0.39**	0.10

1. Plant height
2. Plant girth
3. Canopy spread (E-W)
4. Canopy spread (N-S)
5. Leaf
6. No. of flowers/10cm^2
7. Fruit set percentage
8. No. of fruits/m^2
9. Fruit weight
10. Fruit length
11. Fruit breadth
12. Thickness of pericarp
13. Fresh mace weight
14. Dry mace weight
15. Fresh nut weight
16. Dry nut weight
17. Shell thickness
18. Kernel weight
19. Fruit volume
20. Nut volume
21. Mace volume
22. Kernel volume
23. Nut length
24. Nut breadth
25. No. of fruits/tree
26. Ratio of nut to mace

Fruit length recorded high positive correlation with fruit weight and positive correlation with fruit set percentage and number of fruits per m^2. Fruit breadth showed high significant and positive correlation with fruit weight, fruit length and canopy spread in both E-W directions and also positive correlation with tree height, tree girth, fruit set percentage and number of fruits per m^2.

Thickness of pericarp had high positive correlation with fruit breadth, fruit weight and fruit length and also positive correlation with tree girth, canopy spread in both E-W and N-S directions, leaf area, fruit set percentage and number of fruits per m^2.

Fresh mace weight showed significant and positive correlation with fruit weight, fruit length, fruit breadth and thickness of pericarp. Dry mace weight had highly significant and positive correlation with fresh mace weight, fruit length, fruit breadth, fruit weight and thickness of pericarp.

Fresh nut weight expressed highly significant and positive correlation with fruit weight, fruit length and fruit breadth.

Dry nut weight expressed highly significant and positive correlation with fresh nut weight, dry mace weight, fruit length, fruit weight and negative correlation with leaf area. Shell thickness recorded significant and positive correlation with fruit breadth, fresh mace weight, dry mace weight as well as fruit weight.

Kernel weight recorded highly significant and positive correlation with dry nut weight, fresh nut weight, dry mace weight, fruit set percentage, fruit weight, fruit length as well as fruit breadth.

Fruit volume registered highly significant and positive correlation with fruit weight, fruit breadth, thickness of pericarp and fruit length. Similarly, nut volume registered positive and significant correlation between fresh nut weight, dry nut weight as well as kernel weight.

Mace volume registered highly significant and positive correlation with fresh and dry weights of mace. Kernel volume recorded highly significant and positive correlation with nut volume, fresh nut weight and kernel weight.

Nut length was found to have highly significant and positive correlation with fruit length, nut volume as well as fresh nut weight. High positive correlation was observed between nut breadth, nut weight and nut volume.

Ratio of nut to mace registered positive correlation with number of fruits per m^2 and nut length. It expressed high negative correlation with fresh and dry weight of mace was well as mace volume.

Number of fruits per tree registered high significant and positive correlation with number of fruits per m^2, fruit set percentage as well as canopy spread in both E-W and N-S directions.

4.1.11.2 Phenotypic correlation coefficient analysis

Tree girth exhibited highly significant and positive correlation with tree height. Canopy spread in both E-W and N-S directions showed highly significant and positive correlation with tree girth. Positive correlation was recorded between tree height and canopy spread in N-S as also E-W direction. Leaf area recorded significant and positive correlation with tree height, tree girth and canopy spread in both N-S and E-W directions.

Fruit set percentage expressed significant correlation with canopy spread (E-W). The number of fruits per m^2 revealed a strong significant and positive association with fruit set percentage.

Fruit weight showed significant and positive correlation with fruit set percentage, number of fruits per m^2 as well as canopy spread (E-W). Fruit length recorded significant and positive correlation with fruit weight, fruit set percentage and number of fruits per m^2. Fruit breadth recorded a strong positive correlation with

Table 4.20. Phenotypic correlation coefficients among growth, flower, yield and yield components in nutmeg accessions

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
2	0.56**																								
3	0.53**	0.54**																							
4	0.53**	0.55**	0.92**																						
5	0.35**	0.35**	0.34**	0.35**																					
6	-0.05	-0.10	0.19	0.17	0.0001																				
7	0.08	0.03	0.22*	0.17	-0.0001	-0.19																			
8	0.15	0.09	0.19	0.10	0.05	-0.18	0.77**																		
9	0.06	0.23*	0.24*	0.20	0.09	0.07	0.26*	0.23*																	
10	-0.08	0.04	-0.04	-0.03	-0.17	0.04	0.33**	0.21*	0.65**																
11	0.25*	0.30**	0.40**	0.37**	0.13	0.12	0.30**	0.28**	0.89**	0.58**															
12	0.30**	0.41**	0.41**	0.42**	0.23*	0.09	0.22*	0.24*	0.82**	0.49**	0.84**														
13	-0.13	-0.03	0.17	0.21*	-0.09	0.09	0.19	0.04	0.43**	0.26*	0.43**	0.32**													
14	-0.04	0.10	0.25*	0.29**	-0.03	-0.03	0.22*	0.01	0.35**	0.20	0.35**	0.33**	0.85**												
15	-0.18	-0.12	0.02	0.02	-0.20	0.01	0.38**	0.24*	0.65**	0.61**	0.59**	0.44**	0.39**	0.34**											
16	-0.15	0.02	0.12	0.12	-0.20	-0.08	0.38**	0.22*	0.41**	0.43**	0.39**	0.35**	0.28**	0.49**	0.75**										
17	-0.02	0.04	0.29**	0.29**	0.07	0.18	0.01	-0.05	0.32**	0.29**	0.39**	0.33**	0.31**	0.32**	0.24*	0.24*									

Contd….

Table 4.20. Continued…

18	-0.10	0.05	0.13	0.14	-0.20	-0.11	0.41**	0.23*	0.38**	0.40**	0.35**	0.34**	0.26*	0.48**	0.66**	0.85**	0.15							
19	0.04	0.17	0.17	0.15	0.09	0.09	0.12	0.06	0.86**	0.63**	0.78**	0.72**	0.28**	0.18	0.57**	0.36**	0.33**	0.22*						
20	-0.22*	-0.07	-0.01	-0.01	-0.25*	0.01	0.38**	0.25*	0.64**	0.66**	0.54**	0.40**	0.35**	0.31**	0.88**	0.69**	0.20	0.67**	0.57**					
21	-0.07	-0.04	0.09	0.11	-0.07	0.01	0.12	0.04	0.45**	0.44**	0.48**	0.35**	0.69**	0.49**	0.45**	0.20	0.33**	0.24*	0.40**	0.51**				
22	-0.21*	-0.05	0.02	0.04	-0.16	0.001	0.33**	0.20	0.54**	0.51**	0.52**	0.30**	0.35**	0.33**	0.75**	0.59**	0.11	0.61**	0.47**	0.78**	0.38**			
23	-0.13	-0.11	-0.11	-0.04	-0.19	-0.04	0.42**	0.21*	0.45**	0.73**	0.41**	0.22*	0.27*	0.19	0.69**	0.47**	0.08	0.46**	0.45**	0.73**	0.49**	0.63**		
24	-0.01	0.01	0.23*	0.20	-0.14	0.07	0.39**	0.34**	0.62**	0.58**	0.67**	0.54**	0.39**	0.41**	0.77**	0.70**	0.54**	0.62**	0.49**	0.74**	0.38**	0.63**	0.41**	
25	-0.001	-0.04	-0.15	-0.19	-0.01	-0.10	0.16	0.18	-0.08	0.12	-0.10	-0.12	-0.74**	-0.64**	0.14	0.07	-0.18	0.05	0.06	0.16	-0.43**	0.08	0.20	-0.003
26	0.26*	0.18	0.38**	0.34**	0.08	-0.10	0.60**	0.69**	0.26*	0.16	0.33**	0.35**	0.08	0.18	0.17	0.27**	0.10	0.36**	0.06	0.14	-0.01	0.22*	0.06	0.36**	0.08

1. Plant height
2. Plant girth
3. Canopy spread (E-W)
4. Canopy spread (N-S)
5. Leaf
6. No. of flowers/10cm^2
7. Fruit set percentage
8. No. of fruits/m^2
9. Fruit weight
10. Fruit length
11. Fruit breadth
12. Thickness of pericarp
13. Fresh mace weight
14. Dry mace weight
15. Fresh nut weight
16. Dry nut weight
17. Shell thickness
18. Kernel weight
19. Fruit volume
20. Nut volume
21. Mace volume
22. Kernel volume
23. Nut length
24. Nut breadth
25. No. of fruits/tree
26. Ratio of nut to mace

fruit weight, fruit length and canopy spread (E-W). Thickness of pericarp showed positive and significant correlation with fruit breadth, fruit weight as well as fruit length.

Fresh mace weight expressed strong significant and positive correlation with fruit weight as well as fruit breadth. Dry mace weight expressed strong significant and positive correlation with fresh mace weight, fruit breadth as well as fruit weight.

Fresh nut weight recorded high significant and positive correlation with fruit weight, fruit length and fruit breadth. Dry nut weight showed high positive and significant correlation with fresh nut weight, dry mace weight, fruit length and fruit breadth. Shell thickness recorded positive and significant correlation with fruit breadth as well as thickness of pericarp. Kernel weight had strong significant and positive association with fresh and dry weight of nut as well as dry mace weight.

Fruit volume showed highly significant and positive correlation with fruit weight, fruit breadth and thickness of pericarp. Similarly, nut volume showed strong positive correlation with fresh and dry weights of nut as well as kernel weight. Kernel volume recorded significant and positive correlation with nut volume, fresh nut weight and kernel weight.

Nut length showed high positive correlation with fruit length, nut volume and fresh nut weight, and nut breadth showed high positive correlation with nut weight, nut volume and nut weight.

Ratio of nut to mace showed a strong negative correlation with fresh mace weight, dry weight and mace volume. Number of fruits per tree exhibited a high significant and positive correlation with number of fruits per m^2, fruit set percentage as well as canopy spread in E-W direction.

The association studies have revealed strong association among the different traits of nutmeg. There are certain linear associations among characters that are

concealed, the correlation studies have failed to bring forth these. So as to unravel these concealed relationships the standard technique of path coefficient analysis was performed.

4.1.12 Path coefficient analysis

Path coefficient analysis is an important tool for partitioning the correlation coefficients among set of variables into direct and indirect effects on a pre-distinguished variable as dependent variable and the rest as explanatory variables. The genotypic path coefficient analysis which splits correlation coefficients of different characters into direct and indirect effects on the genotypic the number of fruits per tree, a typical yield attribute are depicted in Table 4.21.

Out of twenty six characters studied, fourteen traits showed positive direct effects on number of fruits per tree. The pertinent data revealed that fruit weight (2.04), mace volume (1.98) and thickness of pericarp (1.29) showed very high genotypic positive direct effect on number of fruits per tree. Kernel volume (0.99), dry weight of mace (0.88) and fruit set percentage (0.82) exhibited high direct effect. The direct effects of fresh mace weight (-2.82), nut volume (-2.25), fruit volume (-1.78), canopy spread in E-W (-0.81), fruit breadth (-0.77) and fruit length (-0.70) were in the negative direction and were at least very high.

Tree height showed high positive indirect effect on number of fruits per tree through nut volume (0.48), thickness of the pericarp (0.45) and fresh mace weight (0.40). Canopy spread in E-W (-0.42) showed high negative indirect effect. Kernel volume (-0.23) and fruit breadth (-0.21) showed moderate negative indirect effect. Tree girth showed high positive indirect effect on number fruits per tree via thickness of the pericarp (0.58) and fruit weight (0.46), and high negative indirect effect via canopy spread in E-W (-0.43), fruit volume (-0.35) and fruit breadth (-0.25).

Canopy spread both in E-W and N-S direction exhibited high positive indirect effect through thickness of pericarp (0.64 and 0.63, respectively), fruit weight (0.53

Table 4.21. Genotypic path coefficient analysis of nutmeg accessions

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	rG
1	**-0.23**	-0.01	-0.42	0.22	-0.10	-0.03	0.1	0.01	0.12	0.10	-0.21	0.45	0.40	-0.03	0.003	0.10	-0.01	-0.10	-0.09	0.48	-0.13	-0.23	-0.05	-0.003	0.00005	0.26
2	-0.13	**-0.02**	-0.43	0.23	-0.10	-0.043	0.023	0.004	0.46	-0.04	-0.25	0.58	0.10	0.09	0.002	-0.005	0.01	0.07	-0.35	0.12	-0.11	-0.03	-0.04	0.008	-0.001	0.16
3	-0.12	-0.01	**-0.81**	0.40	-0.09	0.098	0.20	0.013	0.53	0.02	-0.34	0.64	-0.53	0.25	-0.00003	-0.10	0.11	0.12	-0.35	0.016	0.23	0.04	-0.05	0.12	-0.005	0.38
4	-0.12	-0.01	-0.75	**0.42**	-0.09	0.10	0.15	0.005	0.43	0.02	-0.31	0.63	-0.61	0.29	-0.00003	-0.10	0.11	0.13	-0.31	-0.025	0.27	0.03	-0.02	0.10	-0.00060	0.34
5	-0.09	-0.01	-0.29	0.15	**-0.25**	0.01	-0.001	0.004	0.23	0.13	-0.12	0.39	0.29	-0.03	0.003	0.17	0.01	-0.17	-0.23	0.55	-0.26	-0.24	-0.10	-0.07	0.00002	0.07
6	0.01	0.002	-0.17	0.09	-0.01	**0.45**	-0.17	-0.01	0.18	-0.03	-0.09	0.15	-0.47	0.02	0.001	0.08	0.06	-0.09	-0.15	0.008	0.09	-0.07	-0.02	0.02	-0.005	-0.11
7	-0.02	-0.001	-0.19	0.08	0.00004	-0.10	**0.82**	0.054	0.53	-0.24	-0.24	0.31	-0.57	0.21	-0.01	-0.26	-0.01	0.37	-0.21	-0.90	0.23	0.37	0.18	0.19	0.005	0.62
8	-0.03	-0.001	-0.16	0.03	-0.014	-0.09	0.69	**0.064**	0.47	-0.16	-0.23	0.34	-0.005	-0.013	-0.004	-0.14	-0.01	0.23	-0.16	-0.70	0.10	0.23	0.09	0.18	0.008	0.72
9	-0.01	-0.004	-0.21	0.09	-0.03	0.04	0.21	0.01	**2.04**	-0.47	-0.72	1.16	-1.28	0.32	-0.01	-0.29	0.12	0.35	-1.69	-1.52	1.04	0.59	0.19	0.31	-0.003	0.27
10	0.02	-0.001	0.03	-0.013	0.05	0.02	0.28	0.01	1.36	**-0.70**	-0.46	0.72	-0.81	0.19	-0.01	-0.31	0.11	0.38	-1.24	-1.62	1.01	0.54	0.32	0.29	0.004	0.17
11	-0.06	-0.01	-0.36	0.17	-0.04	0.06	0.25	0.02	1.88	-0.41	**-0.77**	1.18	-1.32	0.33	-0.01	-0.26	0.14	0.33	-1.53	-1.31	1.04	0.56	0.16	0.33	-0.003	0.35
12	-0.08	-0.01	-0.40	0.21	-0.08	0.05	0.19	0.02	1.84	-0.39	-0.71	**1.29**	-1.10	0.32	-0.01	-0.25	0.11	0.39	-1.45	-1.01	0.73	0.36	0.10	0.30	-0.004	0.38
13	0.03	0.001	-0.15	0.09	0.03	0.08	0.16	0.00001	0.93	-0.20	-0.36	0.50	**-2.82**	0.76	-0.01	-0.20	0.13	0.24	-0.61	-0.87	1.65	0.39	0.12	0.20	-0.025	0.08
14	0.01	-0.002	-0.23	0.14	0.008	0.01	0.19	-0.001	0.75	-0.15	-0.29	0.47	-2.43	**0.88**	-0.005	-0.35	0.14	0.43	-0.41	-0.76	1.14	0.38	0.09	0.21	-0.022	0.19
1	0.0	0.00	-	0.0	0.06	-	0.33	0.01	1.4	-	-	0.6	-	0.35	**-0.01**	-	0.1	0.6	-	-	1.1	0.8	0.3	0.40	0.00	0.21

Contd…

Table 4.21. Continued….

5	4	22	0.02	1		0.03		8	3	0.47	0.51	6	1.25			0.55	1	8	1.15	2.23	8	3	1		3	
16	0.04	-0.00001	-0.12	0.06	0.07	-0.05	0.32	0.013	0.89	-0.33	-0.31	0.49	-0.87	0.47	-0.012	**-0.66**	0.08	0.85	-0.68	-1.70	0.49	0.67	0.21	0.36	0.003	0.28
17	0.01	-0.001	-0.28	0.15	-0.008	0.09	-0.02	-0.003	0.79	-0.25	-0.34	0.48	-1.19	0.34	-0.005	-0.18	**0.30**	0.16	-0.56	-0.54	0.65	0.15	0.03	0.32	-0.007	0.10
18	0.03	-0.001	-0.12	0.07	0.05	-0.05	0.37	0.018	0.87	-0.32	-0.30	0.52	-0.82	0.45	-0.011	-0.66	0.06	**0.83**	-0.67	-1.67	0.51	0.71	0.21	0.33	0.002	0.41
19	-0.01	-0.003	-0.16	0.10	-0.032	0.04	0.095	0.006	1.94	-0.49	-0.67	1.05	-0.97	0.20	-0.01	-0.25	0.10	0.32	**-1.78**	-1.42	0.97	0.56	0.21	0.28	0.001	0.06
20	0.10	0.001	0.01	0.005	0.062	-0.002	0.33	0.02	1.38	-0.50	-0.45	0.58	-1.08	0.29	-0.014	-0.49	0.073	0.62	-1.12	**-2.25**	1.09	0.88	0.32	0.38	0.006	0.17
21	0.02	0.001	-0.09	0.06	0.034	0.02	0.10	0.003	1.08	-0.36	-0.41	0.47	-2.36	0.51	-0.008	-0.16	0.10	0.22	-0.87	-1.24	**1.98**	0.48	0.22	0.27	-0.015	-0.004
22	0.06	0.00004	-0.03	0.01	0.061	-0.03	0.30	0.015	1.22	-0.38	-0.44	0.47	-1.13	0.34	-0.011	-0.44	0.05	0.59	-1.01	-2.01	0.97	**0.99**	0.28	0.33	0.003	0.22
23	0.03	0.002	0.10	-0.02	0.062	-0.02	0.35	0.015	0.95	-0.54	-0.32	0.31	-0.79	0.19	-0.01	-0.33	0.024	0.42	-0.92	-1.75	1.05	0.68	**0.41**	0.20	0.007	0.09
24	0.002	-0.00003	-0.21	0.09	0.037	0.022	0.34	0.024	1.32	-0.42	-0.53	0.81	-1.19	0.38	-0.01	-0.48	0.20	0.57	-1.03	-1.76	0.88	0.68	0.17	**0.48**	-0.00002	0.38
25	-0.003	0.001	0.13	-0.08	-0.002	-0.075	0.14	0.015	-0.16	-0.09	0.08	-0.18	2.16	-0.59	-0.001	-0.06	-0.07	0.06	-0.08	-0.39	-0.93	0.09	0.09	-0.003	**0.03**	0.10

Residual value = -0.012 rG= Genotypic correlation coefficient of number of fruits per tree

1. Plant height
2. Plant girth
3. Canopy spread (E-W)
4. Canopy spread (N-S)
5. Leaf
6. No. of flowers/10cm^2
7. Fruit set percentage
8. No. of fruits/m^2
9. Fruit weight
10. Fruit length
11. Fruit breadth
12. Thickness of pericarp
13. Fresh mace weight
14. Dry mace weight
15. Fresh nut weight
16. Dry nut weight
17. Shell thickness
18. Kernel weight
19. Fruit volume
20. Nut volume
21. Mace volume
22. Kernel volume
23. Nut length
24. Nut breadth
25. No. of fruits/tree
26. Ratio of nut to mace

and 0.43, respectively) and high negative indirect effect was evidenced through fresh weight of mace (-0.53 and -0.61, respectively).

Number of flowers per 10 cm^2 exhibited low positive indirect effect on number of fruits per tree through fruit weight (0.18) and thickness of the pericarp (0.15). Fruit set percentage revealed high positive indirect effect on number of fruits per tree through fruit weight (0.53), kernel weight and kernel volume (0.37 each), and thickness of the pericarp (0.31). High negative influence was exhibited through nut volume (-0.90), fresh mace weight (-0.57) and moderate negative influence through dry nut weight (-0.26).

The indirect effect exerted by number of fruits per m^2 on number of fruits per tree was positive and high via fruit set percentage (0.69), fruit weight (0.47) and thickness of pericarp (0.34) Whereas it was high and negative through nut volume (-0.70), moderately negative through effect on fruit breadth (-0.23) and low negative through fruit length (-0.16).

The character fruit weight exhibited very high positive and indirect effect on the number of fruits per tree through thickness of pericarp (1.16) and mace volume (1.04); high positive indirect effect through kernel volume (0.59) and very high negative indirect effect through with fruit volume (-1.69), nut volume (-1.52) and fresh mace weight (-1.28).

Fruit length was noticed to have very high positive indirect effect on number of fruits per tree through fruit weight (1.36), mace volume (1.01), high positive indirect effect via thickness of pericarp (0.72) and very high negative indirect effect through nut volume (-1.62), fruit volume (-1.24) and high negative indirect effect through fresh mace weight (-0.81).

Thickness of pericarp exhibited high positive indirect effect on number of fruit per tree through fruit weight (1.84), high positive indirect effect through mace

volume (0.73) and very high negative indirect effect on fruit volume (-1.45), fresh mace weight (-1.10) and nut volume (-1.01).

The character, fresh nut weight exhibited very high genotypic positive indirect effect on number of fruits per tree via, fruit weight (1.43), mace volume (1.18), high positive indirect effect via kernel volume (0.83) and very high negative indirect effect through nut volume (-2.23), thickness of pericarp (-1.25) and fruit volume (-1.15).

Indirect high positive effect on number of fruits per tree was shown by dry nut weight through fruit weight (0.89), kernel weight (0.85) and kernel volume (0.67). Nut volume (-1.70), thickness of pericarp (-0.87) and fruit volume (-0.68) showed at least high negative indirect effect on number of fruits per tree.

Kernel weight showed high positive indirect effect through fruit weight (0.87) and kernel volume (0.71) and negative indirect effect by nut volume (-1.67), fresh mace weight (-0.82) and dry nut weight (-0.67).

Fruit volume had at least very high positive indirect effect through fruit weight (1.94), thickness of pericarp (1.05) and mace volume (0.97) and at least high negative indirect effect by nut volume (-1.42), fresh mace weight (-0.97) and fruit breadth (-0.67).

Nut volume recorded at least high positive indirect effect through fruit weight (1.38), mace volume (1.09) and kernel volume (0.88) and very high negative indirect effect by fruit volume (-1.12) and fresh mace weight (-1.08).

Mace volume had very high positive indirect effect through fruit weight (1.08) and dry mace weight and very high negative indirect effect via fresh mace weight (-2.36) and nut volume (-1.24).

Kernel volume showed at least high positive indirect effect through fruit weight (1.22), mace volume (0.97) and kernel weight (0.59) and very high negative

indirect effect by nut volume (-2.01), fresh mace weight (-1.13) and fruit volume (-1.01).

Nut length exhibited at least high positive indirect effect on number of fruit per tree through mace volume (1.05) and fruit weight (0.95) and, at least high negative indirect effect by nut volume (-1.75), fruit volume (-0.92) and fresh mace weight (-0.79). Nut breadth exhibited high positive indirect effect on number of fruits per tree through mace volume (0.88), thickness of pericarp (0.81) and kennel volume (0.68) and, very high negative indirect effect by nut volume (-1.76), fresh mace weight (-1.19) and fruit volume (-1.03).

The ratio of nut to mace expressed very high genotypic positive indirect effect on number of fruits per tree via, fresh mace weight (2.16), whereas it had high negative indirect effect on mace volume (-0.93) and negligible negative indirect effect via dry mace weight (-0.59).

All the inter relationships between quantitative characters have been well analysed through multipronged statistical techniques. The most important output that a farmer perceives as regards his produce is its quality attributes, in most sense measured through biochemical constituents. The major biochemical constituents of the end produce of all the accessions under disposal were scientifically and schematically analysed and the results are as follows.

4.2 BIOCHEMICAL CHARACTERIZATION

Biochemical analyses of kernel, mace and pericarp were carried out in all the forty six accessions and the results are presented below.

4.2.1 Contents of volatile oil, oleoresin and fixed oil

Quality attributes such as content of volatile oil and oleoresin in both kernel and mace as well as fixed oil in the kernel were estimated in the forty six accessions and the data are presented in Table 4.22.

4.2.1.1 Volatile oil content of kernel and mace

Significant difference was observed among the accessions for volatile oil content of kernel as also mace. The volatile oil content in kernel ranged from 1.57 to 7.67 per cent. Highest recovery was noticed in Acc. 34 (7.67%) with the recovery from Acc. 8 (7.32%), Acc. 5 (6.99%) and Acc.7 (6.67%) on par with the highest recovery. Lowest volatile oil content was recorded in Acc. 28 (1.57%).

The mace volatile oil content ranged between 2.05 and 9.33 per cent. Maximum recovery of volatile oil was recorded in Acc. 22 (9.33%) which was significantly higher than that of all other accessions. The recovery form Acc.10 (8.99%), Acc.6 (8.66%), Acc.2 (8.33%), Acc. 38 (8.33%), Acc.37 (8.01%) and Acc.34 (8.01%) were all significantly different from each other though the corresponding recoveries were seemingly close. Minimum volatile oil content in mace was recorded in Acc. 20 (2.05%).

Among the accessions under evaluation, Acc. 34, Acc. 5, Acc. 6, Acc. 8, Acc. 9, Acc. 22 and Acc. (H) 1 were found rich in both the kernel and mace volatile oils.

4.2.1.2 Oleoresin content of kernel and mace

Significant difference was observed among the accessions for oleoresin content of kernel as also mace. Oleoresin content in kernel ranged from 18.59 to 36.20 per cent. Significantly, highest recovery of oleoresin was noticed in Acc. 40 (36.20%). The recovery from Acc. 9 (35.20%) was on par with that of Acc. 40. The Lowest oleoresin content was recorded in Acc. 30 (18.59%).

Mace oleoresin content ranged between 11.38 and 31.66 per cent. Maximum recovery of oleoresin was recorded in Acc. 4 (31.66%) and was significantly superior of all the other accessions. Minimum oleoresin content was recorded in Acc. 20 (11.38 %).

Table 4.22. Volatile oil, oleoresin and fixed oil content in kernel and mace of nutmeg accessions

Accessions	Volatile oil (%)		Oleoresin (%)		Fixed oil in kernel (%)
	Kernel	Mace	Kernel	Mace	
Acc. 1	4.32 (2.30)	6.33(2.70)	32.20 (5.76)	26.33(5.22)	28.52 (5.43)
Acc. 2	3.99(2.23)	8.33(3.05)	32.01(5.74)	29.66(5.53)	35.39 (6.03)
Acc. 3	4.24(2.28)	6.67(2.76)	29.40(5.51)	21.99(4.79)	32.80 (5.81)
Acc. 4	3.33(2.08)	6.67(2.76)	30.00(5.56)	31.66(5.71)	29.49 (5.52)
Acc. 5	6.99(2.82)	6.33(2.70)	27.39(5.32)	17.67(4.32)	26.70 (5.26)
Acc. 6	5.00(2.44)	8.66(3.10)	26.01(5.19)	22.20(4.81)	40.69 (6.45)
Acc. 7	6.67(2.76)	4.65(2.37)	26.00(5.19)	26.32(5.22)	40.50 (6.44)
Acc. 8	7.32(2.88)	6.33(2.70)	31.60(5.71)	23.01(4.89)	35.89 (6.07)
Acc. 9	6.00(2.64)	5.67(2.58)	35.20(6.01)	17.33(4.28)	23.67 (4.96)
Acc. 10	4.33(2.30)	8.99(3.16)	31.59(5.70)	28.67(5.44)	33.40 (5.86)
Acc. 11	5.67(2.58)	6.34(2.71)	24.16(5.01)	24.17(5.01)	28.40 (5.42)
Acc. 12	3.26(2.06)	5.40(2.53)	19.60(4.53)	23.60(4.96)	34.60 (5.96)
Acc. 13	3.67(2.16)	7.33(2.88)	26.21(5.21)	15.32(4.04)	26.50 (5.24)
Acc. 14	3.32(2.08)	7.00(2.82)	22.79(4.87)	20.01(4.58)	32.10 (5.75)
Acc. 15	4.67(2.38)	6.67(2.76)	33.40(5.86)	15.99(4.12)	36.99 (6.16)
Acc. 16	2.74(1.93)	2.80(1.94)	22.21(4.81)	12.40(3.65)	26.94 (5.28)
Acc. 17	5.99(2.64)	6.33(2.70)	26.66(5.25)	22.20(4.81)	37.10 (6.17)
Acc. 18	3.33(2.08)	6.33(2.70)	26.60(5.25)	20.32(4.61)	34.50 (5.95)
Acc. 19	3.33(2.08)	3.74(2.17)	29.59(2.53)	23.00(4.89)	24.40 (5.04)
Acc. 20	1.67(1.63)	2.05(1.74)	19.72(4.55)	11.38(3.51)	19.48 (4.25)
Acc. 21	5.67(2.58)	6.25(2.69)	32.80(5.81)	19.33(4.50)	28.89 (5.46)
Acc. 22	4.66(2.38)	9.33(3.21)	31.20(5.67)	23.01(4.89	44.80 (6.76)
Acc. 23	2.99(1.99)	7.37(2.89)	23.80(4.98)	16.67(4.20)	35.59 (6.04)
Acc. 24	2.99(1.99)	6.01(2.64)	23.60(4.96)	20.32(4.61)	23.60 (4.96)
Acc. 25	4.99(2.44)	6.33(2.70)	31.2(5.67)	23.01(4.89)	26.59 (5.25)
Acc. 26	4.99(2.44)	6.64(2.76	22.40(4.83)	29.33(5.50)	25.00 (5.09)
Acc. 27	4.33(2.30)	3.00(2.00)	30.01(5.56)	14.33(3.91)	25.60 (5.15)
Acc. 28	1.57(1.60)	2.41(1.84)	20.23(4.60)	12.27(3.64)	17.79 (4.33)
Acc. 29	2.66(1.91)	6.35(2.71)	19.80(4.56)	22.50(4.84)	38.90 (6.31)
Acc. 30	4.33(2.30)	7.19(2.86)	18.59(4.42)	17.60(4.31)	29.99 (5.56)
Acc. 31	2.29(1.81)	3.03(2.00)	23.26(4.92)	17.75(4.33)	20.65 (4.65)
Acc. 32	1.67(1.63)	4.56(2.35)	28.60(5.44)	19.20(4.49)	25.69 (5.16)
Acc. 33	2.50(1.87)	6.93(2.81)	24.01(5.00)	19.60(4.53)	39.99 (6.40)
Acc. 34	7.67(2.94)	8.01(3.00)	25.33(5.13)	24.80(5.07)	29.49 (5.52)
Acc. 35	3.67(2.16)	6.01(2.64)	28.80(5.45)	14.00(3.87)	37.10 (6.17)
Acc. 36	2.33(1.82)	7.33(2.88)	30.33(5.59)	23.20(4.91)	34.19 (5.93)
Acc. 37	3.33(2.08)	8.01(3.00)	34.80(5.98)	26.33(5.22)	40.69 (6.45)
Acc. 38	1.92(1.70)	8.33(3.05)	26.20(5.21)	17.33(4.28)	41.73 (6.53)
Acc. 39	3.67(2.16)	3.33(2.08)	25.79(5.17)	17.67(4.32)	34.30 (5.94)
Acc. 40	3.00 (2.00)	6.66(2.76)	36.20(6.09)	19.00(4.47)	27.90 (5.37)
Acc. 41	4.33(2.30)	6.33(2.70)	23.79(4.97)	16.67(4.20)	24.13 (5.01)
Acc. 42	3.67(2.16)	6.33(2.70)	26.40(5.23)	16.33(4.16)	28.41 (5.42)
Acc.(H)1	6.33 (2.70)	4.46(2.33)	26.41(5.23)	17.33(4.28)	35.39 (6.03)
Acc. (H)2	2.97(1.99)	2.39(1.84)	21.65(4.75)	14.90(3.98)	24.05 (5.00)
Acc. (H)3	4.00(2.23)	8.14(3.02)	24.33(5.03)	21.40(4.73)	35.70 (6.05)
Acc. (H)4	5.33 (2.51)	6.90(2.81)	27.39(5.32)	22.67(4.86)	39.00 (6.32)
C.D (0.05)	0.15(0.04)	0.16(0.03)	0.92(0.09)	0.75(0.09)	0.92 (0.09)
C.V (%)	1.81 (0.88)	1.34(0.62)	1.69 (0.90)	1.81(1.06)	1.45 (0.78)

*Figures in parenthesis are square root transformed values

In all the accessions, content of oleoresin was high in kernel compared to mace. In Acc. 4, both the kernel and mace oleoresin contents were high.

4.2.1.3 Fixed oil of kernel

Significant difference was observed among the accessions for fixed oil content in the kernel. The fixed oil content ranged between 17.79 to 44.80 per cent. Significantly, highest recovery of fixed oil was recorded in Acc. 22 (44.80 %). The Lowest fixed oil content was recorded in Acc. 28 (17.79 %).

4.2.2 Clustering based on quality attributes (volatile oil, oleoresin and fixed oil)

Agglomerative hierarchical clustering pattern was performed using the Jaccard's similarity coefficient matrix by UPGMA method and the resulting dendrogram and clusters are presented in Figure 4.2 and Table 4.23.

The quality attributes of all the forty six accessions including female and monoecious were used for formulating dendrogram and they could be grouped into 26 clusters at 20 per cent similarity level. The grouping was made at a poor similarity level because the accessions exhibited higher variability for contents of volatile oil, oleoresin and fixed oil. Cluster I was the largest one containing six accessions. Cluster II and VIII included four accessions each. Majority of the accessions remained as independent units.

4.2.3 Inter cluster association of qualitative and biochemical clusters

Analysis based on qualitative characters resulted in 11 clusters and that based on biochemical (volatile oil, oleoresin and fixed oil content) characters resulted in 26 clusters. The parallelism between these two clustering patterns is presented in Table 4.24.

The accessions belonging to each of the qualitative clusters I, III, VIII, X and XI were found to fall exclusively in biochemical clusters I, XI, XII, XIV and XIII

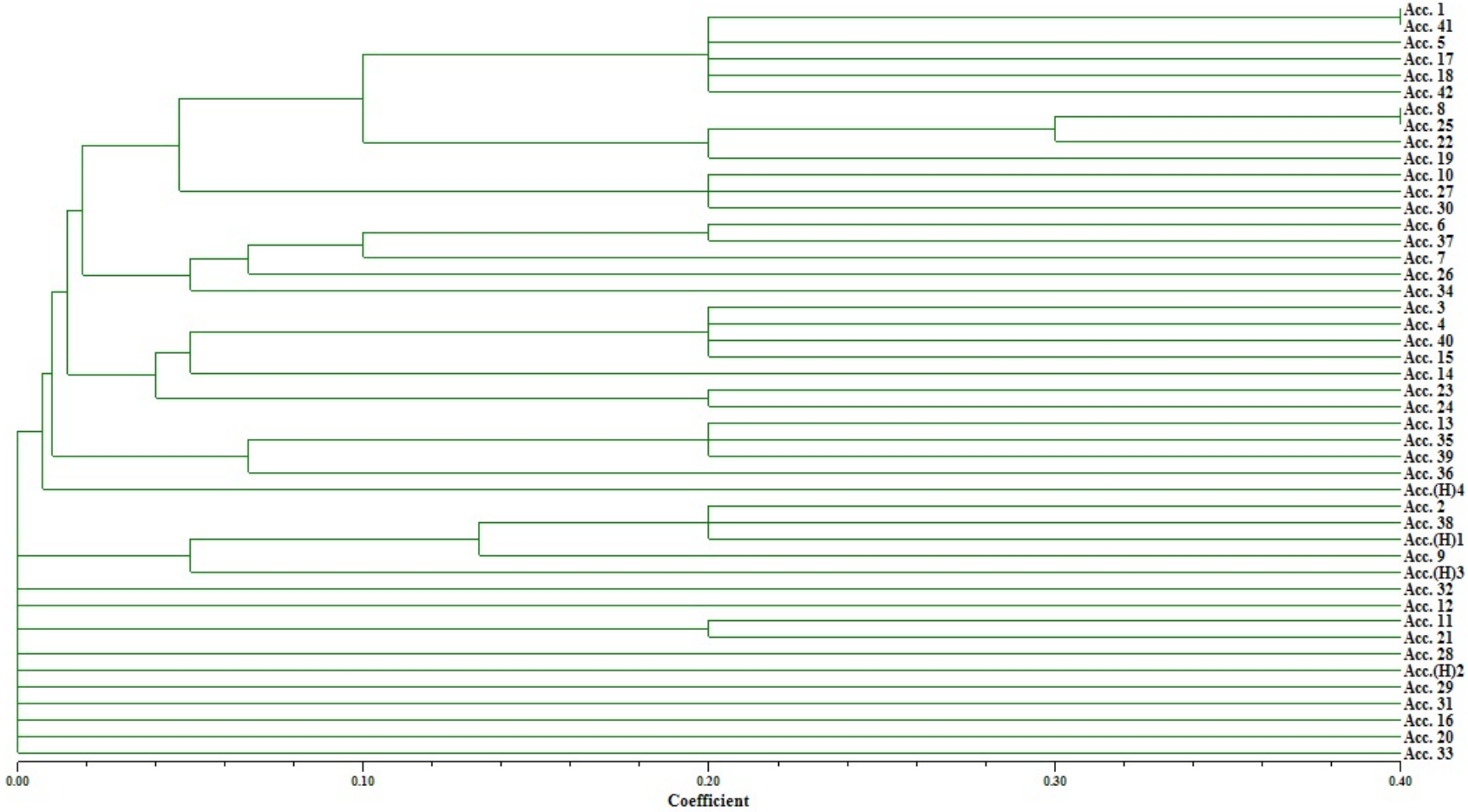


Figure 4.2. UPGMA dendrogram of volatile oil, oleoresin and fixed oil content of nutmeg and mace

Table 4.23. Clustering of nutmeg accessions based on volatile oil, oleoresin and fixed oil content

Cluster number	Number of accessions	Cluster members
I	6	Acc.1, Acc.41, Acc.5, Acc.17, Acc.18, Acc.42
II	4	Acc.8, Acc.25, Acc.22, Acc.19
III	3	Acc.10, Acc.27, Acc.30
IV	2	Acc.6, Acc.37
V	1	Acc.7
VI	1	Acc.26
VII	1	Acc.34
VIII	4	Acc.3, Acc.4, Acc.40, Acc.15
IX	1	Acc.14
X	2	Acc.23, Acc.24
XI	3	Acc.13, Acc.35, Acc.39
XII	1	Acc.36
XIII	1	Acc.(H)4
XIV	3	Acc.2, Acc.38, Acc.(H)1
XV	1	Acc.9
XVI	1	Acc.(H)3
XVII	1	Acc.32
XVIII	1	Acc.12
XIX	2	Acc.11, Acc.21
XX	1	Acc.28
XXI	1	Acc.(H)2
XXII	1	Acc.29
XXIII	1	Acc.31
XXIV	1	Acc.16
XXV	1	Acc.20
XXVI	1	Acc.33

Table 4.24. Inter cluster association between clustering patterns based on qualitative and biochemical characters

Qualitative cluster	Number of accessions	Per cent distribution of accessions in biochemical cluster																									
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XIII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII	XXIII	XXIV	XXV	XXVI
I	1	100	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
II	2	*	*	50	*	*	*	*	*	*	*	*	*	*	*	50	*	*	*	*	*	*	*	*	*	*	*
III	3	*	100	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
IV	20	20	5	10	5	*	5	5	15	5	10	5	*	*	5	*	*	5	*	*	*	5	*	*	5	*	*
V	4	*	*	*	*	*	*	*	*	*		50	*	*	*	*	*	*	*	50	*	*	*	*	*	*	*
VI	4	25	*	25	25	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	25	*	*	*	*
VII	7	*	*	*	*	*	*	*	14.28	*	*	*	*	*	*	*	14.28	14.28	*	*	14.28	14.28	*	14.28	*	14.28	*
VIII	1	*	*	*	*	*	*	*	*	*	*	*	100	*	*	*	*	*	*	*	*	*	*	*	*	*	*
IX	2	*	*	*	*	*	*	*	*	*	*	*	*	*	50	*	*	*	*	*	*	*	*	*	*	*	50
X	1	*	*	*	*	*	*	*	*	*	*	*	*	*	100	*	*	*	*	*	*	*	*	*	*	*	*
XI	1	*	*	*	*	*	*	*	*	*	*	*	*	100	*	*	*	*	*	*	*	*	*	*	*	*	*

respectively. The accessions in qualitative cluster II were found equally distributed in biochemical clusters III and XV. Similarly, the accessions in qualitative cluster V were found equally distributed in the biochemical clusters XI and XIX. The accessions in qualitative cluster IX were also found equally in distributed in biochemical clusters XIV and XXVI.

In case of qualitative cluster IV, maximum number of accessions were distributed in biochemical cluster I (20%) followed by the biochemical cluster VIII (15%). Ten per cent each of the accessions were found distributed in biochemical clusters III and X. The biochemical clusters II, IV, VI, VII, VIII, IX, XI, XIV, XVI, XXI and XXIV were having five per cent each of the accessions of qualitative cluster IV.

Biochemical clusters I, III, IV and XXII had 25 per cent each of the accessions of qualitative cluster VI. The accessions of qualitative cluster VII were falling equally in biochemical clusters VIII, XVI, XVII, XX, XXI, XXIII and XXV.

4.3 PEST AND DISEASE INCIDENCE

Incidence of various diseases was scored as and when it was noticed during field evaluation and Per cent Disease Severity (PDS) calculated (Table 4.25 & Plate 4.22).

Phytopthora leaf fall, the most important and emerging disease was noticed in six accessions. Medium incidence was recorded in Acc. 18 (21%) followed by Acc. 1 (14.50%). Other accessions showed field tolerance to *Phytopthora* leaf fall during the course of evaluation.

Incidence of *Colletotrichum* leaf spot was found medium in Acc. 37 (21%) followed by Acc. 18 (11.50%). Remaining accessions had only low incidence of *Colletotrichum* leaf spot.

Table 4.25. Disease incidence in nutmeg accessions (%)

Accessions	*Phytopthora* leaf fall	*Colletotrichum* leaf spot	*Colletotrichum* fruit rot	*Marasmius* thread blight	*Lasiodiploidia* die back
Acc.1	14.50	4.00	10.00	-	-
Acc.2	-	9.50	-	-	-
Acc.3	-	4.00	-	-	5.00
Acc.4	-	7.00	8.00	-	-
Acc.5	-	5.00	18.00	-	-
Acc.6	-	4.50	6.00	-	-
Acc.7	-	6.50	7.00	-	10.00
Acc.8	-	5.00	5.00	-	-
Acc.9	-	3.50	24.00	-	-
Acc.10	-	4.00	-	-	25.00
Acc.11	-	7.00	-	-	5.00
Acc.12	-	7.50	-	-	-
Acc.13	-	6.50	-	-	10.00
Acc.14	-	6.50	-	-	-
Acc.15	-	8.50	5.00	-	5.00
Acc.16	-	4.50	-	-	-
Acc.17	-	8.50	-	30.00	-
Acc.18	21.00	11.50	-	-	-
Acc.19	-	7.00	-	-	-
Acc.20	-	4.50	-	-	-
Acc.21	-	4.00	-	-	5.00
Acc.22	-	7.00	9.00	-	-
Acc.23	6.00	2.50	-	-	-
Acc.24	9.50	4.00	4.00	-	-
Acc.25	8.00	8.00	-	-	-
Acc.26	7.50	7.50	-	-	-
Acc.27	-	6.50	7.00	-	-
Acc.28	-	2.00	-	20.00	-
Acc.29	-	7.50	-	-	5.00
Acc.30	-	3.00	-	-	-
Acc.31	-	8.50	-	-	5.00
Acc.32	-	7.00	-	-	-
Acc.33	-	6.50	-	-	-
Acc.34	-	5.00	-	-	-
Acc.35	-	4.50	6.00	-	20.00
Acc.36	-	6.00	6.00	-	5.00
Acc.37	-	21.00	5.00	-	-
Acc.38	-	5.00	4.00	-	5.00
Acc.39	-	4.50	-	-	-
Acc.40	-	6.50	7.00	-	-
Acc.41	-	4.00	-	-	-
Acc.42	-	4.50	-	-	-
Acc.(H)1	-	9.50	-	-	5.00
Acc.(H)2	-	5.50	-	-	10.00
Acc.(H)3	-	4.50	-	-	10.00
Acc.(H)4	-	5.00	-	-	-
Acc.(M)1	-	3.00	-	-	-
Acc.(M)2	-	5.00	-	-	-
Acc.(M)3	-	10.00	-	-	-
Acc.(M)4	-	2.50	-	-	-

- No disease

Horse hair blight

Die back

***Phytophthora* leaf fall**

Plate 4.22. Major diseases of nutmeg

Fruit rot incidence was observed medium in Acc. 9 and Acc. 5. A few of the accessions had mild incidence. Remaining accessions could escape from the disease.

Thread blight incidence was moderate in Acc. 17 and medium in Acc. 28 and other accessions were free from thread blight. Very few accessions recorded incidence of dieback. Acc. 10 (25%) and Acc. 35 (20%) recorded medium incidence of dieback.

With regard to pest infestation, all the accessions were free from the infestation by pests during the study period.

4.4 SELECTION OF DISTINCT ACCESSIONS BASED ON MORPHOLOGICAL CHARACTERS

From the nutmeg core germplasm under evaluation, seventeen distinct accessions were selected based on distinctive specific features ensuring adequate representation of all the quantitative clusters. These seventeen distinctly featured accessions only were utilized for further biochemical analysis of pericarp, chemoprofiling and molecular characterization. Details of select accessions are presented in Table 4.26.

4.4.1 Chemoprofiling based on kernel and mace volatiles

GC-MS profile of volatile oil of both mace and kernel of seventeen distinct nutmeg accessions revealed wide variation. Data pertaining to the content of various constituents of volatile oil are furnished in Table 4.27 and Table 4.28. Based on the content of major constituents, seventeen accessions were classified into three groups *viz*., high, medium and low, and they are presented in Table 4.29.

4.4.1.1 Chemoprofiling based on kernel volatiles

A total of twenty constituents were identified from the kernel volatile oils of seventeen distinct nutmeg accessions. The total volatile constituents in the accessions **Table 4.26. Distinct nutmeg accessions selected and their specific characteristics**

Sl. No.	Accessions	Characteristic features
1	Acc. 1	Very early flowering and fruiting, susceptible to *Phytopthora* leaf fall
2	Acc. 5	Partial fruit splitting
3	Acc.8	More number of fruits per tree
4	Acc.9	More number of fruits per tree
5	Acc. 11	Normal tree
6	Acc. 14	Thick and entire mace
7	Acc.18	Very early flowering and fruiting, susceptible to *Phytopthora* leaf fall
8	Acc.21	Orange-red colour of mace
9	Acc. 23	Typical conical shape of tree
10	Acc. 24	Typical conical shape of tree
11	Acc.30	Pronounced kernel grooves
12	Acc.35	No kernel grooves
13	Acc. 36	Higher fruit weight , bold nut
14	Acc. 37	Higher fruit weight, light green mature leaf, susceptible to *Colletotrichum* leaf spot
15	Acc.38	Entire maced type
16	Acc.(H)1	Two seeds and fruit split into three splits
17	Acc.(H)4	Two seeds and fruit split into four splits

ranged between 68.96 to 90.49 per cent. The highest percentage of volatile oil constituents was recorded in Acc. (H) 1 (90.49 %) followed by Acc. 8 (88.26 %), while the lowest was recorded in Acc. 14 (68.96 %).

Principal compounds in the volatile oil were myristicin, elemicin, sabinene and safrole. The highest total percentage of these four compounds was recorded in Acc. 5 (38.51%) and the lowest was recorded in Acc. 35 (18.53%).

The highest percentage of myristicin content was noticed in Acc. 14 (12.82%) followed by Acc. (H) 1 (11.54%). The lowest myristicin content was recorded in Acc. 9 (2.48%).

Table 4.27. Variability in biochemical constituents of volatile oil of kernel in select nutmeg accessions

Sl. No	Compound /accessions	Acc.1	Acc.5	Acc. 8	Acc.9	Acc. 11	Acc. 14	Acc. 18	Acc. 21	Acc. 23	Acc. 24	Acc. 30	Acc. 35	Acc. 36	Acc. 37	Acc. 38	Acc. (H)1	Acc. (H)4	Q1	Q2	Q3
1	Alpha- Thujen	1.45	1.11	2.34	1.28	1.13	0.70	1.16	1.17	2.73	1.37	1.82	1.71	1.56	1.60	0.83	1.02	1.00	1.06	1.28	1.65
2	Alpha-Pinene	4.86	3.84	6.33	5.60	4.04	2.76	3.43	5.27	4.88	3.72	5.85	5.05	6.04	6.12	2.55	4.94	3.72	3.72	4.88	5.72
3	Sabinene	5.32	7.19	11.75	10.07	7.78	5.05	5.57	1.06	6.94	4.40	9.48	7.95	8.24	0.18	3.89	10.44	6.68	4.72	6.94	8.86
4	beta-Pinene	7.32	4.36	6.71	6.36	5.39	3.70	4.70	6.54	5.52	5.56	6.84	7.95	7.68	11.59	3.95	5.67	5.16	4.93	5.67	7.08
5	beta-Myrcene	2.17	1.97	2.64	2.61	1.84	1.28	1.85	2.40	1.90	1.49	2.55	2.40	2.42	6.03	1.14	2.18	1.81	1.82	2.17	2.48
6	(+)-3-Caren	1.46	*	*	*	1.15	*	*	*	*	*	*	*	*	*	*	*	*	0	0	0
7	Alpha-Terpinene	3.18	2.27	3.39	2.64	1.90	1.60	2.43	2.29	3.75	3.08	2.97	3.12	3.19	1.69	1.63	1.91	2.20	1.90	2.43	3.15
8	D-Limonene	5.64	*	*	*	3.30	*	*	*	*	*	*	*	*	*	*	*	*	0	0	0
9	Limonene	*	3.43	5.41	4.56	*	2.44	3.28	4.18	3.25	3.15	4.61	4.26	4.33	1.58	2.09	4.21	3.38	2.26	3.38	4.29
10	Gamma-Terpinene	2.50	3.94	6.02	4.79	*	2.74	4.26	3.89	6.21	5.23	4.99	5.33	5.26	4.45	2.79	3.21	3.83	3.00	4.26	5.24
11	4-Thujanol	*	0.82	3.13	0.78	4.30	1.05	4.69	1.64	5.06	0.57	4.32	4.09	0.68	1.49	2.64	0.84	1.34	0.80	1.49	4.19
12	Terpinolene	2.55	1.94	2.68	2.55	1.45	1.30	2.19	2.06	2.45	2.14	2.51	2.58	2.34	2.33	1.19	1.70	1.77	1.73	2.19	2.53
13	Beta-Linalool	*	1.47	3.59	1.47	4.66	1.86	5.89	2.34	4.61	1.20	4.69	4.79	1.21	2.20	4.83	1.39	2.13	1.43	2.20	4.67
14	P-Menth-2-En-1-ol	*	1.13	1.09	1.36	*	1.07	1.49	1.11	1.32	1.39	1.19	1.64	1.10	1.04	0.75	0.63	1.46	0.89	1.11	1.37
15	L-4-terpineol	24.73	15.90	16.31	21.56	15.61	14.83	19.55	16.58	17.96	25.60	17.65	21.60	18.50	14.56	15.99	10.12	21.14	15.75	17.65	21.35
16	Safrole	3.60	3.28	2.51	4.64	3.60	3.87	4.52	3.54	2.44	4.28	3.73	3.45	3.74	3.74	2.73	3.56	3.37	3.32	3.60	3.80
17	Eugenol methyl ether	3.22	5.41	1.60	2.43	3.01	4.35	3.97	3.11	2.61	2.23	2.32	2.39	3.70	2.36	6.20	*	*	2.27	2.61	3.83
18	Isoeugenol methyl ether	*	1.27	*	*	2.10	1.63	1.43	0.66	1.20	3.72	*	*	1.57	1.41	3.09	4.82	4.60	0.00	1.41	2.59
19	Myristicin	3.66	8.28	3.77	2.48	4.17	12.82	4.27	3.54	3.77	6.15	3.53	2.90	2.79	4.71	7.70	11.54	4.54	3.53	4.17	6.92
20	Elemicin	10.11	19.76	8.99	6.34	17.88	5.91	7.84	15.87	11.02	6.99	4.31	4.23	13.92	12.31	22.43	22.31	16.07	6.66	11.02	16.97
Total		**81.77**	**87.37**	**88.26**	**81.52**	**83.31**	**68.96**	**82.52**	**77.25**	**87.62**	**82.2**	**83.36**	**85.44**	**88.27**	**79.39**	**86.42**	**90.49**	**84.20**	**81.77**	**87.37**	**88.26**

***Not detected**

Table 4.28. Variability in biochemical constituents volatile oil of mace in select nutmeg accessions

Sl. No.	Compound /accessions	Acc. 1	Acc. 5	Acc. 8	Acc. 9	Acc. 11	Acc. 14	Acc. 18	Acc. 21	Acc. 23	Acc. 24	Acc. 30	Acc. 35	Acc. 36	Acc. 37	Acc. 38	Acc. (H)1	Acc. (H)4	Q1	Q2	Q3
1	Alpha- Thujen	2.36	1.17	2.14	1.86	1.52	1.54	3.32	0.60	0.88	1.61	1.11	2.48	1.73	1.84	1.78	1.14	1.84	1.15	1.73	2.00
2	Alpha-Pinene	6.93	5.67	6.99	7.08	6.85	7.96	8.68	5.61	4.49	7.18	4.82	7.59	8.53	9.50	8.54	4.69	8.22	5.64	7.08	8.37
3	(+)-Sabinene	13.00	11.01	13.62	11.90	13.72	14.80	15.04	11.43	8.42	13.00	8.18	15.18	16.32	16.27	16.15	5.59	14.77	11.22	13.62	15.11
4	beta-Pinene	6.05	4.66	5.30	7.00	6.41	6.99	7.28	4.95	5.51	7.04	5.82	5.96	7.66	8.01	6.26	5.29	7.23	5.40	6.26	7.13
5	beta-Myrcene	2.79	2.37	3.16	2.95	2.87	3.06	3.55	2.46	1.88	*	2.20	3.37	3.54	3.52	3.20	1.77	3.32	2.28	2.95	3.34
6	Alpha-Phellandrene	1.00	*	1.16	1.09	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	0
7	(+)-3-Caren	2.45	2.13	2.83	2.78	2.62	2.81	3.33	2.35	1.74	1.02	2.02	3.22	3.35	3.36	3.04	1.72	3.07	2.07	2.78	3.14
8	Alpha-Terpinene	3.60	1.81	3.38	3.60	2.17	2.23	4.50	2.04	2.04	2.83	2.94	3.67	2.34	2.71	2.29	2.47	2.78	2.20	2.71	3.49
9	D-Limonene	*	4.78	6.66	6.44	5.94	6.25	7.43	5.25	4.29	6.59	5.30	7.22	7.40	7.39	6.47	4.01	6.87	5.01	6.44	7.04
10	Limonene	5.83	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	0
11	Gamma-Terpinene	5.99	2.75	5.54	6.12	3.49	3.53	7.53	3.34	3.34	4.65	5.23	6.18	3.65	4.23	3.61	3.94	4.36	3.51	4.23	5.76
12	Alpha-terpinolene	3.06	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	0
13	Terpinolene	*	2.03	3.30	3.35	2.55	2.59	4.14	2.40	2.02	3.12	2.90	3.71	3.02	3.04	2.72	2.02	3.07	2.21	2.90	3.21
14	Beta-Linalool	1.69	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	0
15	P-Menth-2-En-1-ol	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
16	L-4-terpineol	14.87	6.74	11.25	14.69	6.98	8.10	15.58	7.80	8.55	10.35	17.34	11.30	6.75	10.07	8.87	10.23	8.71	7.95	10.07	12.99
17	Safrole	3.53	3.57	3.73	3.66	4.48	3.85	4.00	3.64	3.92	4.30	4.39	4.37	4.89	4.69	3.59	2.96	4.56	3.61	3.92	4.43
18	Eugenol methyl ether	2.35	6.78	3.12	2.90	2.92	4.44	0.89	4.89	4.06	3.19	3.96	4.09	3.63	2.62	9.22	5.24	3.54	2.91	3.63	4.66
19	Isoeugenol methyl ether	*	*	*	*	2.33	0.96	*	*	*	*	*	*	*	*	*	*	*	0	0	0
20	Myristicin	2.93	10.20	3.87	3.19	3.95	5.36	1.57	18.87	13.08	8.20	10.24	4.03	2.60	3.21	3.32	14.51	4.17	3.20	4.03	10.22
21	Elemicin	9.58	25.10	13.38	7.02	23.95	16.96	1.39	7.20	26.86	8.34	11.90	4.25	15.28	8.57	9.65	27.86	11.49	7.77	11.49	20.45
22	t-Caryophyllene	*	*	0.28	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	0
23	Cymene	*	*	*	1.26	1.14	1.15	1.58	1.03	*	2.86	*	*	*	*	*	*	*	0	0	0
24	(E)- Isoeugenol	*	*	*	*	*	*	*	*	*	*	*	1.08	1.28	*	1.81	*	*	0	0	01.14
Total		**88.01**	**90.77**	**89.71**	**86.89**	**93.89**	**92.58**	**89.81**	**83.86**	**91.08**	**84.28**	**88.35**	**87.7**	**91.97**	**89.03**	**90.52**	**93.44**	**88.00**	**88.01**	**90.77**	**89.71**

***Not detected**

Table 4.29. Chemotypes of nutmeg identified based on major volatiles

Volatile oil	Category	Alpha-pinene	Beta-pinene	L-4-terpineol	Gamma-terpinene	Sabinene	Safrole	Myristicin	Elemicin
Nut	**High**	Acc.8, Acc.30, Acc.36, Acc.37	Acc. 35, Acc.36, Acc.37	Acc.1, Acc.9, Acc.24, Acc.35,	Acc.8, Acc.23, Acc.35, Acc.36,	Acc. 8, Acc.9, Acc.30, Acc.(H)1	Acc.9, Acc. 14, Acc.18, Acc.24	Acc.5, Acc.14, Acc.38, Acc.(H)1,	Acc.5, Acc. 11, Acc.38, Acc.(H)1,
	Medium	Acc. 1, Acc.5, Acc.9, Acc.11, Acc.21, Acc.23, Acc.35, Acc.(H)1	Acc.1, Acc.8, Acc.9, Acc.11, Acc.21, Acc.23, Acc.24, Acc.30, Acc.(H)1, Acc.(H)4	Acc.5, Acc.8, Acc.18, Acc.21, Acc.23, Acc.30, Acc.36, Acc.38, Acc.(H)4	Acc.5, Acc.9, Acc.18, Acc.21, Acc.24, Acc.30, Acc.37, Acc.(H)1, Acc.(H)4	Acc.1, Acc.5, Acc.11, Acc.14, Acc.18, Acc.23, Acc.35, Acc.36, Acc.(H)4	Acc. 14, Acc.1, Acc. 11, Acc.21, Acc.30, Acc.35, Acc. 36, Acc.37, Acc.(H)1, Acc.(H)4	Acc.1, Acc.8, Acc.11, Acc.18, Acc.21, Acc.23, Acc.24, Acc.37, Acc.(H)4	Acc.1, Acc.8, Acc.18, Acc.21, Acc.23, Acc.24, Acc.36, Acc.37, Acc.(H)4
	Low	Acc.14, Acc.18, Acc.24, Acc.38, Acc.(H)4	Acc.5, Acc.14, Acc.18, Acc.38	Acc.11, Acc.14, Acc.37, Acc.(H)1	Acc.1, Acc.14, Acc.38	Acc.21, Acc.24, Acc.37, Acc.38	Acc.5, Acc.8, Acc.23, Acc.38	Acc.9, Acc.30, Acc.35, Acc.36	Acc.9, Acc.14, Acc.30, Acc.35
Mace	**High**	Acc.18, Acc.36, Acc.37, Acc.38	Acc.18, Acc.36, Acc.37, Acc.(H)4	Acc.1, Acc.9, Acc.18, Acc.30	Acc.1, , Acc.9, Acc.18, Acc.35	Acc.18, Acc.35, Acc.36, Acc.37, Acc.38	Acc.11, Acc.36, Acc.37, Acc.(H)4	Acc.5, Acc.21, Acc.23, Acc.30, Acc.(H)1	Acc.5, Acc.11, Acc.23, Acc.(H)1
	Medium	Acc.1, Acc.5, Acc.8, Acc.9, Acc.11, Acc.14, Acc.24, Acc.35, Acc.(H)4	Acc.1, Acc.9, Acc.11, Acc.14, Acc.23, Acc.24, Acc.30, Acc.35, Acc.38	Acc.8, Acc.14, Acc.23, Acc.24, Acc.35, Acc.37, Acc.38, Acc.(H)1, Acc.(H)4	Acc.8, Acc.14, Acc.24, Acc.30, Acc.36, Acc.37, Acc.38, Acc.(H)1, Acc.(H)4	Acc.1, Acc.8, Acc.9, Acc.11, Acc.14, Acc.21, Acc.24, Acc.(H)4	Acc.8, Acc.9, Acc.14, Acc.18, Acc.21, Acc.23, Acc.24, Acc.30, Acc.35	Acc.8, Acc.11, Acc.14, Acc.24, Acc.35, Acc.37, Acc.38, Acc.(H)4	Acc.8, Acc.14, Acc.24, Acc.30, Acc.36, Acc.37, Acc.38, Acc.(H)4,
	Low	Acc.21, Acc.23, Acc.30, Acc.(H)1	Acc.5, Acc.8, Acc.21, Acc.(H)1	Acc.5, Acc.11, Acc.21, Acc.36	Acc.5, Acc.11, Acc.21, Acc.23	Acc.5, Acc.23, Acc.30, Acc.(H)1	Acc.1, Acc.5, Acc.38, Acc.(H)1	Acc.1, Acc.9, Acc.18, Acc.36	Acc.1, Acc.9, Acc.18, Acc.21, Acc.35

Wide variation in elemicin content was recorded in kernel volatile oil; it ranged from 4.31 to 22.43 per cent. Maximum content of elemicin was recorded in Acc. 38 (22.43%) followed by Acc. (H) 1 (22.31%). Minimum content of elemicin was recorded in Acc. 30 (4.31%).

Highest safrole content was detected in Acc. 9 (4.64%) followed by Acc. 18 (4.52%), whereas lowest was in Acc. 23 (2.44%). Sabinene content was highest in Acc. 8 (11.75%) followed by Acc. (H) 1 (10.44%) and Acc. 9 (10.07%). The lowest sabinene content was recorded in Acc. 21 (1.06%).

Alpha-pinene content ranged between 2.55 per cent in Acc. 38 and 6.33 per cent in Acc. 8. Beta-pinene content ranged from 3.70 per cent in Acc. 14 to 11.59 per cent in Acc. 37.

L-4-terpineol content in volatile oil of kernel varied from 10.12 to 25.60 per cent. Higher concentration of L-4-terpineol was exhibited by accessions *viz.*, Acc. 1, Acc. 24, Acc. 35 and Acc. (H) 4.

Only two accessions *viz.*, Acc.1 and Acc.11 showed the presence of the (+)-3-caren and D-Limonene compounds in the volatile oil of kernel.

4.4.1.2 Chemoprofiling based on mace volatiles

The volatile oils from the mace of seventeen distinct nutmeg accessions were found to be made up of a total of twenty four compounds, total percentage of which ranged from 83.86 to 93.89 per cent. Highest percentage of volatile oil constituents was identified in Acc. 11 (93.89 %) followed by Acc. (H) 1 (93.44%), while the lowest was indentified in Acc. 21 (83.86%).

The principle compounds in the mace volatile oil were myristicin, elemicin, sabinene and safrole. The highest total percentage of these four compounds was noticed in Acc. 23 (52.28%) followed by Acc. (H) 1 (50.92%) and the lowest was in Acc. 18 (22.00%).

Wide range of variation was observed in myristicin content; it ranged from 1.57 to 18.87 per cent. Highest myristicin content was recorded in Acc. 21 (18.87%) followed by that of Acc. (H) 1 (14.51%). The lowest myristicin content was recorded in Acc. 18 (1.57%). Similarly, wide variation was observed in the elemicin content; it ranged from 1.39 in and 27.86 per cent. The highest elemicin content was recorded in Acc. (H) 1 (27.86%) followed by that of by Acc. 23 (26.86%). The lowest elemicin content was recorded in Acc. 18 (1.39%).

Highest safrole content was recorded in Acc. 36 (4.89 %) followed by that of Acc. (H) 4 (4.56%). The lowest safrole content was recorded in Acc. (H) 1 (2.96%). Sabinene content recorded maximum value in Acc. 36 (16.32%), followed by that of Acc. 37 (16.27%) and Acc. 38 (16.15%).

Alpha-pinene content ranged from 4.49 per cent (Acc. 23) to 9.50 per cent (Acc. 37). Beta-pinene content ranged from 4.66 per cent (Acc. 5) to 8.01 per cent (Acc. 37).

Maximum L-4-terpineol content was found in Acc. 30 (17.34%), followed that of Acc. 18 (15.58%), Acc, 1 (14.87%) and Acc. 9 (14.69%). Minimum was in Acc. 5 (6.74%).

Among the select accessions, a few were found to contain some unique volatile compounds. Three compounds such as limonene, alpha-terpinolene and beta-linalool were recorded only in Acc. 1. Alpha-phellandrene, isoeugenol methyl ether, t-caryophyllene, cymene and (E)- Isoeugenol were also detected sparingly in a few of the accessions.

4.4.1.3 Changes in constituents of kernel and mace volatile oil on storage

Biochemical constituents of volatile oil of kernel and mace in select samples were estimated after one year of storage. Data pertaining to the volatile compounds of

fresh as well as stored oils of kernel and mace are presented in Table 4.30 and Table 4.31.

4.4.1.3.1 Changes in kernel volatiles on storage

GC-MS analysis of stored volatile oil from select three nutmeg accessions identified a total of 29 compounds. Increase as also decrease phenomenon on storage was noticed in the three samples of volatile oil as regards the volatile compounds. Among the three samples of volatile oil, Acc. 5 registered an increase in the total constituents in stored oil (91.83%) compared to fresh oil (87.37%). Remaining two samples *viz*., Acc. 38 and Acc. (H) 1 showed loss of compounds on storage of volatile oil.

Among the seven major components of the oil, myristicin and elemicin contents increased in all the three samples during storage.

Alpha-pinene, sabinene and beta-pinene content decreased in all the three samples upon storage. Percentage of L-4-terpineol and safrole increased in Acc. 5 and L-4-terpineol alone increased in Acc. (H) 1 during storage.

Displacement of volatile oil constituents on storage was observed in all the three samples with respect to limonene content. A few new compounds were identified on storage of kernel volatile oil *viz*., (+)-caren, D-limonene, (R)-(-)-alpha-phellandrine, P-cymene, alpha-terpineol, borneyl acetate, eugenol, eucalyptol, trans(beta)-caryophellene and elemol.

4.4.1.3.2 Changes in mace volatiles on storage

GC-MS analysis of stored volatile oil of mace of four nutmeg accessions showed presence of 34 compounds. Percentage content of the compounds increased in all the four samples on storage. Maximum percentage of change in constituents of volatile oil was recorded in Acc. 21 (14.63%) and minimum was in Acc. 11 (1.92 %).

Table 4.30. Chemical constituents in fresh and stored volatile oil of kernel in select nutmeg accessions

Sl. No.	Compound /accessions	Acc.5			Acc.38			Acc.(H)1		
		Fresh oil	Stored oil	Change in constituent (%)	Fresh oil	Stored oil	Change in constituent (%)	Fresh oil	Stored oil	Change in constituent (%)
1	Alpha- Thujen	1.11	0.1	-90.991	0.83	0.14	-83.1325	1.02	0.12	-88.2353
2	Alpha-Pinene	3.84	0.31	-91.9271	2.55	0.38	-85.098	4.94	0.56	-88.664
3	Sabinene	7.19	1.08	-84.9791	3.89	1.45	-62.7249	10.44	1.99	-80.9387
4	beta-Pinene	4.36	0.87	-80.0459	3.95	1.49	-62.2785	5.67	1.61	-71.6049
5	beta-Myrcene	1.97	0.28	-85.7868	1.14	0.39	-65.7895	2.18	0.67	-69.2661
6	(+)-3-Caren	*	0.17	*	*	0.24	*	*	0.37	*
7	Alpha-Terpinene	2.27	0.3	-86.7841	1.63	0.36	-77.9141	1.91	0.61	-68.0628
8	D-Limonene	*	0.84	*	*	1.08	*	*	1.83	*
9	Limonene	3.43	*	-100	2.09	*	-100	4.21	*	-100
10	Gamma-Terpinene	3.94	0.71	-81.9797	2.79	0.7	-74.9104	3.21	1.52	-52.648
11	4-Thujanol	0.82	1.02	24.39024	2.64	1.44	-45.4545	0.84	0.85	1.190476
12	Terpinolene	1.94	0.35	-81.9588	1.19	0.33	-72.2689	1.7	0.88	-48.2353
13	Beta-Linalool	1.47	1.76	19.72789	4.83	3.4	-29.6066	1.39	1.55	11.51079
14	P-Menth-2-En-1-ol	1.13	0.9	-20.354	0.75	0.67	-10.6667	0.63	0.72	14.28571
15	L-4-terpineol	15.9	18.04	13.45912	15.99	13.76	-13.9462	10.12	11.48	13.43874
16	Safrole	3.28	3.48	6.097561	2.73	2.58	-5.49451	3.56	3.35	-5.89888
17	Eugenol methyl ether	5.41	8.36	54.52865	6.2	8.64	39.35484	*	6.2	*
18	Isoeugenol methyl ether	1.27	1.58	24.40945	3.09	4.64	50.16181	4.82	*	-100
19	Myristicin	8.28	13.3	60.62802	7.7	11.09	44.02597	11.54	15.7	36.04853
20	Elemicin	19.76	34.08	72.46964	22.43	26.67	18.90325	22.31	31.75	42.31286
21	(R)-(-)-Alpha Phellandrine	*	0.04	*	*	0.08	*	*	0.14	*
22	P-Cymene	*	1.17	*	*	1.45	*	*	1.47	*
23	(+)-Alpha-Limonene	*	*	*	*	*	*	*	*	*
24	Alpha-Terpineol	*	1.74	*	*	1.4	*	*	1.07	*
25	Borneyl acetate	*	0.42	*	*	0.46	*	*	0.21	*
26	Eugenol	*	0.41	*	*	0.32	*	*	0.39	*
27	Eucalyptol	*	*	*	*	*	*	*	*	*
28	Trans(Beta)-Caryophellene	*	*	*	*	0.92	*	*	*	*
29	Elemol	*	*	*	*	0.61	*	*	*	*
Total		**87.37**	**91.83**	**5.10**	**86.42**	**84.69**	**-2.00**	**90.49**	**85.04**	**-6.02**

***Not detected**

Table 4.31. Chemical constituents in fresh and stored volatile oil of mace in select nutmeg accessions

Sl. No.	Compound /accessions	Acc.11			Acc.21			Acc.23			Acc.(H)1		
		Fresh oil	Stored oil	Change in constituent (%)	Fresh oil	Stored oil	Change in constituent (%)	Fresh oil	Stored oil	Change in constituent (%)	Fresh oil	Stored oil	Change in constituent (%)
1	Alpha- Thujen	1.52	0.52	-65.78	0.6	0.19	-68.33	0.88	0.29	-67.04	1.14	0.49	-57.01
2	Alpha-Pinene	6.85	0.76	-88.90	5.61	1.05	-81.28	4.49	2.15	-52.11	4.69	2.95	-37.10
3	(+)-Sabinene	13.72	2.83	-79.37	11.43	2.99	-73.84	8.42	4.85	-42.39	5.59	5.26	-5.90
4	beta-Pinene	6.41	1.08	-83.15	4.95	2.03	-58.98	5.51	3.32	-39.74	5.29	3.91	-26.08
5	beta-Myrcene	2.87	0.35	-87.80	2.46	0.58	-76.42	1.88	0.82	-56.38	1.77	1.09	-38.41
6	Alpha-Phellandrene	*	*	*	*	*	*	*	*	*	*	*	*
7	(+)-3-Caren	2.62	0.37	-85.87	2.35	0.51	-78.29	1.74	0.78	-55.17	1.72	1.06	-38.37
8	Alpha-Terpinene	2.17	1.45	-33.17	2.04	0.38	-81.37	2.04	0.32	-84.31	2.47	0.69	-72.06
9	D-Limonene	5.94	*	-100	5.25	*	-100	4.29	*	-100	4.01	*	-100
10	Limonene	*	*	*	*	*	*	*	*	*	*	*	*
11	Gamma-Terpinene	3.49	2.61	-25.21	3.34	0.77	-76.94	3.34	0.61	-81.73	3.94	1.5	-61.92
12	Alpha-terpinolene	*	*	*	*	*	*	*	*	*	*	*	*
13	4-Thujanol	*	0.22	*	*	0.27	*	*	0.32	*	*	0.1	*
14	Terpinolene	2.55	1.02	-60	2.4	0.47	-80.41	2.02	0.45	-77.72	2.02	0.97	-51.98
15	Beta-Linalool	*	0.86	*	*	0.71	*	*	0.76	*	*	0.4	*
16	P-Menth-2-En-1-ol	*	0.6	*	*	0.31	*	*	0.42	*	*	0.31	*
17	L-4-terpineol	6.98	13.04	86.81	7.8	7.79	-0.128	8.55	8.84	3.39	10.23	9.21	-9.97
18	Safrole	4.48	4.07	-9.15	3.64	3.44	-5.49	3.92	3.64	-7.14	2.96	3.02	2.02

19	Eugenol methyl ether	2.92	6.28	115.06	4.89	6.56	34.15	4.06	5.26	29.55	5.24	6.84	30.53
20	Isoeugenol methyl ether	2.33	2.82	21.03	*	1.07	*	*	0.92	*	*	0.99	*

Contd...

Table 4.31 Continued...

21	Myristicin	3.95	12.65	220.25	18.87	9.04	-52.09	13.08	17.84	36.39	14.51	17.22	18.67
22	Elemicin	23.95	40.57	69.39	7.2	46.42	544.72	26.86	39.52	47.13	27.86	34.74	24.69
24	(R)-(-)-Alpha Phellandrine	*	0.15	*	*	0.11	*	*	0.11	*	*	0.21	*
25	Cymene	1.14	*	*	1.03	*	*	*	*	*	*	*	*
26	P-Cymene	*	0.41	*	*	1.28	*	*	1.84	*	*	1.79	*
27	(+)-Alpha-Limonene	*	1.22	*	*	1.67	*	*	2.2	*	*	2.81	*
28	Alpha-Terpineol	*	1.43	*	*	0.91	*	*	0.91	*	*	0.93	*
29	Borneyl acetate	*	0.22	*	*	0.42	*	*	0.25	*	*	0.2	*
30	Eugenol	*	0.17	*	*	6.77	*	*	0.13	*	*	*	*
31	Eucalyptol	*	*	*	*	0.09	*	*	0.11	*	*	*	*
32	Trans(Beta)-Caryophellene	*	*	*	*	0.3	*	*	0.23	*	*	0.17	*
33	Copaene	*	*	*	*	*	*	*	*	*	*	0.12	*
34	Elemol	*	*	*	*	*	*	*	*	*	*	0.87	*
Total		**93.89**	**95.70**	**1.92**	**83.86**	**96.13**	**14.63**	**91.08**	**96.89**	**6.37**	**93.44**	**97.85**	**4.71**

***Not detected**

Among the seven major components of the oil, elemicin per cent increased in all the four samples of mace oil upon storage, whereas myristicin content recorded increased values in these samples and it decreased on storage in Acc. 21.

Content of alpha-pinene, sabinene and beta-pinene decreased in all the four samples on storage. Percentage of L-4-terpineol increased in Acc. 11 and Acc. 21. Similarly slightly higher safrole content was observed in Acc. (H) 1 on storage.

Displacement of volatile oil constituents on storage was recorded in all the accessions with respect to D-limonene and cymene content.

A few new compounds were detected on storage of mace volatile oil *viz*., 4-thujanol, beta-linalool, P-menth-2-en-l-ol, (R)-(-)-alpha-phellandrine, P-cymene, (+)-alpha-limonene, caren, alpha-terpineol, borneyl acetate, eugenol, eucalyptol, trans(beta)-caryophellene, copaene and elemol.

4.4.2 Biochemical analysis of pericarp of select accessions

Data on biochemical parameters of pericarp of seventeen accessions are furnished in Table 4.32.

4.4.2.1 Moisture content

Significant difference was observed among the accessions for the moisture content in the pericarp. Moisture content ranged between 87.13 and 89.41 per cent. Acc. 5 (89.41%) recorded the highest moisture content followed by Acc. 30. Among the accessions, Acc. 11 (87.13%) had the lowest moisture content in the pericarp.

4.4.2.2 Acidity

Accessions varied significantly for acidity content in the pericarp. Acidity content ranged from 1.28 per cent to 1.92 per cent. Acc. 8 and Acc. 9 showed the highest value for acidity and Acc. (H) 4 recorded the lowest value for acidity.

4.4.2.3 Ascorbic acid

Accessions differed significantly for ascorbic acid content and it ranged between 4.54 and 9.52 (mg/100g). Acc. 9 had maximum ascorbic acid content. Lowest value was recorded in Acc (H) 1.

4.4.2.4 Pectin content

Pectin content in the pericarp ranged from 0.205 to 1.083 (% Calcium pectate). Acc. 9 (1.083 % Calcium pectate) recorded significantly higher pectin content, which was closely followed by Acc (H) 4 and Acc. 11. Acc.24 (0.201 % Calcium pectate) recorded the lowest pectin content.

4.4.2.5 Protein

Protein content in the pericarp ranged from 0.213 to 1.853 (g/100g). Significantly higher protein content was recorded in Acc. 23 (1.853 g/ 100g) followed by Acc. 36. Lowest protein content was recorded in Acc. 18 (0.213 g/100g).

4.4.2.6 Starch

Starch content in the pericarp ranged between 0.30 and 1.233 (g/ 100g). Significantly highest starch content was recorded in Acc. 23 (1.233 g/ 100g) followed by Acc. 24. The lowest starch content was in Acc. 37 and Acc. 8 (0.30 g/ 100g each).

4.4.2.7 Total phenol

Total phenol content differed significantly among different accessions. Total phenol ranged ranged between 27.77 and 57.55 (mg/100g). Maximum total phenol content was recorded in Acc. 18 (57.55 mg/100g) and minimum in Acc. 21 (27.77 mg/100g).

Table 4.32. Biochemical constituents of pericarp in select nutmeg accessions

Accessions	Moisture (%)	Acidity (%)	Ascorbic acid (mg/100g)	Pectin (% Calcium pectate)	Protein (g/100g)	Starch (g/100g)	Total Phenol (mg/100g)	Tannin (mg/100g)	Total mineral (%)	Crude fibre (%)
Acc.1	87.145^{e}	1.680^{ab}	7.627^{ab}	0.585^{efg}	1.280^{c}	0.782^{cde}	42.775^{e}	400.50^{e}	2.147^{d}	2.412^{ef}
Acc.5	89.406^{a}	1.726^{ab}	7.140^{ab}	0.316^{hi}	0.963^{f}	0.456^{fg}	44.333^{e}	143.30^{j}	2.066^{d}	2.466^{ef}
Acc.8	88.443^{cd}	1.920^{a}	8.330^{a}	0.856^{bc}	1.243^{cd}	0.300^{g}	31.000^{fg}	216.60^{i}	2.286^{bc}	2.750^{cd}
Acc.9	87.233^{e}	1.920^{a}	9.520^{a}	1.083^{a}	1.300^{c}	0.633^{ef}	43.330^{e}	293.30^{fg}	2.320^{c}	3.650^{a}
Acc.11	87.130^{e}	1.603^{ab}	8.123^{a}	0.906^{abc}	0.780^{h}	0.720^{de}	44.186^{e}	313.30^{f}	2.090^{d}	2.660^{de}
Acc.14	87.240^{e}	1.600^{ab}	7.573^{ab}	0.516^{fgh}	0.350^{j}	0.880^{cd}	49.443^{cd}	436.60^{c}	2.500^{a}	2.750^{cd}
Acc.18	87.283^{e}	1.600^{ab}	9.090^{a}	0.796^{bcd}	0.213^{k}	0.860^{cd}	57.553^{a}	276.60^{g}	2.173^{cd}	2.450^{ef}
Acc.21	88.436^{cd}	1.600^{ab}	4.710^{bc}	0.783^{bcd}	0.900^{fg}	0.913^{cd}	27.776^{g}	253.30^{h}	2.053^{d}	2.416^{f}
Acc.23	88.153^{d}	1.600^{ab}	9.090^{a}	0.356^{hi}	1.853^{a}	1.233^{a}	51.000^{bc}	430.00^{cd}	2.500^{a}	3.483^{a}
Acc.24	87.375^{e}	1.600^{ab}	9.090^{a}	0.205^{i}	1.125^{e}	1.140^{ab}	51.660^{bc}	750.00^{a}	2.465^{ab}	2.150^{g}
Acc.30	89.376^{a}	1.653^{a}	7.140^{ab}	0.620^{def}	0.970^{f}	0.860^{cd}	46.663^{de}	230.00^{hi}	2.206^{cd}	2.216^{g}
Acc.35	89.073^{b}	1.656^{ab}	6.946^{ab}	0.410^{gh}	0.923^{fg}	0.956^{bc}	45.190^{e}	388.40^{e}	2.100^{d}	2.060^{g}
Acc.36	88.580^{c}	1.600^{ab}	7.826^{ab}	0.416^{gh}	1.463^{b}	0.723^{de}	33.330^{f}	710.00^{b}	2.013^{d}	2.166^{g}
Acc.37	87.116^{e}	1.653^{ab}	7.140^{ab}	0.726^{cde}	0.836^{gh}	0.300^{g}	28.330^{g}	230.60^{hi}	2.040^{d}	2.650^{de}
Acc.38	87.270^{e}	1.600^{ab}	9.090^{a}	0.513^{fgh}	0.653^{i}	0.770^{cde}	55.000^{ab}	410.00^{de}	2.080^{d}	3.050^{b}
Acc.(H)1	88.213^{d}	1.600^{ab}	4.540^{c}	0.400^{gh}	1.143^{de}	0.906^{cd}	42.216^{e}	406.60^{de}	2.570^{a}	2.933^{bc}
Acc.(H)4	88.886^{b}	1.280^{c}	9.413^{a}	0.963^{ab}	0.943^{fg}	0.726^{de}	31.660^{fg}	313.30^{f}	2.013^{d}	2.466^{ef}

4.4.2.8 Tannin

Considerable variation was noticed among the accessions with respect to tannin content. Tannin content ranged from 143.30 to 750.00 (mg/ 100g). Highest tannin content was recorded in Acc. 24 (750.00 mg/ 100g) and lowest in Acc. 5 (143.30 mg/ 100g). Accessions, 8, 30 and 37 also contained less tannin and were on par with each other.

4.4.2.9 Total minerals

Accessions varied significantly with respect to content of total minerals. The highest total mineral content was observed in Acc. (H) 1 (2.57 %) which was closely followed by Acc.14, Acc. 23 and Acc. 24. Remaining accessions were observed to have significantly lower total mineral content.

4.4.2.10 Crude fibre

Significantly higher crude fibre content was noticed in Acc. 9 (3.65 %) which was closely followed by Acc. 23. The accessions, Acc. 24, Acc. 30, Acc. 35 and Acc. 36 were noticed to possess significantly lower crude fibre content.

4.4.3 Isozyme profiling of select accessions

All the seventeen distinct accessions were analysed for variation in isozyme banding pattern using two biochemical markers, peroxidase (POX) and polyphenol oxidase (POP) enzyme.

Total of four isoforms were observed in the accessions screened for peroxidase enzyme (Plate 4.23). Among the four accessions, Acc. 21 showed maximum of three isoforms. Accessions, 1, 5, 8, 9, 11, 18, 23, 24, 30 and 35 exhibited two isoforms each. Acc. 14 and 23 exhibited a unique band in the second position.

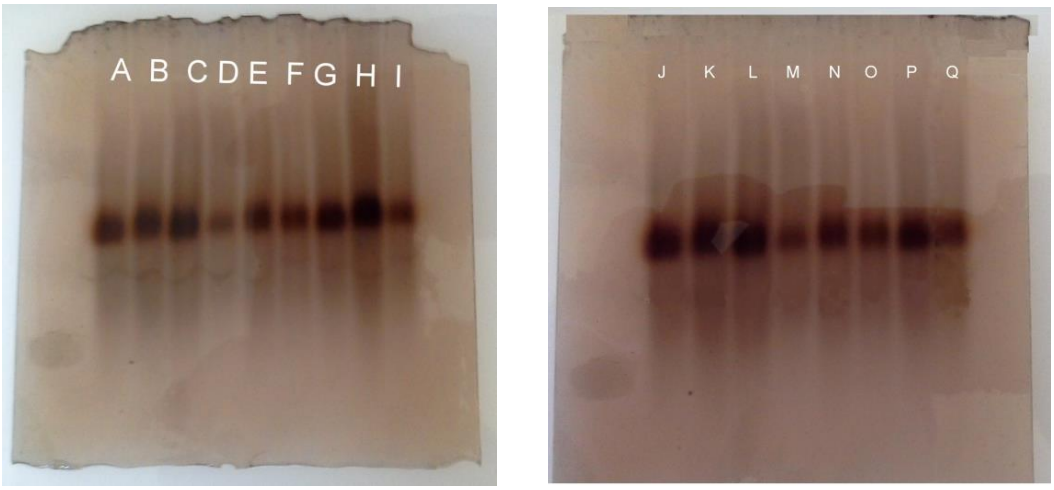


Plate 4.23. Peroxidase isozyme profile of select nutmeg accessions

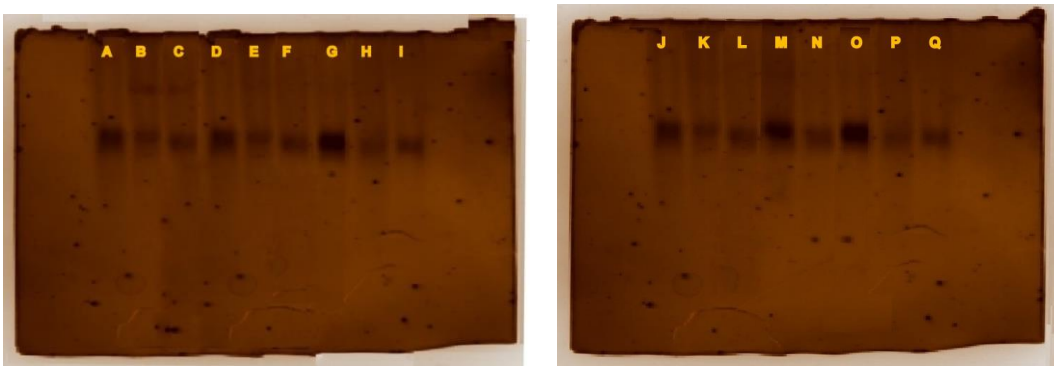


Plate 4.24. Polyphenol oxidase isozyme profile of select nutmeg accessions

A:Acc.1, B:Acc.5, C:Acc.8, D:Acc.9, E:Acc.11, F:Acc.14, G:Acc.18, H:Acc.21, I:Acc.23, J:Acc.24, K:Acc.30, L:Acc.35, M:Acc.36, N:Acc.37, O:Acc.38, P:Acc(H).1, Q:Acc(H).4

All the seventeen accessions produced a total of three different polyphenol oxidase forms (Plate 4.24). Among the accessions, Acc. 5 exhibited all the three bands. Accessions, 1, 8, 9, 11, 18, 21, 24, 30, 36 and 38 exhibited two bands each. Remaining accessions displayed only faint minor single band.

Agglomerative hierarchical clustering was performed on the Jaccard's similarity co-efficient matrix utilizing the UPGMA method using seven isoforms in the seventeen distinct accessions and the resulting dendrogram generated is presented in Figure 4.3. From the dendrogram, seventeen accessions could be grouped into eight clusters at 85 per cent similarity (Table 4.33).

4.4.3.1 Inter cluster association of qualitative and isozyme clusters

The cluster analysis of qualitative characters resulted in 11 clusters and that based on isozyme markers resulted in eight clusters. The association between these two clustering patterns is presented in Table 4.34.

The accessions of qualitative clusters I, II, III, V, VI, VIII, X and XI aligned 100 per cent into isozyme clusters I, III, I, IV, II, VII, V and V respectively. The clusters IV, V, VI, VII and VIII respectively had 12.5, 37.5, 12.5, 12.5 and 25 per cent of the accessions of qualitative cluster IV.

4.4.4 Enzyme activity

The activity of peroxidase and polyphenol oxidase was carried out and results are given in Table 4.35.

Peroxidase enzyme activity ranged from 0.128 (Acc. 8) to 1.727 (Acc. 30). Maximum peroxidase enzyme activity was noticed in Acc. 30 (1.727) followed by Acc. 5. Minimum activity was noticed in Acc. 8 (0.128).

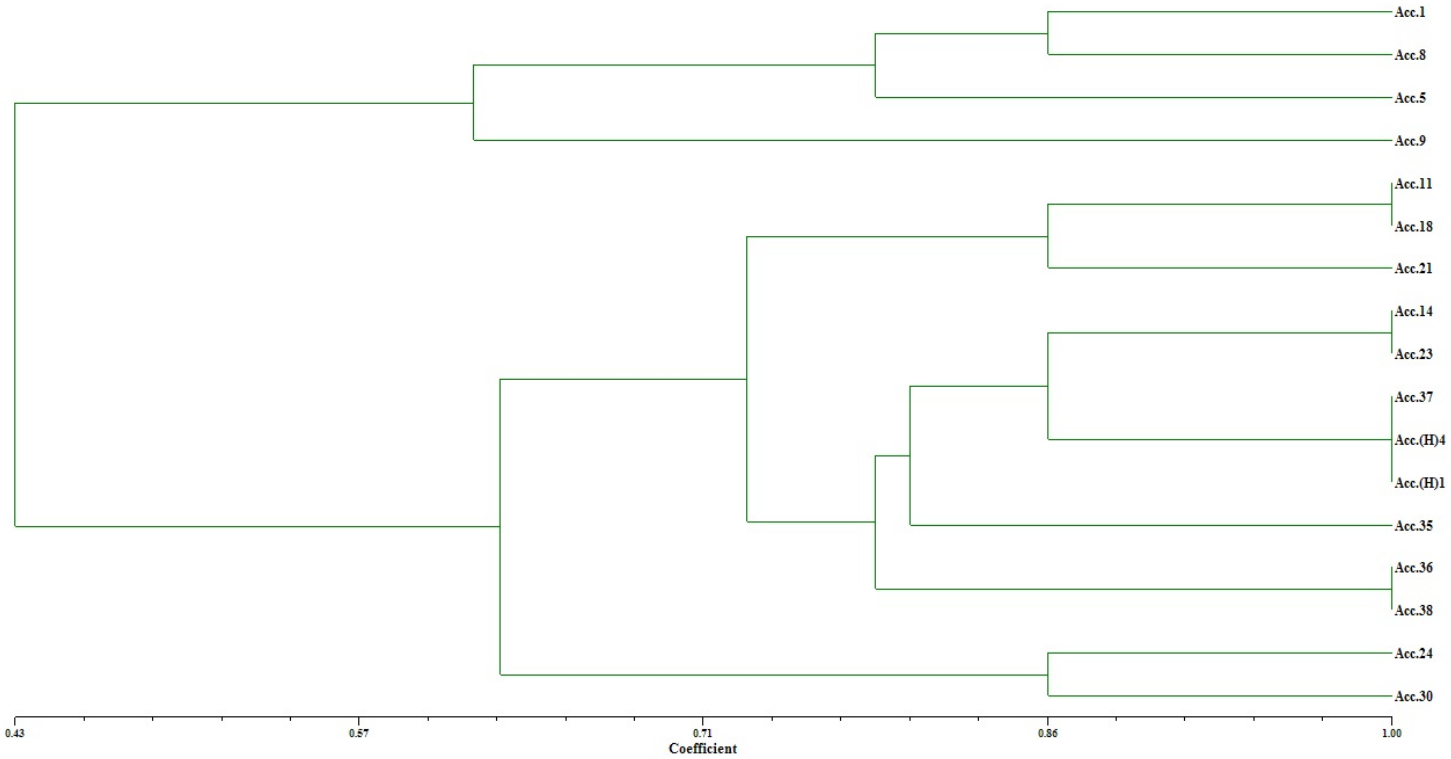


Fig. 4.3 UPGMA dendrogram of isozyme profiling of select nutmeg accessions

Table 4.33. Clustering based on isozyme profiling of nutmeg accessions

Cluster number	Number of accessions	Cluster members
I	2	Acc.1, Acc.8
II	1	Acc.5
III	1	Acc.9
IV	3	Acc.11, Acc.18, Acc.21
V	5	Acc.14, Acc.23, Acc.37, Acc.(H)1, Acc.(H)4
VI	1	Acc.35
VII	2	Acc.36, Acc.38
VIII	2	Acc.24, Acc.30

Table 4.34. Inter cluster association of qualitative and isozyme clusters

Qualitative cluster	Number of accessions	Per cent distribution of accessions in different isozyme clusters							
		I	II	III	IV	V	VI	VII	VIII
I	**1**	100	*	*	*	*	*	*	*
II	**2**	*	*	100	*		*	*	*
III	**3**	100	*	*	*	*	*	*	*
IV	**20**	*	*	*	12.5	37.5	12.5	12.5	25
V	**4**	*	*	*	100	*	*	*	*
VI	**4**	*	100	*	*	*	*	*	*
VII	**7**	*	*	*	*	*	*	*	*
VIII	**1**	*	*	*	*	*	*	100	*
IX	**2**	*	*	*	*	*	*	*	*
X	**1**	*	*	*	*	100	*	*	*
XI	**1**	*	*	*	*	100	*	*	*

Table 4.35. Peroxidase and polyphenol oxidase activity in select nutmeg accessions

Sl. No.	Accessions	Peroxidase (units/min)	Polyphenol oxidase (units/min)
1	Acc.1	0.373	0.227
2	Acc.5	1.420	0.116
3	Acc.8	0.128	0.107
4	Acc.9	0.402	0.329
5	Acc.11	0.325	0.111
6	Acc.14	0.635	0.119
7	Acc.18	0.491	0.121
8	Acc.21	0.313	0.139
9	Acc.23	0.687	0.145
10	Acc.24	0.289	0.207
11	Acc.30	1.727	0.119
12	Acc.35	0.959	0.113
13	Acc.36	0.264	0.098
14	Acc.37	0.381	0.103
15	Acc.38	0.441	0.188
16	Acc.(H)1	0.483	0.136
17	Acc.(H)4	0.889	0.114

The polyphenol oxidase activity ranged between 0.098 (Acc. 36) and 0.329 (Acc. 9). Maximum polyphenol oxidase activity was observed in Acc.9 followed by Acc. 1. Minimum activity was recorded in Acc. 36.

4.4.5 Molecular characterization of nutmeg accessions

An investigation of the characteristics of a member of plant kingdom will be complete and fool proof only when a clear picture of the exact genetic make up is obtained. For this very purpose, molecular characterization was attempted to.

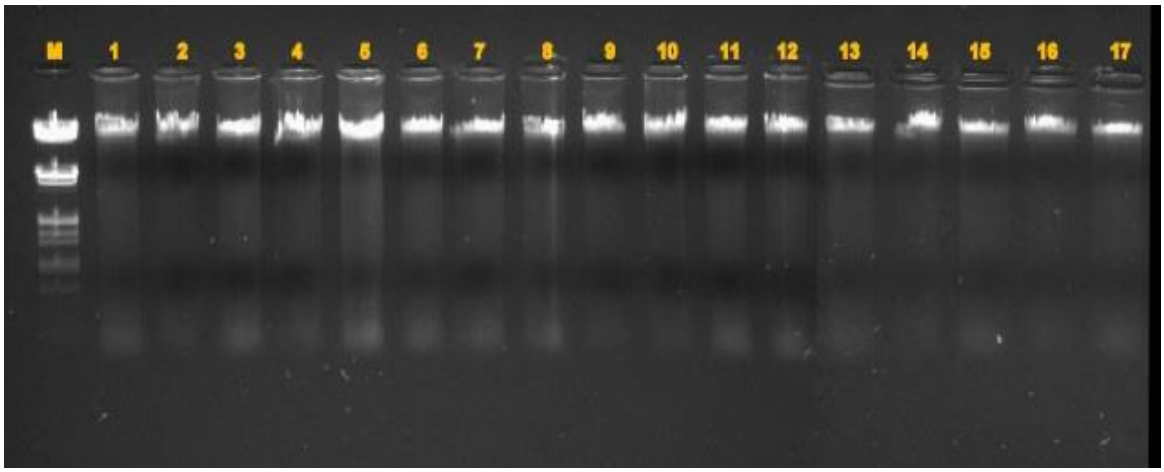
4.4.5.1 Isolation, purification and quantification of DNA

Genomic DNA was isolated from young, tender leaves of seventeen select accessions using CTAB protocol (Doyle and Doyle, 1987) with slight modifications as mentioned in methodology. After the DNA isolation, it was treated with RNase which resulted in good quality DNA (Plate 4.25). The agarose gel electrophoresis showed clear and intact band with no RNA contamination and NanoDrop^{R} ND-1000 spectrophotometer analysis gave the acceptable ratio of absorbance (A_{260}/A_{280}) between 1.70 and 1.97 (Table 4.36). The DNA of 30ng/µl dilution was used as template for RAPD and ISSR marker assay.

4.4.5.2 RAPD analysis

Forty three random primers were screened initially with all the 17 distinct accessions of nutmeg to check polymorphism and reproducibility. Based on clear banding pattern and polymorphism, 21 decamer primers were selected for RAPD assay.

Twenty one decamer primers used for the study have yielded a total of 164 scorable bands with an average polymorphism of 63.21 per cent. The number of bands resolved per amplification was primer dependent and varied from a minimum



M: ladder (EcoRI/Hind III, 1000bp), 1:Acc.1, 2:Acc.5, 3:Acc.8, 4:Acc.9, 5:Acc.11, 6:Acc.14, 7:Acc.18, 8:Acc.21, 9:Acc.23, 10:Acc.24, 11:Acc.30, 12:Acc.35, 13:Acc.36, 14:Acc.37, 15:Acc.38, 16:Acc(H).1, 17:Acc(H).4

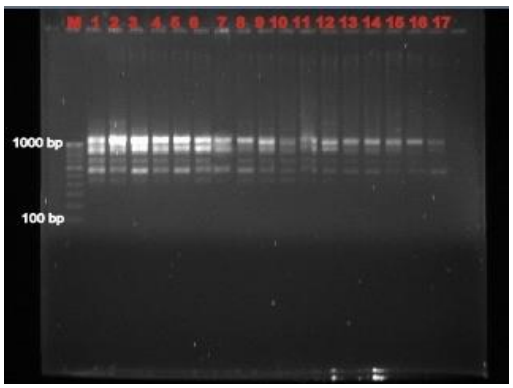
Plate 4.25. Intact DNA isolated from seventeen select nutmeg accessions (after RNase treatment)

Table 4.36. Quantity and quality of DNA isolated from nutmeg accessions

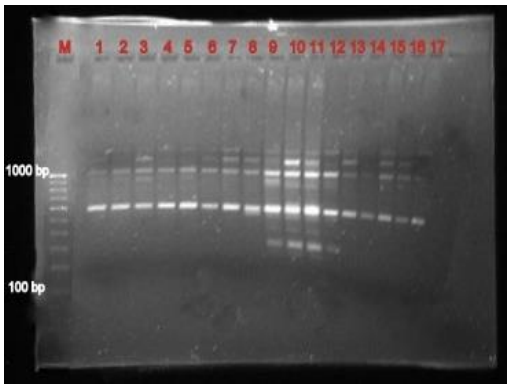
Sl. No.	Accessions	A_{260}/A_{280}	Quantity of DNA (ng/µl)
1	Acc. 1	1.78	301.80
2	Acc. 5	1.71	452.80
3	Acc. 8	1.80	516.72
4	Acc. 9	1.91	565.54
5	Acc. 11	1.79	320.00
6	Acc. 14	1.77	371.40
7	Acc. 18	1.79	676.12
8	Acc. 21	1.91	524.50
9	Acc. 23	1.83	403.40
10	Acc. 24	1.70	317.10
11	Acc. 30	1.81	418.33
12	Acc. 35	1.96	255.04
13	Acc. 36	1.90	236.95
14	Acc. 37	1.72	295.63
15	Acc. 38	1.70	253.32
16	Acc. (H) 1	1.81	289.60
17	Acc. (H) 4	1.97	265.87

Table 4.37. Amplification pattern in nutmeg accessions with RAPD primers

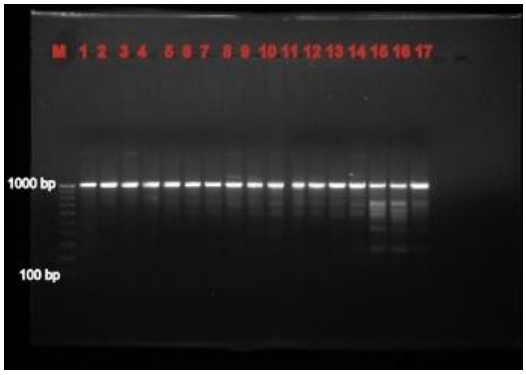
Sl. No.	Primers	Total no. of amplicons	No. of polymorphic amplicons	No. of monomorphic amplicons	Polymorphism (%)	Size of amplicons (range-bp)
1	OPA 01	7	5	2	71.43	450- >1000
2	OPA 07	10	9	1	90.00	300- >1000
3	OPA 08	9	5	4	55.56	250- >1000
4	OPA 09	9	4	5	44.44	150- >1000
5	OPE 14	8	5	3	62.50	300- >1000
6	OPE 15	6	4	2	66.67	350- 700
7	OPE 16	6	2	4	33.33	350- 850
8	OPA 02	5	4	1	80.00	550 -900
9	OPB 06	5	4	1	80.00	400- 950
10	OPB 07	10	5	5	50.00	350- >1000
11	OPB 09	7	4	3	57.14	500- >1000
12	OPB 10	8	5	3	62.50	200- >1000
13	OPB 19	10	7	3	70.00	300- >1000
14	OPC 05	10	8	2	80.00	300- >1000
15	OPC 07	6	4	2	66.67	350- 1000
16	OPC 08	8	6	2	75.00	200- 800
17	OPD 08	8	4	4	50.00	350- 1000
18	OPL 12	8	2	6	25.00	250- >1000
19	OPL 18	10	5	5	50.00	450- >1000
20	OPP 13	7	6	1	85.71	700- >1000
21	OPY 02	7	5	2	71.43	300- >1000



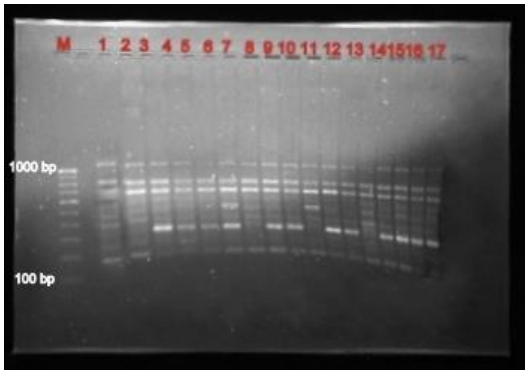
a. Primer OPA 01



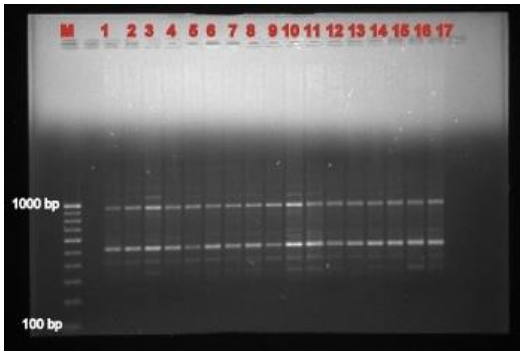
b. Primer OPA 07



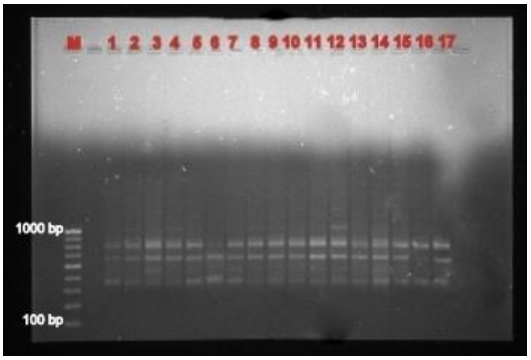
c. Primer OPA 08



d. Primer OPA 09



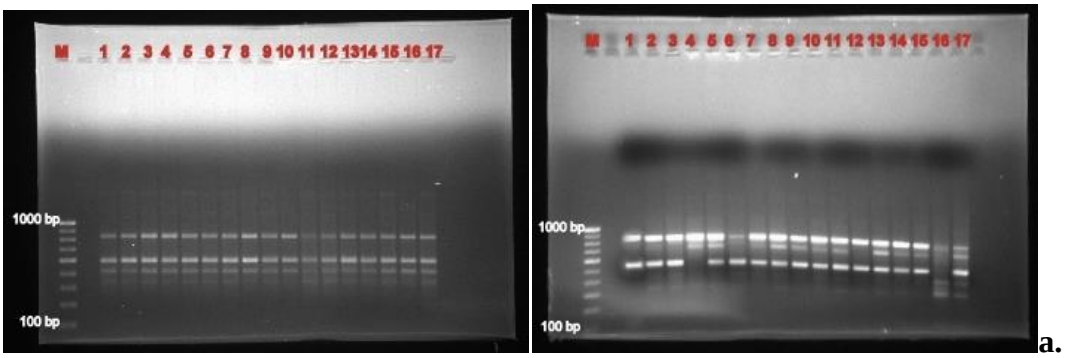
e. Primer OPE 14



f. Primer OPE 15

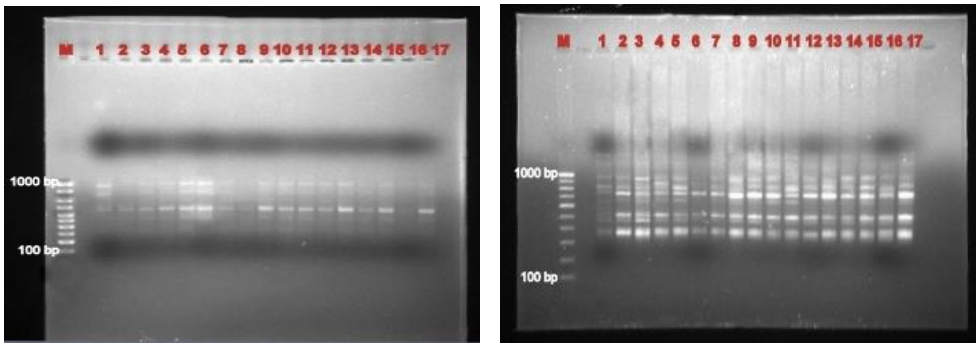
M: ladder (EcoRI/Hind III, 1000bp), 1:Acc.1, 2:Acc.5, 3:Acc.8, 4:Acc.9, 5:Acc.11, 6:Acc.14, 7:Acc.18, 8:Acc.21, 9:Acc.23, 10:Acc.24, 11:Acc.30, 12:Acc.35, 13:Acc.36, 14:Acc.37, 15:Acc.38, 16:Acc(H).1, 17:Acc(H).4

Plate 4.26. Amplification pattern in select nutmeg accessions with RAPD primers



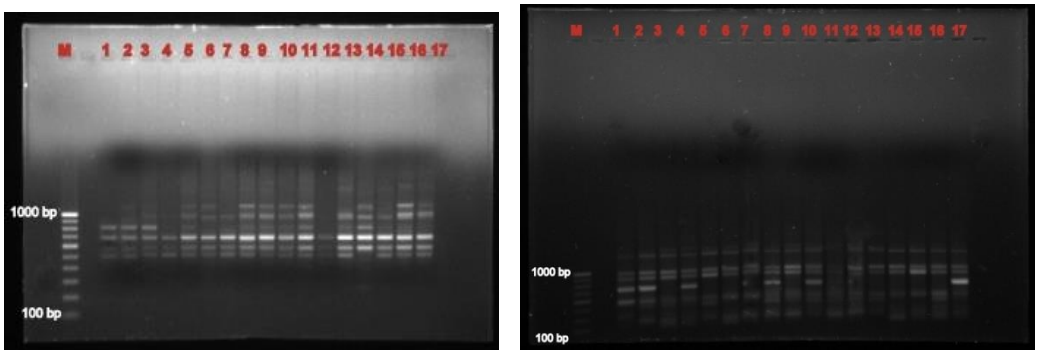
a. Primer OPE 16

b. Primer OPA 02



c. Primer OPB 06

d. Primer OPB 07



e. Primer OPB 09

f. Primer OPB 10

M: ladder (EcoRI/Hind III, 1000bp), 1:Acc.1, 2:Acc.5, 3:Acc.8, 4:Acc.9, 5:Acc.11, 6:Acc.14, 7:Acc.18, 8:Acc.21, 9:Acc.23, 10:Acc.24, 11:Acc.30, 12:Acc.35, 13:Acc.36, 14:Acc.37, 15:Acc.38, 16:Acc(H).1, 17:Acc(H).4

Plate 4.27. Amplification pattern in select nutmeg accessions with RAPD primers

of five to a maximum of ten. The highest number of scorable bands (10 bands) was given by the primes, OPA 07, OPB 07, OPB 19, OPC 05 and OPL 18. The primers, OPA 08 and OPA 09 gave nine bands each. The details of amplification pattern of 21 RAPD primers are provided in Table 4.37.

The primer OPA 01 produced a total of seven scorable bands and amplicons ranging in size from 450 bp to 1100 bp. Five polymorphic bands were identified with a 71.43 per cent polymorphism (Plate 4.26a).

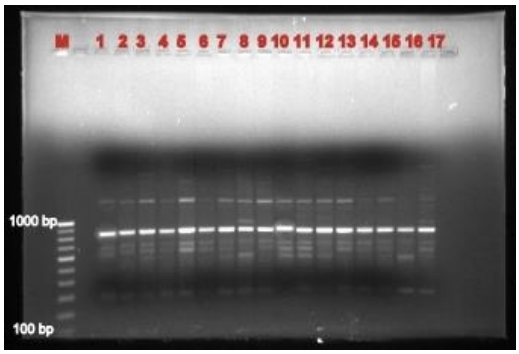
Using OPA 07 primer, ten amplicons ranging in size from 300 bp to 1200 bp were produced. It has generated nine polymorphic bands out of ten amplicons (Plate 4.26b) and the percentage of polymorphism was 90.00 per cent. Three loci of size 850 bp, 900 bp and 950 bp were found in Acc. 23, Acc. 24, Acc. 30 and Acc. 35, respectively.

OPA 08 produced nine amplicons ranging in size from 250 bp to 1100 bp (Plate 4.26c). Five bands were polymorphic and the percentage of polymorphism was 55.56.

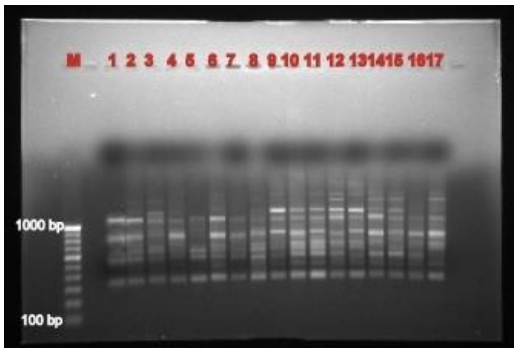
Nine clear and reproducible bands were produced by primer OPA 09 (Plate 4.26d) and their size ranged from 150 bp to 1100 bp. It has generated four polymorphic amplicons out of nine and the percentage of polymorphism was 44.44.

The primer OPE 14 has generated a total of eight reproducible bands (300 bp to 1300 bp). Five were polymorphic bands and the percentage of polymorphism was 62.50. The amplification profile is given in Plate 4.26e.

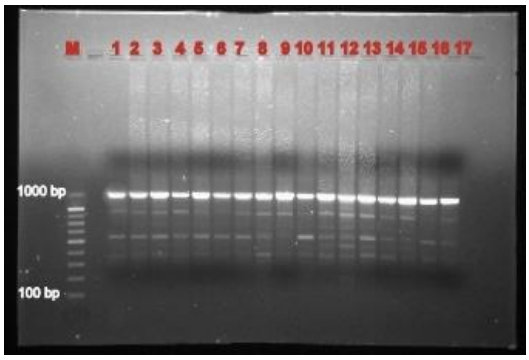
The primer OPE 15 generated six amplicons (Plate 4.26f) ranging in size from 350 bp to 700 bp. Amplifications were produced in all the accessions except in Acc. (H) 1 at two loci (450 bp and 550 bp).



a. Primer OPB 19

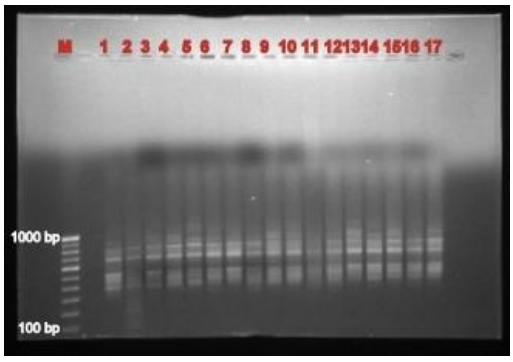


b. Primer OPC 05

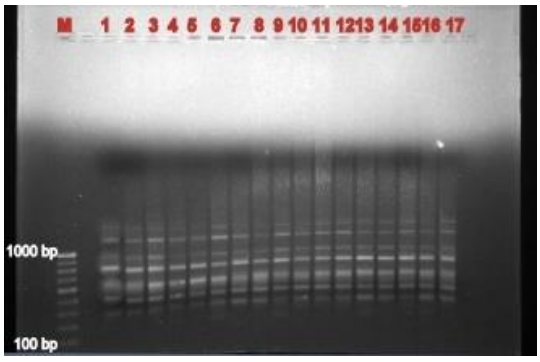


c. Primer OPC 07

d. Primer OPC 08



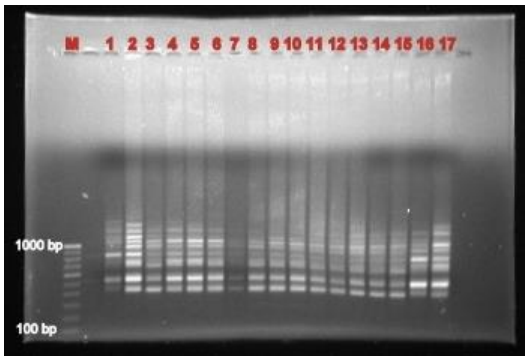
e. Primer OPD 08



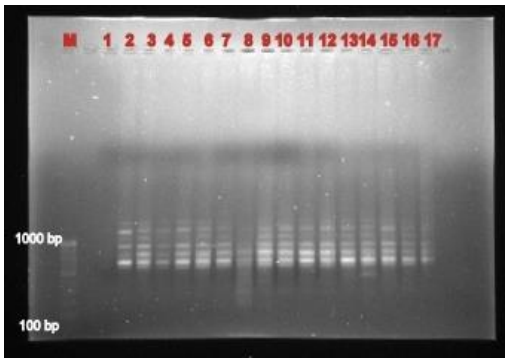
f. Primer OPL 12

M: ladder (EcoRI/Hind III, 1000bp), 1:Acc.1, 2:Acc.5, 3:Acc.8, 4:Acc.9, 5:Acc.11, 6:Acc.14, 7:Acc.18, 8:Acc.21, 9:Acc.23, 10:Acc.24, 11:Acc.30, 12:Acc.35, 13:Acc.36, 14:Acc.37, 15:Acc.38, 16:Acc(H).1, 17:Acc(H).4

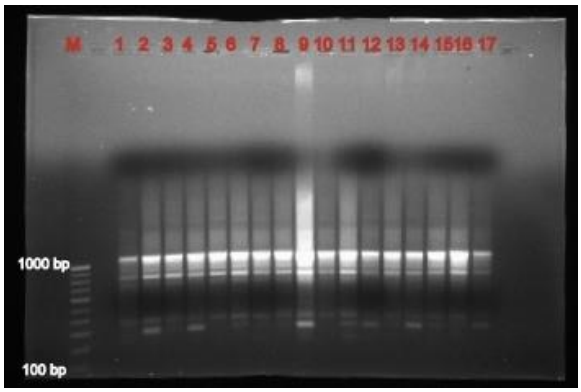
Plate 4.28. Amplification pattern in select nutmeg accessions with RAPD primers



a. Primer OPL 08



b. Primer OPP 13



c. Primer OPY 02

M: ladder (EcoRI/Hind III, 1000bp), 1:Acc.1, 2:Acc.5, 3:Acc.8, 4:Acc.9, 5:Acc.11, 6:Acc.14, 7:Acc.18, 8:Acc.21, 9:Acc.23, 10:Acc.24, 11:Acc.30, 12:Acc.35, 13:Acc.36, 14:Acc.37, 15:Acc.38, 16:Acc(H).1, 17:Acc(H).4

Plate 4.29. Amplification pattern in select nutmeg accessions with RAPD primers

Amplification of nutmeg accessions with the primer OPE 16 has yielded six amplicons (Plate 4.27a) and their sizes ranged from 350 bp to 850 bp. Two were polymorphic bands. One unique band of size 200 bp was detected in Acc. (H) 4.

The primer OPA 02 has produced five clear, distinct and reproducible amplicons (Plate 4.28b) ranging in size from 550 bp to 900 bp. Four were polymorphic bands and the polymorphism was found to be 80.00 per cent.

OPB 06 has generated a total of five amplicons (Plate 4.27c) of size range 400 bp to 950 bp. Out of five bands, four were polymorphic across the accessions and polymorphism was found to be 80 per cent.

The primer OPB 07 has generated ten scorable bands and the band size ranged from 350 bp to 1300 bp (Plate 4.27d). The percentage of polymorphism and monomorphism was 50 per cent each.

Seven clear and reproducible bands were produced by primer OPB 09 (Plate 4.27e) ranging in size from 500 bp to 1200 bp. It could detect five polymorphic amplicons out of seven and the percentage of polymorphism was 57.14.

OPB 10 has generated eight scorable bands and the band size ranged from 200-1100 bp (Plate 4.27f). Five were polymorphic bands and the percentage of polymorphism was 62.50.

The primer OPB 19 has generated a total of ten clear, distinct and reproducible bands (300-1300 bp). Seven were polymorphic bands (Plate 4.28a) and the percentage of polymorphism was 70. One loci of size 900 bp was found unique in Acc. 21 and Acc. (H) 4.

Amplification of nutmeg accessions with OPC 05 primer has produced ten clear and reproducible bands which ranged in size from 300-1300 bp (Plate 4.28b). Eight of the ten bands were polymorphic and the percentage of polymorphism was 80.

The primer OPC 07 has produced a total of six scorable bands (350-1200 bp). Four bands were polymorphic and the percentage of polymorphism was 66.67. The amplification profile is given in Plate 4.28c.

When OPC 08 was used for amplification, a total of eight scorable bands were generated (200- 800 bp). Six bands were polymorphic and the percentage of polymorphism was 75. The amplification profile is presented in Plate 4.28d.

Amplification with OPD 08 has produced eight bands in all the accessions with primer (350-1000 bp). Out of them four bands were polymorphic. The amplification profile is given in Plate 4.28e.

The primer OPL 12 has amplified eight scorable bands (Plate 4.28f) which ranged in size from 250 bp to 1200 bp. Two of them were polymorphic across the genotypes and the polymorphism calculated was 25 per cent.

The primer OPL 18 has produced a total of ten clear, distinct and reproducible bands (450-1400 bp), out of which five were polymorphic and the percentage of polymorphism was 50. The amplification profile is given in Plate 4.29a.

Seven amplicons were produced by primer OPP 13 (Plate 4.29b) ranging from 700 bp to 900 bp. It could detect six polymorphic amplicons out of seven and the percentage of polymorphism was 85.71.

The primer OPY 02 has generated seven amplicons (Plate 4.29c) ranging in size from 300 bp to 1200 bp. Five amplicons were polymorphic and the percentage of polymorphism was 71.43.

4.4.5.2.1 Cluster analysis based on RAPD data

Agglomerative hierarchical clustering was performed by UPGMA method using Jaccard's similarity coefficient matrix. Molecular profiles of seventeen

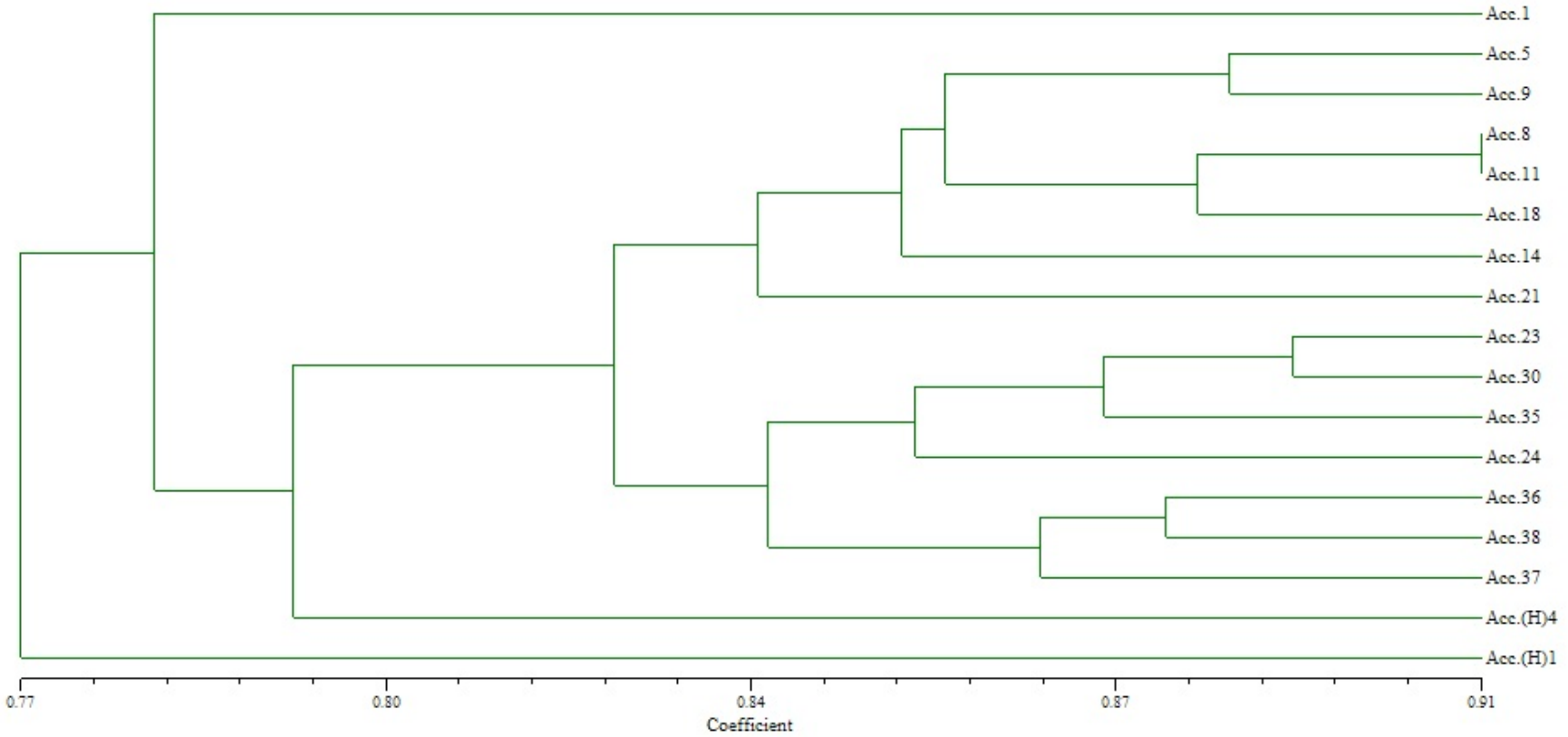


Figure 4.4 UPGMA dendrogram of RAPD profiling of select nutmeg accessions

Table 4.38. Clustering based on RAPD profiling of nutmeg accessions

Cluster number	Number of accessions	Cluster members
I	1	Acc.1
II	6	Acc.5, Acc.8, Acc.9, Acc.11, Acc.14, Acc.18
III	1	Acc. 21
IV	4	Acc. 23, Acc. 24, Acc.30, Acc.35
V	3	Acc.36, Acc.37, Acc. 38
VI	1	Acc.(H)4
VII	1	Acc.(H)1

Table 4.39. Inter cluster association of qualitative and RAPD clusters

Qualitative cluster	Number of accessions	Per cent distribution of accessions in RAPD clusters						
		I	II	III	IV	V	VI	VII
I	**1**	100	*	*	*	*	*	*
II	**2**	*	100	*	*	*	*	*
III	**3**	*	100	*	*	*	*	*
IV	**20**	*	25	*	50	25	*	*
V	**4**	*	50	50		*	*	*
VI	**4**	*	100	*	*	*	*	*
VII	**7**	*	*	*	*	*	*	*
VIII	**1**	*	*	*	*	100	*	*
IX	**2**	*	*	*	*	*	*	*
X	**1**	*	*	*	*	*	100	*
XI	**1**	*	*	*	*	*	*	100

accessions of nutmeg were generated using 21 polymorphic random primers and dendrogram was constructed on the basis of binary data (Fig. 4.4).

These 17 accessions formed into seven clusters at 86 per cent of similarity level. Maximum of six accessions were included in cluster II. Cluster I, III, VI and VII had one accession each. The seven clusters along with the accessions in each cluster are presented in Table 4.38.

4.4.5.2.2 Inter cluster association of qualitative and RAPD clusters

The cluster analysis of qualitative characters resulted in 11 clusters and that based on RAPD markers resulted in seven clusters. The parallelism between these two clustering patterns is presented in Table 4.39.

The accessions of qualitative clusters I, II, III, VI, VIII, X, and XI fell completely into RAPD clusters I, II, V, VI and VII respectively. The 20 accessions in qualitative cluster IV were found to be distributed in three different RAPD clusters, II, IV and V. In this case, maximum numbers of accessions (50%) fell in RAPD cluster IV. The accessions in qualitative cluster V were found equally distributed in the RAPD clusters II and III.

4.4.5.3 ISSR analysis

Preliminary screening of 18 ISSR primers using 17 distinct nutmeg accessions was done for confirmation of the polymorphism and reproducibility. Based on primer amplification, 12 primers were selected for further ISSR assay.

Twelve ISSR primers used for the characterization gave a total of 87 amplicons with an average polymorphism of 69.44 per cent. The number of bands resolved per amplification was primer dependent and varied from a minimum of four to a maximum of ten. Highest number of amplicons was scored in UBC 864 (10 bands) followed by UBC 810 and ISSR 25 (9 bands). Lowest number of amplicons

Table 4.40. Amplification pattern in nutmeg accessions with ISSR primers

Sl. No.	Primers	Total no. of amplicons	No. of polymorphic amplicons	No. of monomorphic amplicons	Polymorphism (%)	Size of amplicons (range-bp)
1	ISSR 22	8	6	2	75.00	450- >1000
2	ISSR 25	9	6	3	66.67	200- 950
3	ISSR 26	6	5	1	83.33	150- 1000
4	$(TC)_7C$	6	4	2	66.67	500- >1000
5	$(CT)_7AC$	6	6	0	100.00	450- 1000
6	UBC 809	8	5	3	62.50	500- >1000
7	UBC 810	9	7	2	77.78	400->1000
8	UBC 812	8	7	1	87.50	350- 950
9	UBC 816	7	4	3	57.14	550- >1000
10	UBC 857	4	2	2	50.00	350- 1000
11	UBC 864	10	4	6	40.00	300->1000
12	UBC 893	6	4	2	66.67	800- >1000

was scored in UBC 857. Details of amplification pattern of 12 ISSR primers are provided in Table 4.40.

The primer ISSR 22 has produced eight scorable bands which ranged in size of 450 bp to 1100 bp. Bands were clear and reproducible (Plate 4.30a) with a polymorphism percentage of 75.

A total of nine amplifications ranging between 200 bp and 950 bp were produced by the primer ISSR 25 (Plate 4.30b). It has generated six polymorphic

amplicons with a polymorphism percentage of 66.67. One unique locus of size 300 bp was found in Acc. 1. A single locus of size 400 bp was found in Acc. 1 and Acc. 37.

The primer ISSR 26 has generated a total of six clear (Plate 4.30c), distinct and reproducible bands (150 bp to 1000 bp), out of which five were polymorphic bands and the percentage of polymorphism was 83.33. One locus of size 300 bp was found in Acc.1, Acc. 5, Acc. 9, Acc. 21 and Acc. 24.

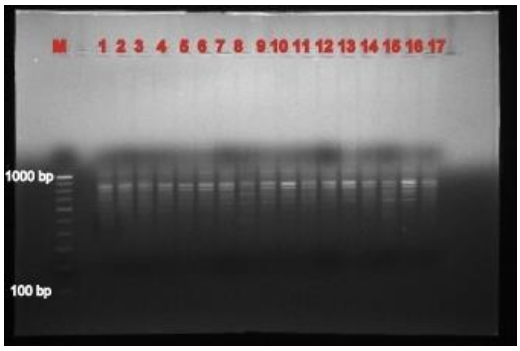
The primer $(TC)_7C$ has produced six clear, distinct and reproducible amplicons which ranged in size from 500 bp to 1200 bp (Plate 4.30d). Out of six bands, four were polymorphic and the polymorphism was to the tune of 66.67 per cent. One locus of size 850 bp was found in Acc. 38 and Acc. (H) 1.

A total of six amplicons ranging in size between 450 bp and 1000 bp were produced by the primer $(CT)_7AC$. All the bands were polymorphic (Plate 4.30e). A single locus of size 750 bp was found in Acc. Acc. 14 and Acc. 38, both of which were entire mace types.

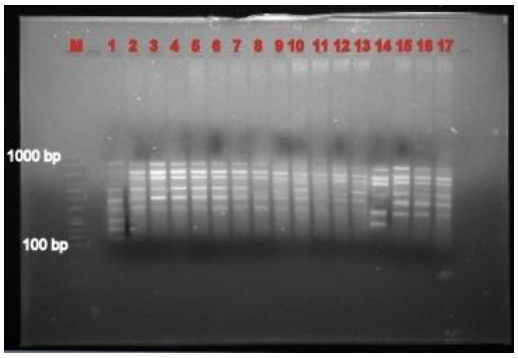
Amplification of nutmeg accessions with the primer UBC 809 resulted in eight clear, distinct and reproducible amplicons which ranged from 500 bp to 1200 bp (Plate 4.30f). Of the eight amplicons, five were polymorphic bands and the polymorphism was 62.50 per cent.

Nine clear, distinct and reproducible amplicons were produced by primer UBC 810 (Plate 4.31a) which had a size range of 400 to 1200 bp. It has yielded seven polymorphic amplicons out of nine and the percentage of polymorphism was 77.78.

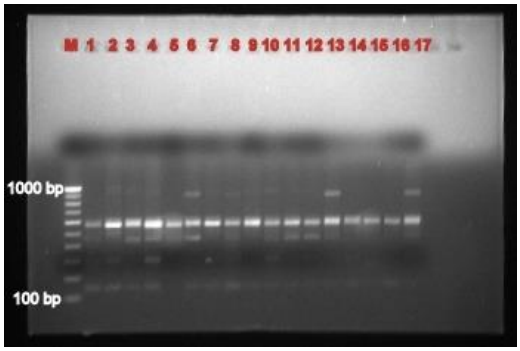
UBC 812 has generated a total of eight clear, distinct and reproducible amplicons (Plate 4.31b) of size 350 bp to 950 bp. Of the eight bands, seven were polymorphic across the accessions and the per cent polymorphism was 87.50.



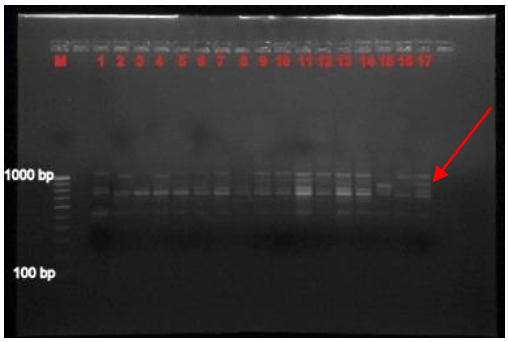
a. Primer ISSR 22



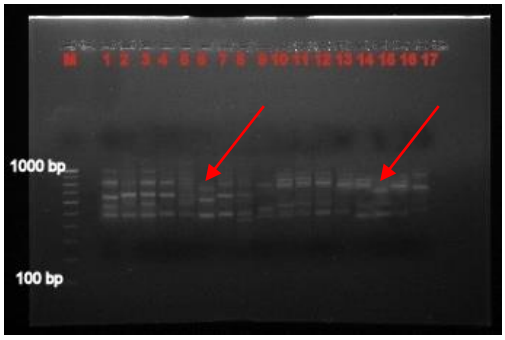
b. Primer ISSR 25



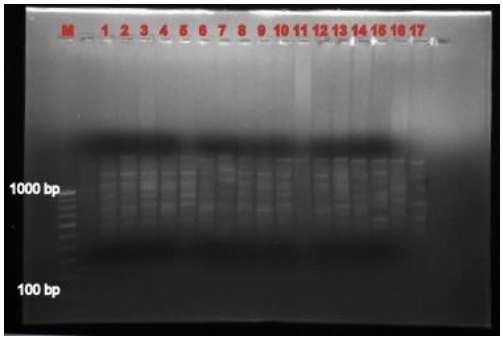
c. Primer ISSR 26



d. Primer P 6



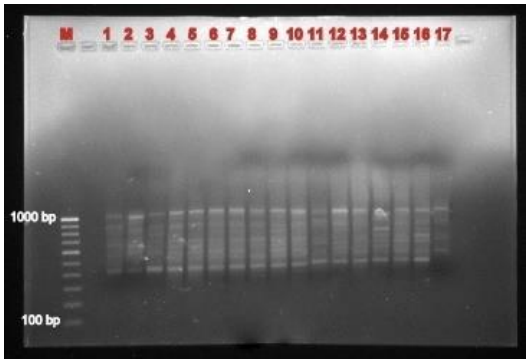
e. Primer P 9



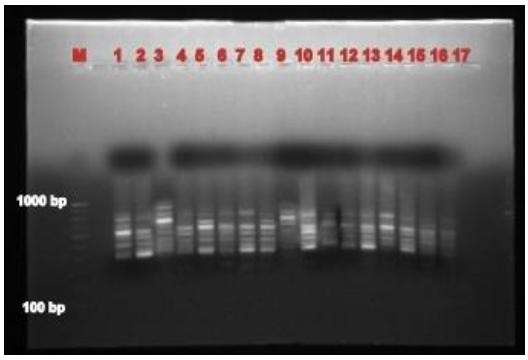
f. Primer UBC 809

M: ladder (EcoRI/Hind III, 1000bp), 1:Acc.1, 2:Acc.5, 3:Acc.8, 4:Acc.9, 5:Acc.11, 6:Acc.14, 7:Acc.18, 8:Acc.21, 9:Acc.23, 10:Acc.24, 11:Acc.30, 12:Acc.35, 13:Acc.36, 14:Acc.37, 15:Acc.38, 16:Acc(H).1, 17:Acc(H).4

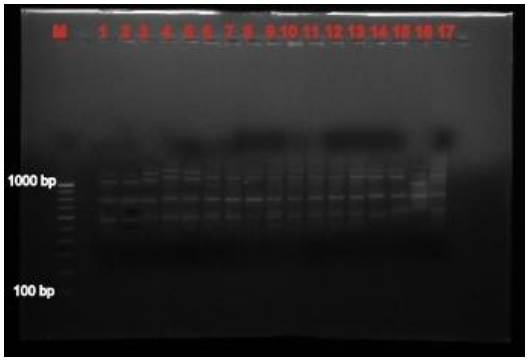
Plate 4.30. Amplification patterns in select nutmeg accessions with ISSR primers



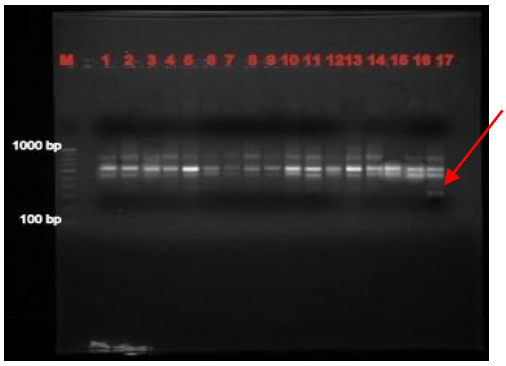
a. Primer UBC 810



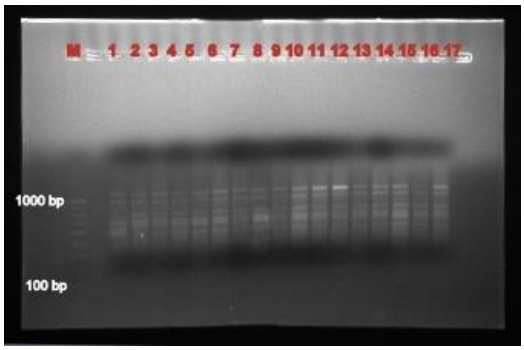
b. Primer UBC 812



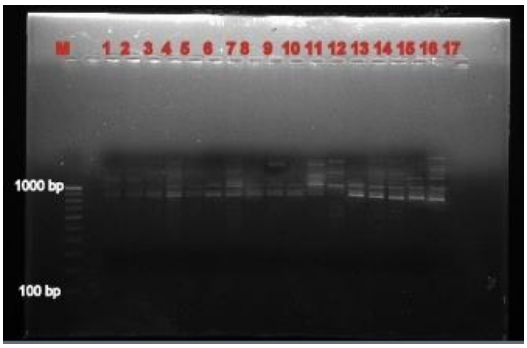
c. Primer UBC 816



d. Primer UBC 857



e. Primer UBC 864



f. Primer UBC 893

M: ladder (EcoRI/Hind III, 1000bp), 1:Acc.1, 2:Acc.5, 3:Acc.8, 4:Acc.9, 5:Acc.11, 6:Acc.14, 7:Acc.18, 8:Acc.21, 9:Acc.23, 10:Acc.24, 11:Acc.30, 12:Acc.35, 13:Acc.36, 14:Acc.37, 15:Acc.38, 16:Acc(H).1, 17:Acc(H).4

Plate 4.31. Amplification pattern in select nutmeg accessions with ISSR primers

A total of seven amplicons which ranged in size 550 to 1100 bp were produced by the primer UBC 816. It has generated four polymorphic bands (Plate 4.31c) and the percentage of polymorphism was 57.14.

Four clear, distinct and reproducible amplicons were produced by primer UBC 857 (Plate 4.31d) and their size ranged from 350-1000 bp. It has detected two polymorphic amplicons out of four and the percentage of polymorphism was 50. A locus of size 320 bp was found only in Acc. (H) 4.

UBC 864 has generated a total of ten clear, distinct and reproducible amplicons (Plate 4.31e) which ranged from 300-1200 bp. Of the ten bands, four were polymorphic and the polymorphism was found to be 40 per cent.

Six scorable amplicons were produced by primer UBC 893 (Plate 4.31f) which ranged in size from 800-1300 bp and above. It has detected four polymorphic amplicons out of six and the percentage of polymorphism was 66.67.

4.4.5.3.1 Cluster analysis based on ISSR data

Agglomerative hierarchical clustering was performed based on the Jaccard's similarity coefficient using UPGMA method with the binary data of 12 ISSR primers and the resultant dendrogram is presented in Figure 4.5.

The seventeen accessions were separated into 11 clusters with a similarity of 85 per cent. Cluster III and VII had maximum of three accessions each. Cluster I, II, V, VI, VIII, X and XI had only one accession each (Table 4.41).

4.4.5.3.2 Inter cluster association of qualitative and ISSR clusters

The cluster analysis of qualitative characters resulted in 11 clusters and that based on IISR markers resulted in 11 clusters. The likeness between these two clustering patterns is presented in Table 4.42.

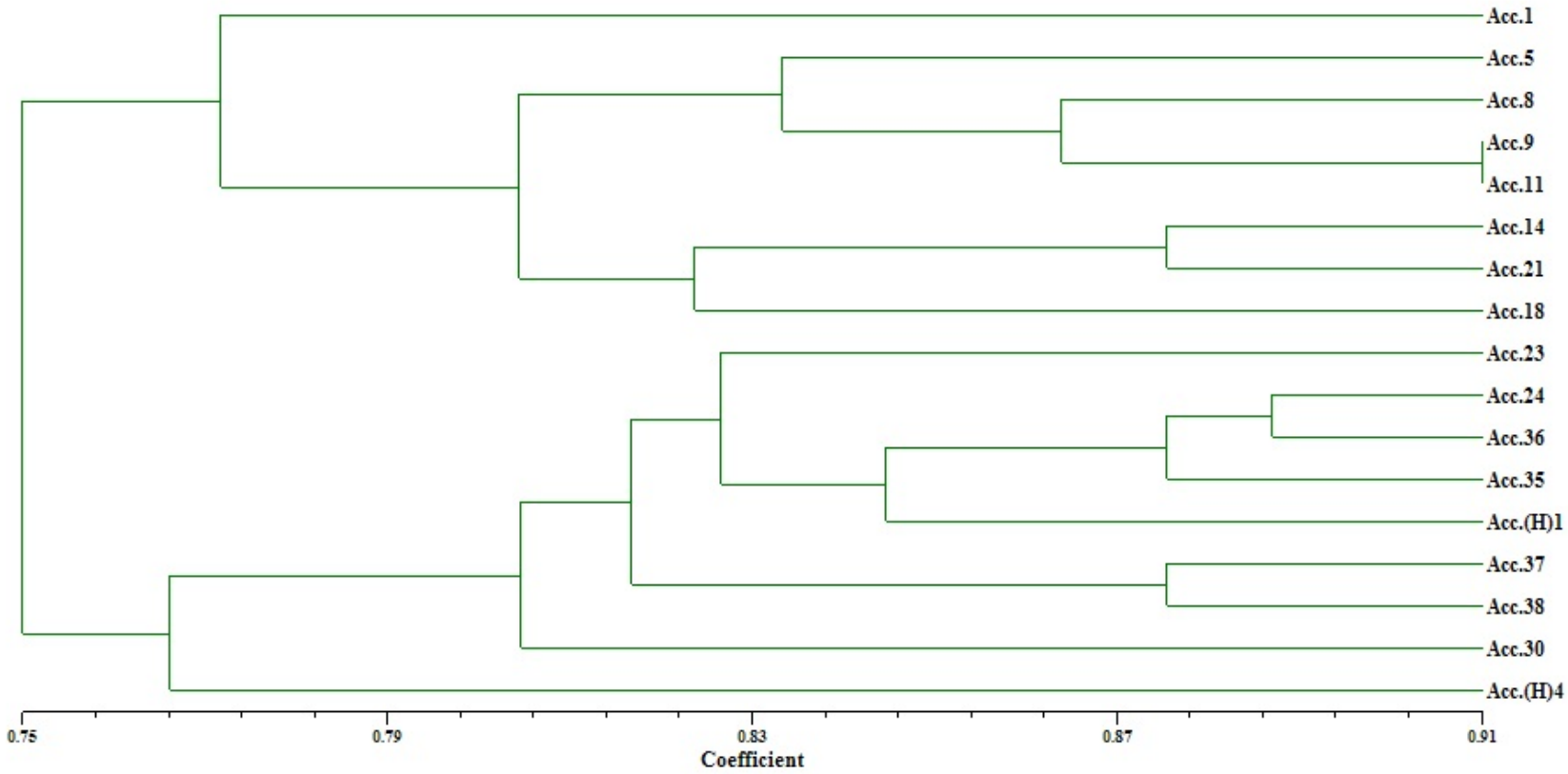


Figure 4.5. UPGMA dendrogram of ISSR profiling of select nutmeg accessions

Table 4.41. Clustering based on ISSR profiling of nutmeg accessions

Cluster number	Number of accessions	Cluster members
I	1	Acc.1
II	1	Acc.5
III	3	Acc. 8, Acc.9, Acc.11
IV	2	Acc. 14, Acc. 21
V	1	Acc. 18
VI	1	Acc.23
VII	3	Acc.24, Acc.35, Acc.36
VIII	1	Acc.(H)1
IX	2	Acc.37, Acc.38
X	1	Acc.30
XI	1	Acc.(H)4

Table 4.42. Inter cluster association of qualitative and ISSR clusters

Qualitative cluster	Number of accessions	Per cent distribution of accessions in different ISSR clusters										
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	**1**	100	*	*	*	*	*	*	*	*	*	*
II	**2**	*	*	100	*	*	*	*	*	*	*	*
III	**3**	*	*	100	*	*	*	*	*	*	*	*
IV	**20**	*	*	*	12.5	12.5	12.5	25	*	25	12.5	*
V	**4**	*	*	50	50	*	*	*	*	*	*	*
VI	**4**	*	100	*	*	*	*	*	*	*	*	*
VII	**7**	*	*	*	*	*	*	*	*	*	*	*
VIII	**1**	*	*	*	*	*	*	100	*	*	*	*
IX	**2**	*	*	*	*	*	*	*	*	*	*	*
X	**1**	*	*	*	*	*	*	*	100	*	*	*
XI	**1**	*	*	*	*	*	*	*	*	*	*	100

The accessions of qualitative clusters I, II, III, VI, VIII, X and XI were distributed 100 per cent into ISSR clusters I, III, II, VII, VIII and XI respectively. In the case of qualitative cluster IV, 25 per cent of accessions were distributed in IISR clusters VII and IX. 12.5 per cent of accessions fell in IISR clusters IV, V, VI and X.

4.4.5.4 Cluster analysis based on combined data of RAPD and ISSR

Cluster analysis was performed based on the Jaccard's similarity coefficient matrices using UPGMA method by taking both RAPD and ISSR markers. The dendrogram generated using both the markers is presented in Figure 4.6. The combined analysis of RAPD and ISSR markers depicted the genetic relationships better than individual analyses.

Seventeen accessions got grouped into nine clusters at 85 per cent of similarity level (Table 4.43). Cluster II had the highest number of four accessions. Cluster VI included three accessions. Acc. 1, Acc. 21, Acc. (H) 4 and Acc. (H) 1 remained single in clusters I, IV, VIII and IX, respectively.

4.4.5.4.1 Inter cluster association of qualitative and molecular clusters (combined RAPD & ISSR)

The cluster analysis of qualitative characters resulted in 11 clusters and that based on both RAPD and IISR markers together resulted in nine clusters (Fig.4.6). The parallelism between these two clustering patterns is presented in Table 4.44.

The accessions of qualitative clusters I, II, III, VI, VIII, X and XI were distributed solely in the combined RAPD and ISSR clusters I, II, II, II, VI, X and XI respectively. The accessions in qualitative cluster IV fell equally in the combined RAPD and IISR clusters III, V, VI and VII. Similarly, the accessions in qualitative cluster V were also found equally distributed in two different RAPD and IISR combined clusters (clusters, II and IV).

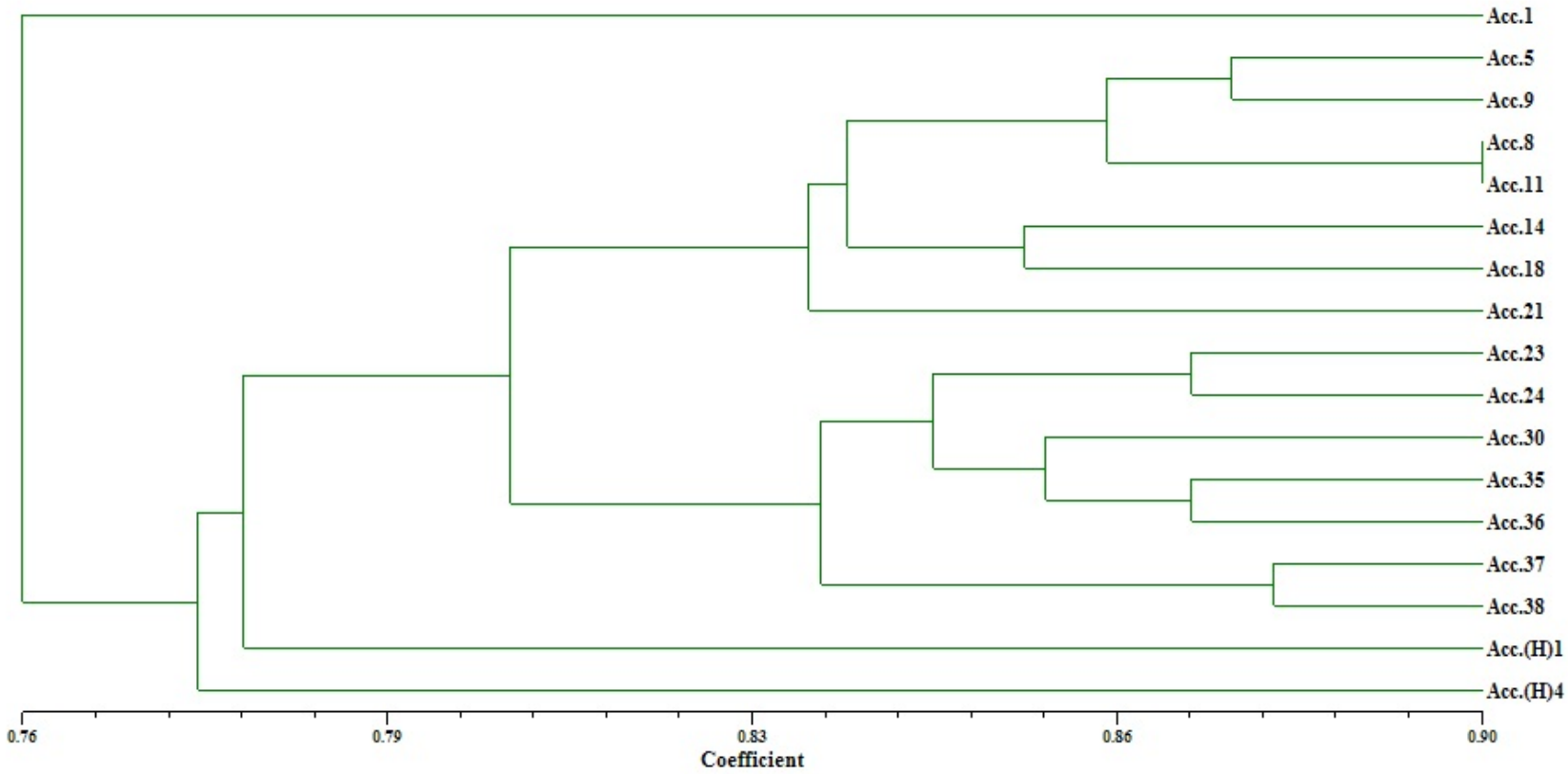


Fig 4.6. UPGMA dendrogram of RAPD and ISSR profiling of select nutmeg accessions

Table 4.43. Clustering pattern based on RAPD and ISSR profiling of nutmeg accessions

Cluster number	Number of accessions	Cluster members
I	1	Acc.1
II	4	Acc.5, Acc.8, Acc.9, Acc.11
III	2	Acc. 14, Acc.18
IV	1	Acc. 21
V	2	Acc. 23, Acc.24
VI	3	Acc. 30, Acc.35, Acc.36
VII	2	Acc. 37, Acc. 38
VIII	1	Acc. (H)1
IX	1	Acc. (H)4

Table 4.44. Inter cluster association of qualitative and combined RAPD and ISSR clusters

Qualitative cluster	Number of accessions	Distribution of accessions in different RAPD and ISSR clusters								
		I	II	III	IV	V	VI	VII	VIII	IX
I	**1**	100	*	*	*	*	*	*	*	*
II	**2**	*	100	*	*	*	*	*	*	*
III	**3**	*	100	*	*	*	*	*	*	*
IV	**20**	*	*	25	*	25	25	25	*	*
V	**4**	*	50	*	50	*	*	*	*	*
VI	**4**	*	100	*	*	*	*	*	*	*
VII	**7**	*	*	*	*	*	*	*	*	*
VIII	**1**	*	*	*	*	*	100	*	*	*
IX	**2**	*	*	*	*	*	*	*	*	*
X	**1**	*	*	*	*	*	*	*	100	*
XI	**1**	*	*	*	*	*	*	*	*	100

The multidimensional analysis of a nutmeg tree has brought forth the ultimate conclusion that the variability is a phenomenon which may be regarded as a continuous variable, very difficult to net down. Whatever number of nutmeg trees the researcher may investigate will not conclusively siphon out the mystery of nutmeg. But a miniature attempt is made towards identification of an elite nutmeg tree with the limited information that was churned out in this study.

4.5 KEY FOR IDENTIFICATION OF ELITE NUTMEG TREE

Thirteen key quantitative characters were selected based on their impact on yield as well as commercial importance. Characters *viz*., tree height, canopy spread in the E-W and N-S direction, number of flowers per 10 cm^2, fruit set percentage, number of fruits per m^2, fruit weight, thickness of pericarp, dry mace weight, dry nut weight, kernel weight, ratio of nut to mace, and number of fruits per tree were the identified key characters. Using these key characters, a statistical key was developed for identifying an elite nutmeg tree. The qualitative clusters were ranked based on relative best performance of the perceived key characters. Data base was generated for the key characters and from this data base, plausible value of each key character was predicted (Table 4.45). An overall assessment of the predicted key characteristics will entail an elite nutmeg tree as detailed below.

Sl. No.	**Characteristics**	**Desirable measure**
1.	Tree height	: 8 m
2.	Canopy spread E-W	: 7 m
3.	Canopy spread N-S	: 8 m
4.	Number of flowers per 10 cm^2	: 6
5.	Fruit set percentage	: 37
6.	Number of fruits per m^2	: 19

7. Fruit weight : 81 g
8. Thickness of pericarp : 14 mm
9. Dry mace weight : 2 g
10. Dry nut weight : 10 g
11. Kernel weight : 7 g
12. Ratio of nut to mace : 6.63
13. Number of fruits per tree : 3342

Table 4.45. Data base for visualization of elite nutmeg tree

Sl. No.	Characters	Cluster II	Cluster VIII	Cluster IV	Cluster X
1	Plant height (m)	7.99	6.27	8.17	8.16
2	Canopy spread (E-W)-(m)	6.23	7.18	5.88	5.18
3	Canopy spread (N-S)-(m)	6.15	7.67	5.85	4.78
4	Number of flowers per 10 cm^2	4.34	6.13	4.81	3.92
5	Fruit set percentage	33.65	36.54	26.00	32.58
6	Number of fruits per m^2	18.15	18.50	16.27	24.00
7	Fruit weight (g)	81.12	71.78	67.41	64.62
8	Thickness of pericarp (mm)	13.74	13.04	12.25	11.99
9	Dry weight of mace (g)	1.75	2.09	1.32	0.97
10	Dry weight of nut (g)	9.63	8.39	7.13	6.05
11	Kernel weight (g)	7.28	6.14	5.24	4.23
12	Ratio of nut to mace	6.63	2.86	4.22	4.24
13	Number of fruits per tree	3341.93	1744.25	1710.15	1525.00

Discussion

5. DISCUSSION

Nutmeg (*Myristica fragrans* Houtt.) the two in one spice is valued for its flavouring and medicinal properties. A native of Moluccas Islands in Indonesia, it was introduced to India about two centuries ago. Although an introduced crop, there exists tremendous variability for this crop in Kerala, which is the major nutmeg growing state in the country. Variability can be seen with respect to sex forms, growth characters *viz*., tree shape, branching pattern, size and shape of leaf, floral characters, fruit and quality characters (Haldankar *et al*., 2004a; Sasikumar, 2009; Miniraj *et al*., 2015). Assessment of the existing variability is necessary for taking up successful crop improvement programmes. It is also important to know the association of various yield contributing components in order to identify promising accessions.

In the present study, a basic work on characterization was done using fifty select nutmeg accessions from a core germplasm planted in Chalakudy river basin in Kerala was used for the study. These accessions belonging to the age group of fifteen years comprised of forty two females, four monoecious and four males. The accessions were evaluated to assess the extent of existing genetic variability.

Detailed morphological characterization was done using all the fifty accessions. As traditional morphological markers are not very effective in discriminating closely related genotypes, molecular as well as biochemical markers were also employed in the characterization of select accessions. Results pertaining to the present investigations are discussed here in this chapter.

5.1 MORPHOLOGICAL CHARACTERIZATION

Morphological markers describe the observable characters of an organism. In India, only little work has been done on identification of nutmeg genotypes based on morphological markers. Both qualitative and quantitative observations were used in the present study for morphological characterization of nutmeg accessions.

5.1.1 Descriptor for qualitative evaluation

Basic unit in plant genetic resources information management is the descriptor. In the absence of a standard descriptor for nutmeg, efforts were made to develop a descriptor for nutmeg using fifty one qualitative variable characters recorded in the study (Table 4.1). The descriptor was developed by scoring in a 0 to 9 scale, for each character under study and recording the data from the accessions based on the mode of expression of each character. The fifty one characters considered were categorised as sex form, foliage characters, flowering and fruiting characters as well as mace and nut characters. These characters contribute to yield directly or indirectly. Tremendous variation in the morphological traits were observed which was used in the development of relevant descriptors and descriptor states. The descriptor list, so developed as part of this study would be helpful on development of a data sheet of the collected material towards utilization in the crop improvement programmes. Similar efforts to develop descriptors have already been attempted in tropical and sub tropical fruit crops (Mahajan *et al.,* 2002) and medicinal and aromatic plants (Singh *et al.,* 2003). Sunil *et al.* (2013) developed a minimal descriptor for characterization and evaluation of jatropha germplasm. Mathew *et al.* (2007) developed a set of pictorial descriptors for morphological characterization of seabuckthorn germplasm.

5.1.2 Qualitative evaluation

In the present investigation, fifty accessions were studied for vegetative and floral characters, and forty six accessions were studied for fruit characters. The minimal descriptor developed in the study was utilised for characterization.

5.1.2.1 Sex form and tree characters

Among the accessions studied, there were two sex forms (monoecious and dioecious) and three types of trees namely gynoecious, androecious and monoecious. Dioecy exist in other perennial crops like garcinia and allspice. Family Myristicaceae is a primitive one and the genus *Myristica* is predominantly

dioecious. But, sex reversion as reported in papaya could be noticed in nutmeg too. Male trees if retained for a longer period may produce fruits although in limited numbers in the later years. It could be taken as a change during the course of natural evolution of the species. In this study, dioecy dominated over monoecy. Krishnamoorthy (2000) has identified five different types of trees *viz*., pure male, pure female, bisexual male, bisexual female and hermaphrodite in nutmeg. Very recently, existence of monoecious and dioecious sex forms in Kerala have been reported by Miniraj *et al*. (2012) and Sasikumar *et al*. (2015), and in Karnataka by Rema *et al*. (2015). An improved variety, Konkan Sugandha with bisexual flowers has been developed at Konkan Krishi Vidyapeeth, Dapoli, Maharashtra (Parthasarathy, 2010).

The branching pattern, leaf and leaf characters are the main components of the canopy that ultimately bears the load of fruits. In nutmeg too, these canopy components exhibited wide variation.

Among the three stem colours exhibited by the select accessions, grey was found to be the dominant trait. Based on the canopy shape, the fifty accessions were grouped into four *viz*., conical, pyramidal, oblong and globular (Fig. 5.1). Majority of the accessions had pyramidal canopy shape (64%) followed by globular (16%) and oblong (14%). Conical shape (6%) was exhibited by accessions 10, 23 and 24. Conical trees usually grow high with less horizontal spread. This helps in the accommodation of more number of plants per unit area. They have an added advantage of ornamental value and suit well to homesteads and high density planting systems.

Abundant foliage density was observed in majority of the evaluated accessions followed by intermediate and sparse foliage density. In nutmeg, canopy architecture is a very important factor, this is not only indicative of bearing capacity of the tree but also the spacing and population density per unit area.

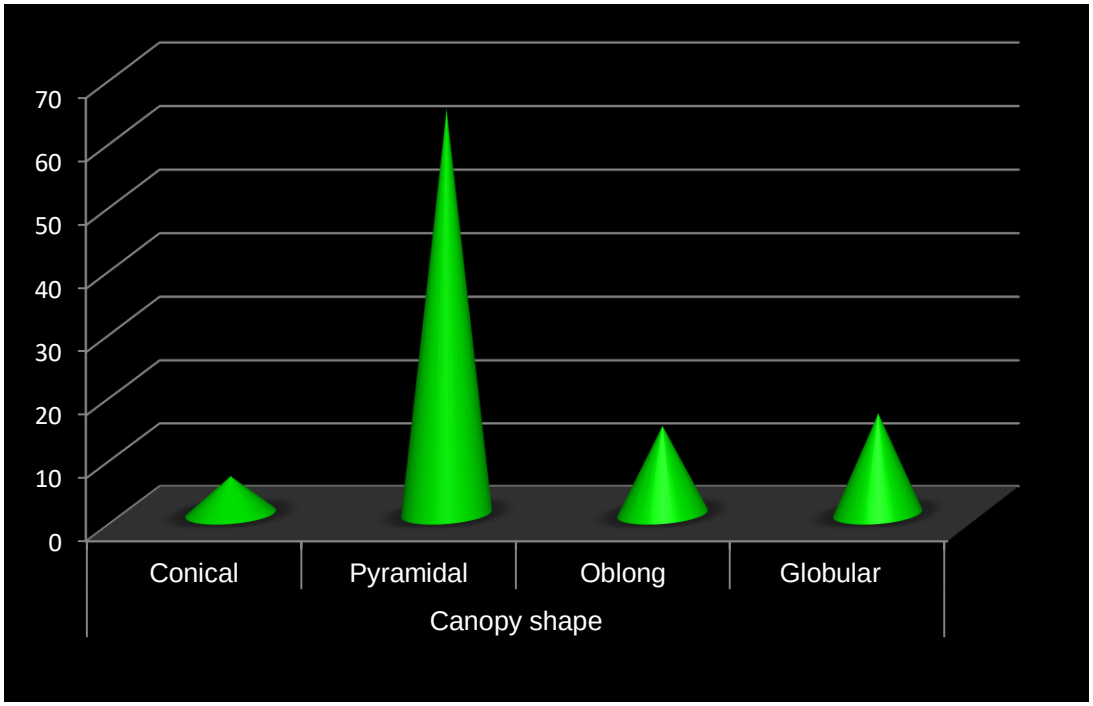


Fig. 5.1. Distribution of canopy shape among nutmeg accessions

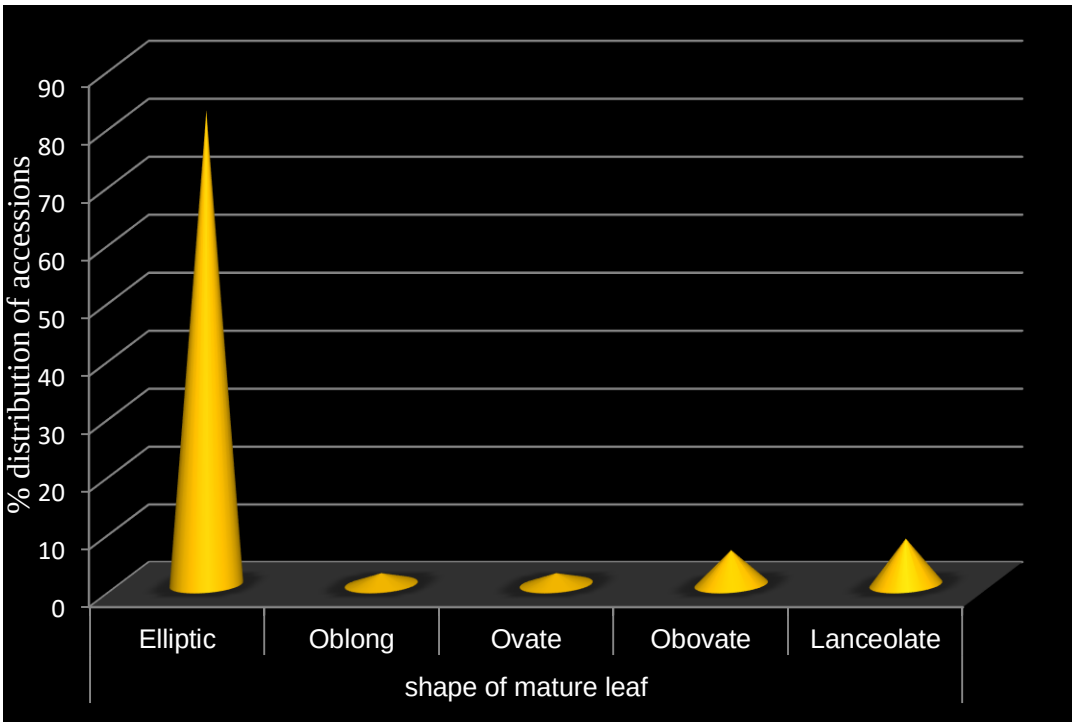


Fig. 5.2. Distribution of shape of mature leaf among nutmeg accessions

Branches are the skeletal structures of the tree. Two branching patterns were observed among the accessions studied, in which the spreading types dominated the over erect types. Nature of branching is also important in deciding the population density in utilising the horizontal as well as vertical space.

Nutmeg tree grows in flushes. There is a period of active growth followed by quiescence. Variation in flushing pattern was observed among accessions with mid flushing types having high frequency followed by early flushing ones. Late flushing was noticed in accessions 20 and 21. Predominant flush colour was either yellowish green or greenish yellow. The flushing pattern and colour of flushes are largely decided by genetic makeup of the tree, even though chances of environmental parameters playing a role cannot be ruled out as reported by Muthulakshmi (2003) in jack.

5.1.2.2 Leaf characters

Wide variation was noticed among the accessions for leaf characters. Nutmeg accessions differed in the visual morphology with respect to leaf shape, mature leaf colour and leaf apex. Elliptic leaf was noticed in 82 per cent of accessions followed by other shapes *viz*., lanceolate, obovate, ovate and oblong (Fig. 5.2). The accessions differed in having acuminate, acute and obtuse leaf apex. The obtuse leaf apex was rare and it was exhibited by Acc. 18 alone. The leaf shape and leaf apex are genetically controlled and hence, they could be good be taken as morphological markers. Among the accessions evaluated leaf colour varied from green to dark green.

5.1.2.3 Floral characters

Flowering time eventually decides the harvesting time in nutmeg. Flower characters are the key factors that lead to the total yield. In view of this, the observations on floral characters of different accessions were recoded (Table 4.4 and 4.5).

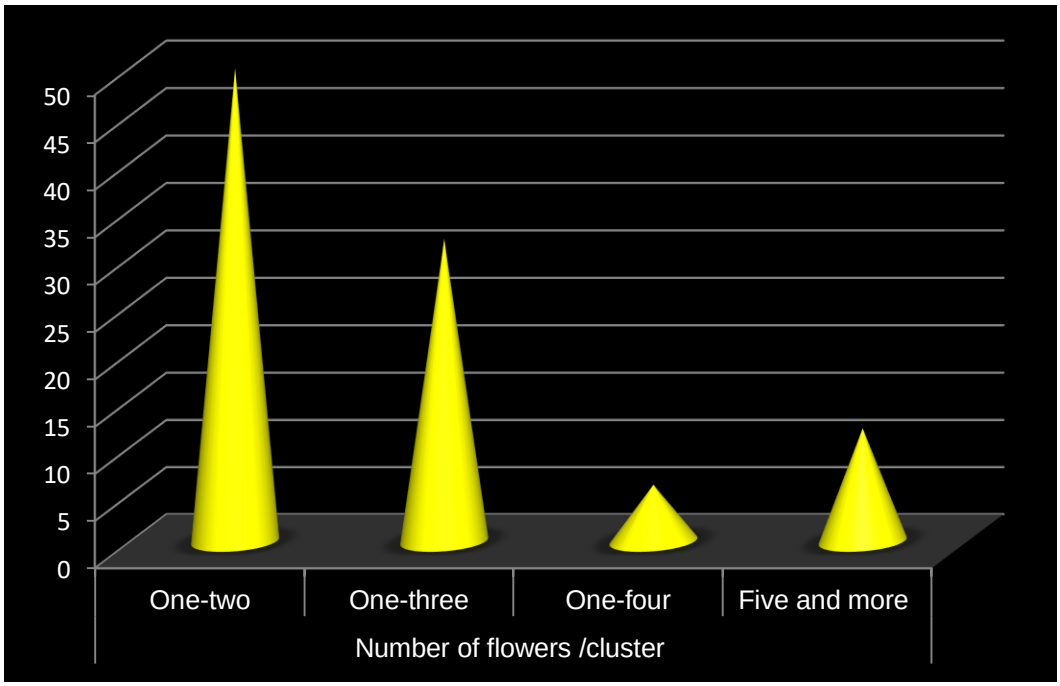


Fig. 5.3. Distribution of no. of flowers/cluster among nutmeg accessions

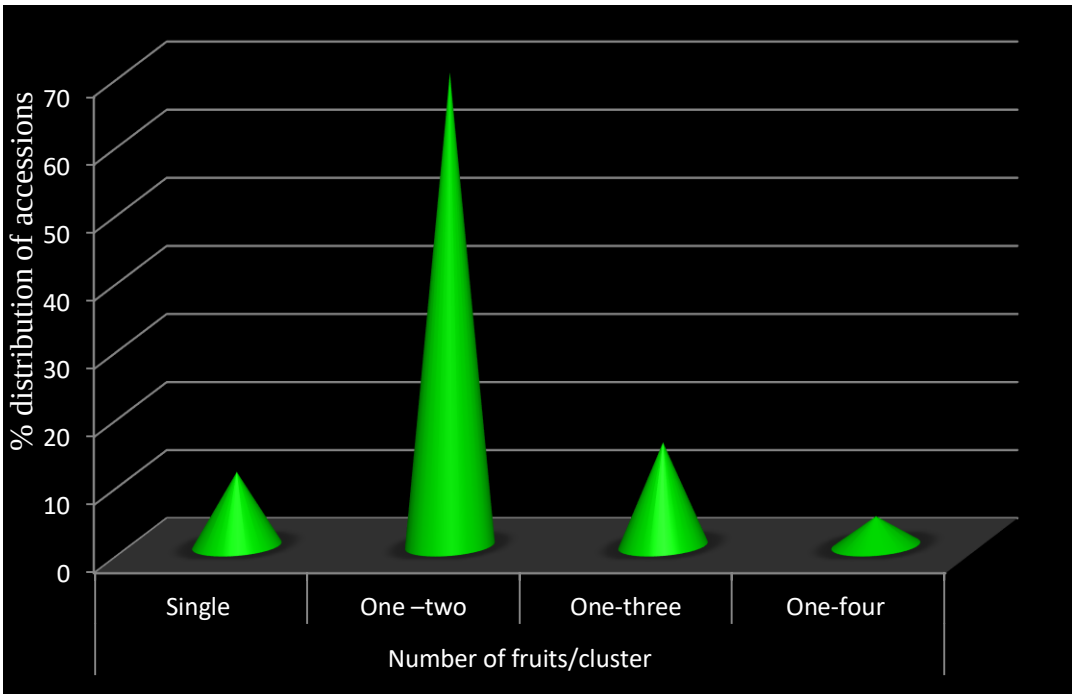


Fig. 5.4. Distribution of no. of fruits/cluster among nutmeg accessions

Flowering pattern of female and male nutmeg trees differ (Nazeem, 1979). Among the forty two female accessions, forty accessions exhibited seasonal flowering with three peaks during July-August, November and late January - February. Two accessions 8 and 22 exhibited both peak flowering during the aforesaid periods as well as lean flowering during the intervening period. All the monoecious and male accessions recorded flowering throughout the year. In an earlier study, Nazeem (1979) also reported round the year flowering of male nutmeg trees.

Variation in flowering season was observed among the accessions evaluated in the study. In the main flowering season, the accessions could be grouped as early flowering (July), mid flowering (late July to early August) and late flowering (late August) types. Early flowering was noted in female accessions 1 and 18. Early flowering leads to early fruiting and thus early harvest, avoiding the harvest during rainy period. Summer harvest has definite advantages in nutmeg, in getting uniform drying leading to best quality kernel and mace. Mango is another crop where varietal difference with respect to flowering is obvious. Simi (2006) observed frequent flowering and fruiting in some South Indian mango types.

Two types of inflorescences were observed in nutmeg, female tree bearing racemose inflorescence in axils of leaves and monoecious and male accessions having umbellate cyme inflorescence in axils of leaves with further continuation on branches. The type of inflorescence produced depends on the sex form of the tree. Earlier, Joseph (1980) also observed branched inflorescence in male trees and racemose inflorescence in female trees of nutmeg.

In female genotypes, the flowers were borne in the leaf axils either solitary or in small groups (1 to 3 flowers). Monoecious and male accessions were producing large number of flowers per cluster (five and more) when compared to the female accessions (Fig. 5.3). Haldankar *et al.* (2004) recorded one to three flowers per cluster in female nutmeg trees under Maharashtra conditions.

Interestingly, frequency of flower clusters in female trees was either profuse or intermediate in most of the accessions; whereas profuse frequency of flower clusters was noticed in monoecious and male trees. This clearly indicates that frequency of flower clusters vary according to genotypes as well as sex forms.

Very close observations were made on flower characters such as colour of pedicel, colour of perianth, shape of perianth, colour of filament and colour of anther. Four types of perianth colour *viz*., creamy colour (74%), creamy white, creamy yellow and greenish creamy were observed among the accession. Joseph (1980) characterized nutmeg flowers as drooping, creamy yellow, with slight fragrance. The shape of perianth was observed to be either bell shaped in as many as 98 per cent of accessions and cylindrical in only one male accession *i.e.* Acc (M) 3. All the female flowers had bell shaped perianth. Association between floral shape and floral colour showed no significant relationship. Nazeem and Nair (1981) observed bell shaped flower in female trees of nutmeg.

5.1.2.4 Fruit characters

Superiority of nutmeg genotypes mainly depends on fruit characters. Abundant variation was noticed for fruit characters among the forty six nutmeg accessions studied (Table 4.4 and 4.6).

The accessions showed wide variation with respect to number of fruits per cluster (Fig. 5.4). Majority of the accessions observed were routinely bearing one-two fruits in a cluster. This was followed by one to three and single fruit bearing types. The accessions 14 and 19 had up to four fruits per cluster. The frequency of fruit clusters per tree was either intermediate or profuse in majority of the accessions evaluated. With respect to density of bearing, intermediate bearing was noticed in fifty per cent of accessions followed by sparse and profuse bearing.

The immature fruit colour varied among the accessions as pale green, green-pale yellow and green. Pale green colour was present in majority of the

accessions. No generalisation could be made on the colour of the mature fruits. Mature fruit colour varied as yellow, yellow light green and light yellow.

High variability was noticed for fruit shape, in the present study. Four different fruit shapes were observed *viz*., round, oval, ovoid and pyriform (Fig. 5.5). Most of the accessions were having round fruits. Fruit shape is a genetic trait and could be used as morphological marker in nutmeg. Das *et al*. (2012) in North Moluccas identified oblong, oval and round shaped fruits in nutmeg. Thomas (2008) observed two types of fruit shape *viz*., pyriform and round shaped fruits under Kerala conditions. With respect to fruit base and fruit apex also variation was noticed among the accessions. Round or pointed fruit base and round, obtuse or acute fruit apex was noticed in the different accessions.

On ripening, nutmeg fruit splits on the tree itself exposing the aril and seed. Full and partial fruit splitting was observed among the accessions. All the accessions except Acc. 5 exhibited the full fruit splitting. Only in Acc. 5 partial fruit splitting was observed. Fruits with full splitting are desirable because in such types, early dehiscence of fruit occur and easy harvest is possible. In fruits with partial splitting, on the other hand rain water gets stagnated which can lead to delayed dehiscence, and mould growth inside the fruit leading to quality loss and damage of fruits.

Ripened nutmeg fruits usually split into two equal halves. In the present study also, all the female accessions had fruits splitting into equal halves. In Acc. (H) 1 and (H) 4, fruit splits into three and four halves respectively. In the present study, all the female trees had single seed per fruit. Two monoecious accessions (Acc. (H) 1 and (H) 4) were found to have double seeds in a fruit. It could be concluded that double seededness and multiple splitting are generally associated with monoecious types. Occurrence of double seeded nutmeg type has been reported from central parts of Kerala by Miniraj *et al*. (2015).

The bearing is seasonal in nutmeg. In the present study, staggered fruiting round the year was recorded in two female accessions (Acc. 8 and 22) and in one

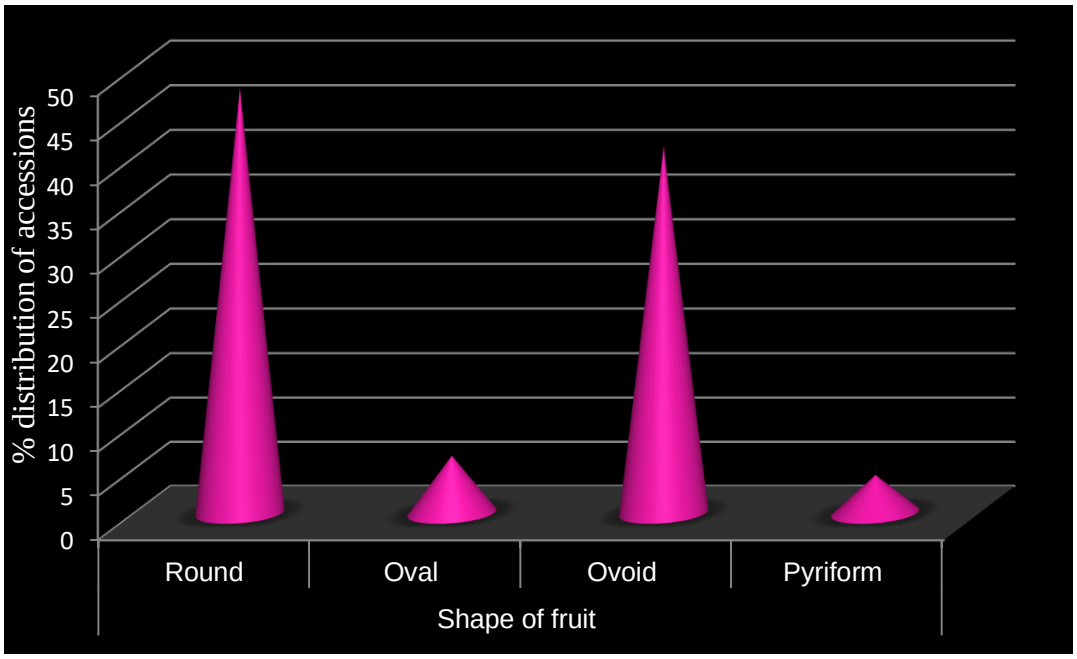


Fig. 5.5. Distribution of shape of fruit among nutmeg accessions

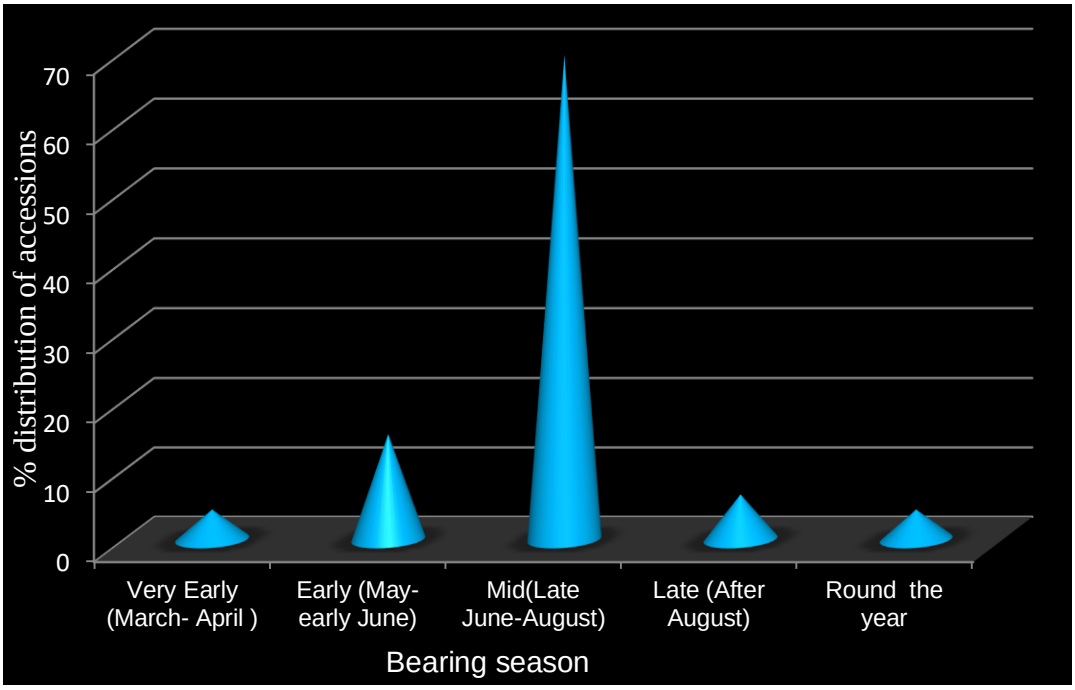


Fig. 5.6. Distribution of bearing season among nutmeg accessions

monoecious tree (Acc. (H) 1). In all other accessions there was peak fruiting during June to August (Fig. 5.6).

Even though three peak flowerings were observed in majority of the accessions, maximum fruit set was observed in the first peak, which resulted in peak fruiting during June to August. It could also be seen that the accessions which exhibited staggered flowering had staggered fruiting. In crop like mango, off season bearing is of economical importance (Naik, 1963). But in nutmeg, it is not desirable due to involvement of high cost of labour in harvesting as well as processing. The fruits of the secondary bearing are under sized than main season fruiting and may not match the quality of the main season fruits.

The peak harvesting season of nutmeg is from June to August in Kerala (Anandraj *et al*., 2005). In the present study, different bearing seasons could be noticed among the accessions *viz*., very early bearing, early bearing, mid bearing, late bearing and round the year bearing. Very early as also early bearing are advantageous as rainy season harvesting could be avoided. This also helps in easy drying and reduction in fungal load in the processed produce, thus avoiding the chance of aflatoxins during storage.

Various immature fruit colours were observed among the accessions namely; pale green, green-pale yellow and green, of which pale green was the most dominant. In mature fruits, yellow, yellow light green and light yellow colour could be observed.

5.1.2.5 Mace and nut characters

The most interesting economic parts in nutmeg are the nut and mace. The nut and mace characters also exhibited high variation. Physical properties of nut and mace are having practical significance in determining yield and quality of the crop (Omokhafe and Alika, 2004).

Three types of mace and nut shapes observed in the present study were oval, round and oblong. Majority of the accessions exhibited oval shape, followed by round and oblong ones.

The fresh mace colour varied from deep red, red to orange red, which upon drying turned to red, scarlet red and orange red respectively. On drying mace of most of the accessions changed to scarlet red colour. Scarlet red is the most predominant colour in nutmeg all over the world. Mace colour is a trait contributed by both heredity and environment. In a crop like nutmeg wherein the market value is decided by the colour and nature of mace, these traits assumes importance in the selection of accessions. Variations in mace colour with other unique characters are reported from Kerala. Yellow mace types have been reported by Rahul *et al.* (2014) and Miniraj *et al.* (2015). Senthilkumar *et al.* (2010) reported deep red and rose coloured mace from the nutmeg growing tracts of Karnataka.

Fresh nut colour was either shining black or dark brown in majority of the accessions, which upon drying turned dark brown. Nut is sold usually in the fresh form, where shining black is the most preferred colour. As in the case of mace, the external appearance could get special advantage in fresh nut marketing.

Nature of mace could be classified into four groups namely, entire, slightly dissected, intermediate and highly dissected. Most of the accessions had slightly dissected mace whereas accessions 14 and 38 possessed entire mace. It could be noted that accessions with entire mace recorded higher mace weight also. Even though thick mace is difficult to dry, maximum recovery of flower grade or first quality mace with high dry weight is obtained from this category, this makes up an ideal trait in the selection of super trees.

Another unique feature noticed among the accessions was beakness of mace which was exhibited only in Acc. 39. Mace attachment to nut was compact in most of the accessions and very few had loosely attached mace. Loose

attachment of mace to nut helps in easy separation and recovery of unbroken mace, which again help to fetch higher market price.

Shallow grooved or pronounced grooved nuts were produced by equal per cent of accessions. But, when it comes to kernel, it was mostly shallow grooved or smooth kernel without any grooves. Grooveness is also a genetic trait which has definite bearing on final appearance of the produce. Smooth and shallow grooveness are positive traits as it aids in uniform drying and prevent the build up of moulds during storage. Colour of dried kernel was grey in majority of accessions and light brown kernels could be seen in accessions 36 and (H) 1.

5.1.3 Cluster analysis based on qualitative characters

Cluster analysis based on forty seven qualitative characters revealed that all the forty six accessions including female and monoecious fell into 11 clusters at 66 per cent similarity.

Among the 11 clusters, Cluster IV was the largest one, including nearly 43 per cent of accessions. All members of this cluster belonged to dioecious sex form, possessing identical and close values in growth parameters, flowering, fruiting pattern and fruit, nut and mace characters. Acc. 15, Acc. 20, Acc. 28, Acc.31, Acc. 32, Acc. (H) 2 and Acc. (H) 3 were grouped in cluster VII. In this cluster, five accessions were dioecious and remaining two were monoecious. These accessions were similar in growth, fruiting pattern and mace characters.

Accessions in cluster V were dioecious with similar branching pattern, canopy shape, fruit and nut characters. Cluster VI members were not identical in tree characters but showing identical sex form, floral and fruit characters.

From the dendrogram shown in Fig. 4.1, it can be seen that the Acc. 9 and 27 belonging to cluster II were exactly identical. Similarly, accessions 2 and 33 falling in the same qualitative cluster were also identical. The solitary accessions included in clusters I, VIII, X and XI possessed qualitative traits distinct from all other accessions.

5.1.4 Quantitative evaluation

The select population of forty two female, four monoecious and four male accessions evaluated for thirty eight quantitative parameters differed significantly except for shelling percentage.

5.1.4.1 Growth parameters

Tree height an important parameter having profound influence on yield. Significant variation was observed for height among the accessions. Tree height varied from 3.20 m to 12.35 m. Accessions such as Acc. 21, Acc. 42, Acc. 16 and Acc. 10 were very tall. Tree girth ranged from 20.33 cm to 63.51 cm.

Tree canopy is another determining factor in the growth of any crop. Significant difference was observed among the accessions for canopy spread in both E-W and N-S directions. Canopy spread E-W varied from 3.11 m (Acc. 28) to 9.02 m (Acc. 42). There are reports in nutmeg which states that percentage of fruit set varied with different aspects. Highest fruit set in trees was on western and eastern aspects primarily due to optimal exposures to sunlight (Nazeem and Nair, 1981). Canopy spread N-S ranged from 3.11 m to 9.30 m.

The internodal length of accessions recorded revealed widely spaced, and closely spaced branching pattern of the accessions. Internodal space between the branches assumes importance as it decides the light penetration, air movement, production of orthotrops, spread of diseases *etc*.

Nutmeg tree exhibits dimorphic growth pattern. The accessions varied with respect to the number of orthotrops, ranging from 1 to 29. Only orthotropic scion shoots are recommended for vegetative propagation in nutmeg. Hence, eliteness for economic characters coupled with production of more number of orthotrops are desirable for rapid multiplication of super trees as reported by Haldankar *et al.* (2013).

Leaf area is one of the parameters which influences the photosynthesis and any factor influencing this trait ultimately influence the biomass out put including

yield. In the present study, highest leaf length was recorded in Acc. 37 and lowest was in Acc. 4. Widest leaf was observed in Acc. (H) 4 and narrowest in Acc. 7. Highest leaf area was observed in Acc. (H) 4 and Acc. 39, whereas smallest leaf area was observed in Acc. 7. Both genetic as well as phenotypic elements have role in determining leaf size. Occurrence of varied leaf characters in nutmeg was reported by Krishnamoorthy *et al.* (1996) and Senthilkumar *et al.* (2010) from nutmeg growing tracts of Kerala and Karnataka respectively.

Among the accessions evaluated, highest chlorophyll content was recorded in Acc. 27 followed by accessions 9, 5 and 36. Lowest chlorophyll content was recorded in Acc. 37. High chlorophyll content contributed to dark green leaves and lower chlorophyll content was associated with light green leaves.

5.1.4.2 Flower characters and fruit set

Inflorescence characters studied included number of flowers per 10 cm^2, length of flower, breadth of flower, length of tepal, breadth of tepal, length of pistil, breadth of pistil and fruit set (Table 4.10). Significant variation was observed for flowers per 10 cm^2. In female accessions, number of female flowers per 10 cm^2 varied from 2.50 to 6.75. In monoecious accessions, number of flowers per 10 cm^2 included female as well as male flowers and their total number ranged from 5.75 to 10.25. In male accessions, number of male flower per 10 cm^2 varied from 8.0 to 14.0. Since monoecious and male trees bear male flowers in clusters, it is quite natural that normal flower counts are very high compared to female trees.

The variation observed was only marginal as regards to length of flower, breadth of flower, length of tepal, breadth of tepal, length of pistil and breadth of pistil.

Earlier, Nazeem and Nair (1981) reported that fruit set percentage in nutmeg varied from 29 to 41.60 per cent among the trees. But in the present study, a higher percentage of fruit set was recorded. The lowest fruit set noted was 6.45 per cent in Acc. 28 and the highest noted was 44.15 per cent in Acc. 8. Sangadji *et*

al. (2015) reported a fruit set of 22.63 to 47.53 per cent from Moluccas Islands, the natural home of nutmeg. Haldankar *et al.* (2004) reported varying degree of fruit set in nutmeg, ranging from 2 to 41 per cent under Maharashtra conditions.

5.1.4.3 Fruit parameters

The variability noticed in fruit characters among the accessions was also reflected in the yield of nutmeg accessions. Wide variability was noticed in biometric characters of fruits including fruit weight, fruit length, fruit breadth and thickness of pericarp (Table 4.11). Weight of fruit varied between 39.33 g to 99.56 g. Similar variation was noticed in fruit volume also. Length of fruits varied from 42.11 mm to 66.25 mm, breadth from 35.19 mm to 57.43 mm and thickness of pericarp from 0.8 mm to 1.42 mm. Senthilkumar *et al.* (2010) reported wide range in fruit characters of nutmeg under Karnataka conditions. An elite nutmeg should have only medium fruit size with thin pericarp, bold nut and entire mace.

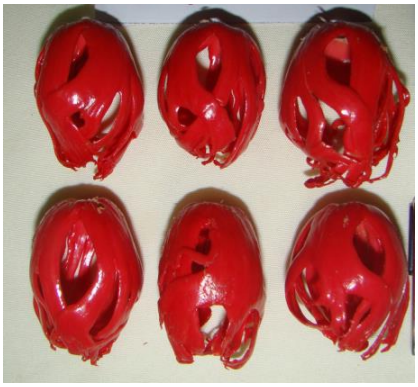
The accessions in the present study showed significant variation in the number of fruits per m^2, which depict the density of fruit bearing of the tree. Higher fruit density indicates the yield potential of the tree. In the present study, it ranged from 4.00 to 31.50. Accessions, 22, 23, 8, 21 and 32 recorded higher number of fruits per m^2.

In the present study, fresh mace weight varied from 0.91 g to 5.27 g. The dried mace weight ranged from 0.46 g to 2.61 g.

Fresh nut weight ranged from 4.41 g to 13.67 g. Upon drying, the weight of nut ranged from 3.56 g to 11.01 g. The lowest values for fresh nut weight, dried nut weight, fruit volume, shell thickness and kernel weight was noticed in Acc. (H) 4. The single kernel weight ranged from 2.65 g to 8.04 g. The accessions with high mace and kernel weight are promising as they contribute much to the quality and grade of final produce.

The variation noticed was high as regards to volume of mace; nut as well as kernel (Table 4.11). Biometric characters of nut studied, included length and

Thin and small mace



Thick mace

Small nut

Bold nut

Small kernel

Bold kernel

Plate 4.21. Variability in mace, nut and kernel

breadth, significant variation was noticed among the accessions, except in the case of shelling percentage.

5.1.5 Yield and number of fruits per tree

Fruit yield in nutmeg is a complex phenomenon. Fruit yield per tree is the targeted quantitative parameter which is dependent on several yield related parameters. All the inherent yield related characteristics, at a given point of time, associate with one or another to eventually decide the yield of the tree. The resultant fruit yield is reported to vary with individual genotype or variety.

Number of fruits per tree was recorded for two consecutive years, 2013 and 2014. Accessions *viz*., Acc. 9, Acc. 8, Acc. 22, Acc. 21 Acc. 18 and Acc. 12 recorded significantly higher values of more than 3000 fruits per tree, during 2013. During 2014 harvest, accessions 9, 8, 22 repeated the same pattern of more than 3000 fruits per tree. The pooled means of number of fruits per tree for two consecutive years put accessions 9, 8 and 22 in the high bearing category of more than 3000 fruits per tree per year. The variation in yield could be due to inherent genetic make up, seasonal weather parameters and management practices. In nutmeg, yield recorded over a longer period is more reliable than single records at early stages. A full bearing tree producing 3000 fruit per year along with other economic characters is considered as a high yielder as reported by Miniraj *et al.* (2015). Haldankar *et al*. (2003) studied the consistency of yield in nutmeg genotypes for six years under Konkan region of Maharashtra and observed variation due to genotypes as well as environmental factors.

In the present study, accessions 9 and 14 recorded significantly higher values of fresh as well as dry mace yield per tree per year. Accessions 9, 22 and 8 were promising as they recorded significantly superior values for fresh and dry nut yield per tree per year as also kernel yield. Considering the economic parameters together accessions 9, 8 and 22 were rated as promising types (Plate 5.1, 5.2 & 5.3).

Plate 5.1. Promising accession 9 (Inset: bearing branch, mace, and nut)

Plate 5.2. Promising accession 8 (Inset: bearing branch, mace, and nut)

Plate 5.3. Promising accession 22 (Inset: bearing branch, mace, and nut)

5.1.6 Clustering based on quantitative characters

In the process of genetic improvement of any crop, genetic diversity among germplasm plays a major role, since it opens the way to determine the most divergent parents based on the contribution of different traits, for further utilization in any hybridization programme. Extent and magnitude of genetic divergence were determined for the purpose of identifying the more diverse parents (Pande and Singh, 2011).

In the present study, D^2 analysis was carried out using 26 characters. The presence of high variability among the accessions studied for different characters were further confirmed through the pattern of distribution of 46 nutmeg accessions in ten clusters. Cluster I with ten accessions formed the largest group. Cluster II, III and IV were having six accessions each. Cluster VI had five accessions. Cluster VII included four accessions and cluster IX with three accessions. Cluster V, VIII and X had two accessions each with identical quantitative characters.

The maximum inter cluster distance was observed between clusters V and III, which indicated maximum divergence between these two clusters. Members of these two clusters could be used for hybridization programme for obtaining a broad spectrum of variability for the genetic improvement of nutmeg. The lowest inter cluster distance was observed between clusters II and I. This indicates the close genetic association of the accessions belonging to these clusters. Haldankar *et al.* (2007) studied the genetic divergence using 34 nutmeg genotypes and grouped them into 12 clusters based on the quantitative characters.

5.1.7 Genetic variability studies

The degree and extent of genetic variability in nutmeg accessions was worth investigating. Analysis of variance for different characters under study revealed that the accession effects were highly significant suggesting existence of high genetic variability in the population. Higher the variability in the genetic resources, better will be the options for meaningful and planned breeding

programmes. The results showed that all the 26 genetic characters exhibited significant difference indicating the presence of sufficient genetic variability among nutmeg accessions (Table 4.13). Among the characters, number of fruits per tree, number of fruits per meter square, fruit set percentage, dry and fresh weights of mace, mace volume and ratio of nut to mace exhibited the high values for PCV and GCV. All the characters recorded higher values for heritability. Highest heritability was recorded for the characters such as fruit set percentage and fruit weight. All the characters except shell thickness and fruit length exhibited high genetic gain, whereas these two characters showed moderate genetic gain. Among the traits, number of fruits per tree (161.28), fruit set percentage (93.36), number of fruits per meter square (84.99), fresh mace weight (77.29), dry mace weight (72.37), ratio of nut to mace (65.92), mace volume (62.97), fruit weight (45.04) and kernel weight (44.17) showed higher genetic gain coupled with higher heritability, thereby indicating their importance in selection. Selection based on these characters can bring about the enhancement of the genetic strength of the accessions. High amount of variability for growth, fruit and yield related characters in nutmeg were reported by Krishnamoorthy *et al.* (1996) and Shinde *et al.* (2006). Parthasarathy *et al.* (2010) opined that optimum number of fruits and moderately good seed weight are the two traits that offer great scope for selection in nutmeg.

The number of flowers per 10 cm^2 and fresh and dry weights of mace exhibited highly positively skewed distribution and kurtosis. Ratio of nut to mace as well as number of fruits per tree also showed highly positive skewed distribution with no kurtosis. Moderately positive skewness and kurtosis were observed in shell thickness. Leaf area recorded moderately positive skewness with no kurtotic distribution. Moderately negative skewness with no kurtotic distribution was recorded in characters *viz*., fruit length, fruit breadth, nut volume, kernel volume and nut length.

In the light of above discussions based on third degree statistics it could be noted that majority of characters possessed positively skewed distribution and

were controlled by additive gene action with complementary epistasis. The traits *viz*., plant girth, fruit length, fruit breadth, fresh nut weight, fruit volume, nut volume, kernel volume and nut length showed additive gene action with duplicate epistasis. Similarly, majority of the characters possessed leptokurtic (positive) graphic distribution and were by controlled little genes. Characters such as plant girth, fruit set percentage, number of fruits per m^2, fruit weight, thickness of pericarp, kernel weight, mace volume and nut length showed platykurtic (negative) graphic distribution and were under the influence of many genes. For all the genetic parameters, prediction of gene action was made as per suggestion given by Roy (2000). Similar results were reported by Shobha *et al.* (2013) in cashew and Sihaloho *et al.* (2014) in soyabean.

5.1.8 Association studies

Yield is a complex entity associated with a number of component characters. All the changes in yield must be accompanied by the changes in one or more component characters. This is due to varying degrees of positive and negative correlations between yield and its components, and among components themselves. A study of association of these characters helps in selection of genotypes and also suggests the advantage of a selection scheme for more than one character at a time. Improvement of one character results in the simultaneous improvement of all positively related characters (Table 4.19 and 4.20).

The above discussions on inter correlations can be summarized as follows. As regards nutmeg a robust tree should have all the tree characters as high as possible with the exception that the tree height should be only in proportion to tree girth. It is the spread of the canopy that is ultimately decisive of the floral, fruit and yield characteristics. The leaf area is found to have a negative influence on fresh and dry nut weight, kernel weight, nut length and nut volume (Appendix I & Appendix II).

The very characterization of fruit setting orientation of flowers and fruit set is linked with the exterior side of canopy. A higher leaf area may shield

negatively towards the above phenomenon. A modern way of pruning of intermediary branches may be thought of. As regards yield characters, especially the fruit characters, the inter correlations existing is a normal phenomenon beyond discussion. The findings of Krishnamoorthy *et al.* (1991) in nutmeg have brought out significant and positive correlation of fruit number with mace weight, fruit weight with seed weight and mace weight and seed weight with mace weight.

5.1.9 Path coefficient analysis

The correlation coefficients indicate only the quantum and nature of association between yield and its attributes, but does not show the direct and indirect effects of different yield attributes which are mutually associated. This in turn will impair the true association existing between a component and yield and a change in any one of these component is likely to disturb the whole network of cause and effect. Thus, each component has two paths of action *viz.*, direct influence on yield and indirect effects through components which are not revealed from the correlation studies. In this context, the path analysis was carried out to provide an effective measure of direct and indirect causes of association and to depict the relative importance of each factor involved.

Among the traits subjected to path analysis, fruit weight, mace volume and thickness of pericarp exerted very high genotypic positive direct effect on number of fruits per tree. Kernel volume, dry weight of mace and fruit set percentage showed high direct effect on number of fruits per tree and very high negative direct effect was exerted by fresh mace weight, nut volume and fruit volume. Canopy spread in E-W, fruit breadth and fruit length showed high negative effect (Appendix III). This is in conformity with earlier studies by Thondaiman and Rajamani (2014) in cocoa. The residual effect was 0.011 in the present path analysis suggesting that the number of characters considered for path analysis was appropriate. This finding, again strongly confirms the reliability of the characters *viz*., fruit weight, mace volume, thickness of pericarp, kernel volume, dry weight

of mace, fruit set percentage and number of fruits per tree towards selection of superior and high yielding genotypes.

5.2 BIOCHEMICAL CHARACTERIZATION

Remarkable variations in the biochemical constitution of the kernel, mace and pericarp of nutmeg were observed among the accessions evaluated in the study.

5.2.1 Contents of volatile oil, oleoresin and fixed oil

The principal constituents of nutmeg are volatile oil, oleoresin and fixed oil. The percentages of constituents of nut and mace differed within as also between the accessions. Quality and quantity of constituents depend on growing environment, time of harvest, method of extraction and genetic make up of the respective accessions (Leela, 2008).

In the present study, volatile oil content in kernel ranged from 1.57 to 7.67 per cent. Accession 5, 7 and 8 had kernel volatile oil on par with Acc. 34. Similarly, mace volatile oil content ranged between 2.05 and 9.33 per cent. Among the accessions, Acc.34, 5, 6, 8, 9, 22, (H) 1 were found rich in both the volatile oils. Abdurrasheed and Janardanan (2009) reported the average content of volatile oil in kernel and mace collected from Kannur, Kerala as 8.7 and 15.8 per cent respectively. Maya *et al*. (2004) had reported that, the volatile oil content in nutmeg from South India ranged from 3.9 to 16.5 per cent, whereas in mace it varied from 6.0 to 26.1 per cent.

Oleoresin content in kernel varied from 18.59 to 36.20 per cent. Higher recovery of kernel oleoresin was recorded in Acc. 40. In mace, oleoresin content varied from 11.38 to 31.66 per cent. Maximum recovery of mace oleoresin was recorded in Acc. 4. In all the accessions, content of oleoresin was maximum in kernel compared to mace. In Acc. 4, both the kernel and mace, oleoresin contents were high. Naveen (2013) observed 22.09 to 29.09 per cent oleoresin in nutmeg under different processing methods. Fixed oil content in the kernel of accessions

varied from 17.79 to 44.80 per cent. Higher recovery of fixed oil was recorded in Acc. 22, which was closely followed by accession 38, 37, 6 and 7. A fixed oil content of 33.80 per cent was recorded in nutmeg collected from northern Kerala (Abdurrasheed and Janardanan, 2009).

5.2.2 Clustering based on quality characters (volatile oil, oleoresin and fixed oil)

Wide genetic variability was observed in the contents of volatile oil, oleoresin and fixed oil and the accessions fell into twenty six clusters. Cluster I was the largest one with six accessions. All the accessions in cluster I were moderate yielders of oil, oleoresin and fixed oil. Cluster II and VIII included four accessions each.

Accessions in cluster II yielded same quantity of oleoresin but varied in volatile oil and fixed oil yield. Cluster VIII members recorded higher mace oil and fixed oil but varied in oleoresin yield. Majority of the accessions remained as monotypic clusters indicating the diversity among them for volatile oil, oleoresin and fixed oil contents.

5.3 DISEASES OF NUTMEG

Nutmeg is affected by several diseases. During the course of field evaluation, the most important and emerging disease, *Phytopthora* leaf fall was noticed in six accessions. Highest incidence was recorded in Acc. 18 followed by Acc. 1. Since these two accessions were early bearing types, fruits were relatively unaffected. Other accessions showed field tolerance to *Phytopthora* leaf fall. Mathew and Beena (2012), Mathew and Miniraj (2013) and Sumbula (2015) have reported occurrence of *Phytopthora* leaf fall of nutmeg in Kerala. Field tolerance to *Phytopthora* leaf fall and other fungal diseases is a highly preferred character in nutmeg both in selection as well as hybridization programme. Incidence of *Colletotrichum* leaf spot was noticed in all the accessions with Acc. 37 and Acc. 18 recorded medium incidence. Incidence of *Colletotrichum* leaf spot was found maximum in Acc. 37 followed by Acc. 18. Fruit rot incidence was observed

medium in Acc. 9 and Acc. 18 and other accessions could escape from fruit rot. Thread blight was moderately high in Acc. 17 and Acc. 28 and other accessions were free from thread blight. Acc. 10 and Acc. 35 recorded moderately high incidence of dieback.

5.4 SELECTION OF DISTINCT ACCESSIONS BASED ON MORPHOLOGICAL CHARACTERS

Seventeen accessions were selected from the initial forty six accessions, based on distinctive characteristic features ensuring adequate representation of all the quantitative clusters. These seventeen distinctly featured accessions only were further employed for biochemical analysis of pericarp, chemoprofiling and molecular characterization.

5.4.1 Chemoprofiling based on kernel and mace volatiles

The volatile oil present in the kernel and mace is responsible for the flavour and distinct aroma of nutmeg. Thus, the volatile oil composition determines the quality of nutmeg. In the present study, volatile oil profile of kernel and mace indicated great variability with regard to volatile constituents. Variation in per cent composition of constituents may be due to different and distinct genotypes. Based on the content of major constituents *viz*., myristicin, elemicin, safrole, sabinene, alpha-pinene, beta-pinene, L-4-terpene and gamma-terpene, the seventeen accessions could be classified into three groups as high, medium and low (Table 4.29).

Volatile oil composition of seventeen kernel and mace samples had twenty and twenty four constituents respectively. Total volatile oil constituents in kernel ranged from 68.96 to 90.49 per cent and in mace from 83.86 to 93.89 per cent (Fig. 5.7). The major compounds in the oil were myristicin, elemicin, sabinene and safrole. Apart from these compounds, accessions also contained higher percentages of alpha-pinene, beta-pinene and L-4-terpineol in kernel and mace oil.

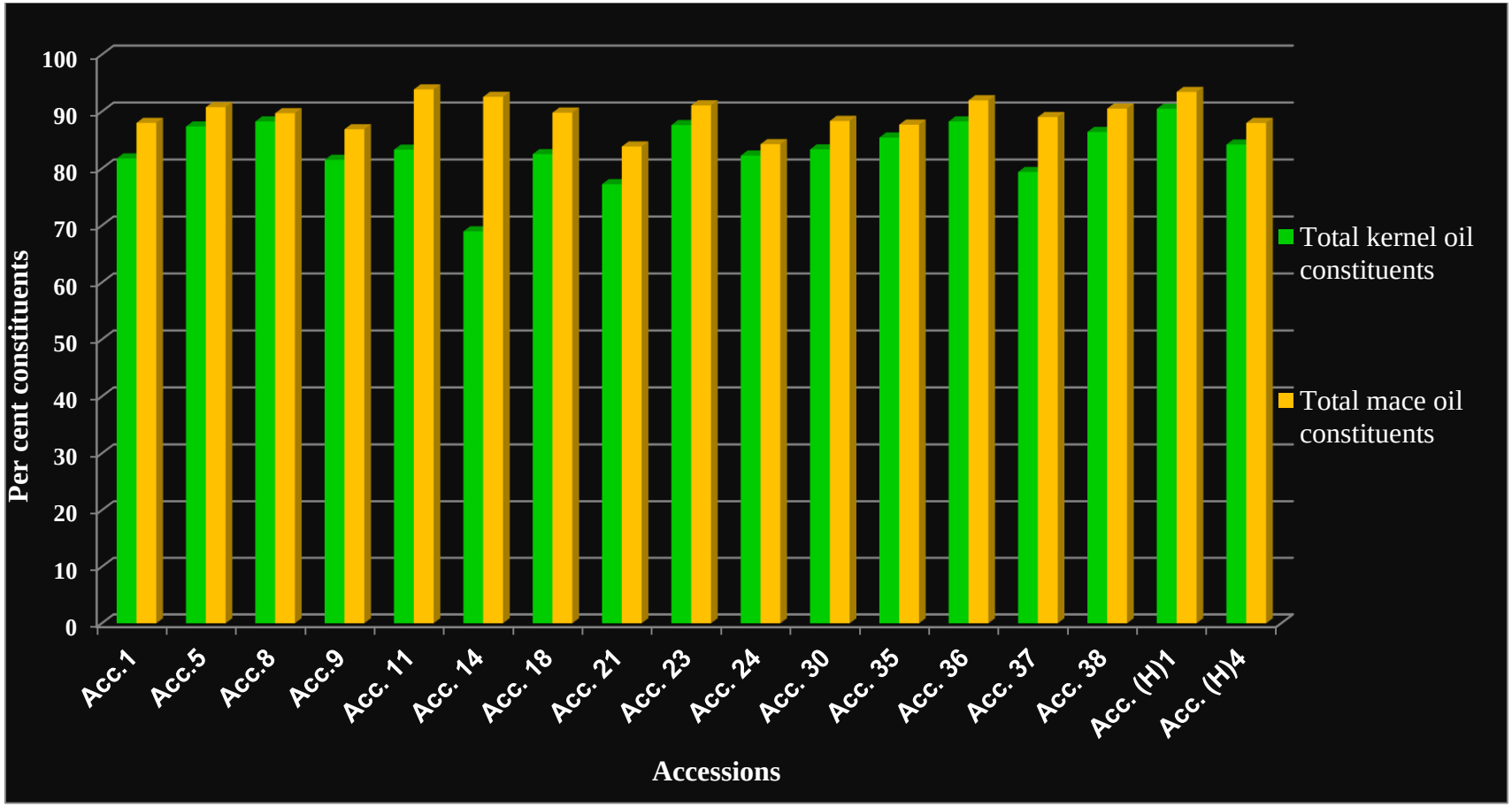


Fig. 5.7. Total constituents in volatile oil of kernel and mace

Chirathaworn *et al.* (2007) identified myristicin, eugenol, isoeugenol and elemacin as the major bioactive compounds in nutmeg oil.

Myristicin content ranged from 2.98 to 12.84 per cent in kernel oil and 1.57 to 18.87 per cent in mace oil. Elemacin content ranged from 4.31 to 22.48 per cent in kernel oil and 1.39 to 27.86 per cent in mace oil. Though, both myristicin and elemicin are synthesized separately in the plant (Khosla and Bhasin, 2000), it is interesting to observe that monoecious accession, Acc. (H) 1 had significantly higher myristicin and elemicin in both kernel as well as mace oil (Fig. 5.8).

Safrole content ranged from 2.44 to 4.64 per cent in kernel oil and 2.96 to 4.89 per cent in mace oil. Sabinene content ranged from 1.06 to 11.75 per cent in kernel oil and 4.89 to 16.20 per cent in mace oil. Accession 8 had significantly highest sabinene in both kernel and mace oil (Fig. 5.9). One notable difference between the kernel oil and mace oil was that, kernel oil contained higher level of L-4-terpineol, whereas it was low to medium in mace oil.

Based on the per cent content of major volatiles in the oils, the select accessions were categorised into low, medium and high groups. Accessions 5 and (H) 1 were high myristicin as well as high elemicin containing chemotypes. Accessions 9 and 36 were in the low myristicin group. Acc. 9 was also low in elemicin along with Acc. 35. High sabinene combined with low myristicin was the intrinsic quality attribute of accession 9 (Fig. 5.10 & Fig. 5.11). Based on the relative content of these major volatile constituents in the kernel and mace oils; the accessions can be utilised for food or pharmacological applications. Myristicin, elemicin and safrole are hallucinogenic compounds in nutmeg oil whereas, sabinene imparts sweetness to the products (Varghese, 2001). Findings of the present study has brought out high as also low hallucinogen containing accessions along with high sabinene containing accessions. Higher content of myristicin, elemicin and safrole is preferred by the pharmaceutical sector while sweet nutmegs with high sabinene content is preferred in the food sector. Maya *et*

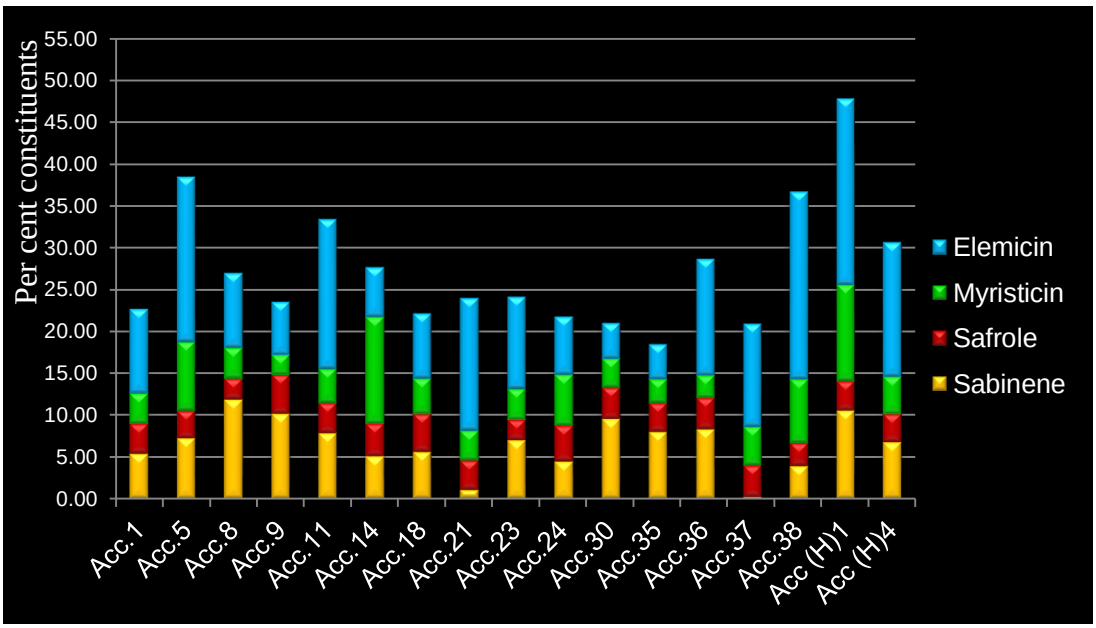


Fig. 5.8. Major volatile oil constituents of kernel in select nutmeg accesions

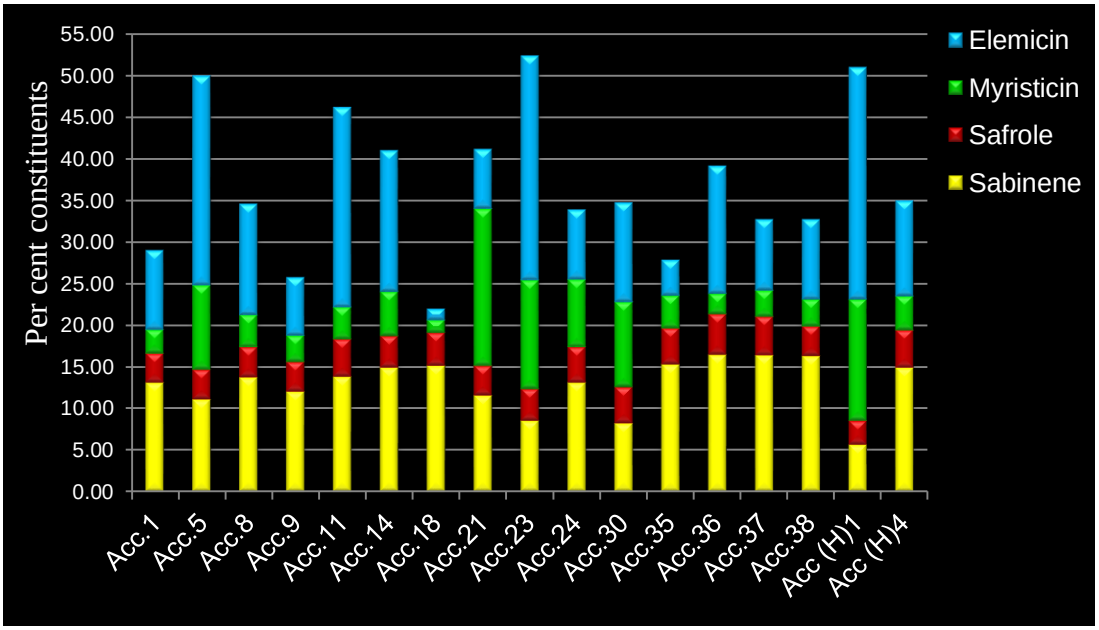


Fig. 5.9. Major volatile oil constituents of mace in select nutmeg accesions

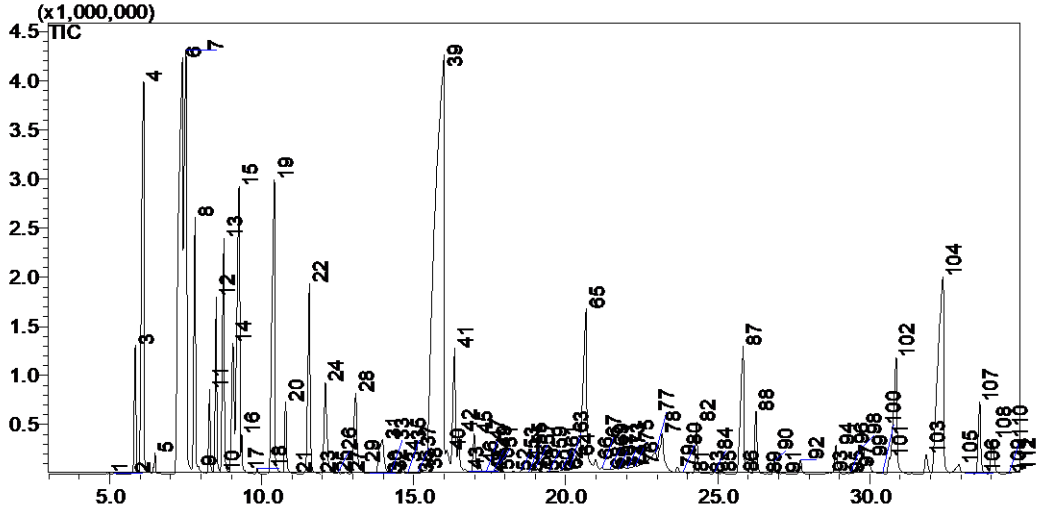


Fig. 5.10. GC-MS profile of kernel volatile oil of accession 9

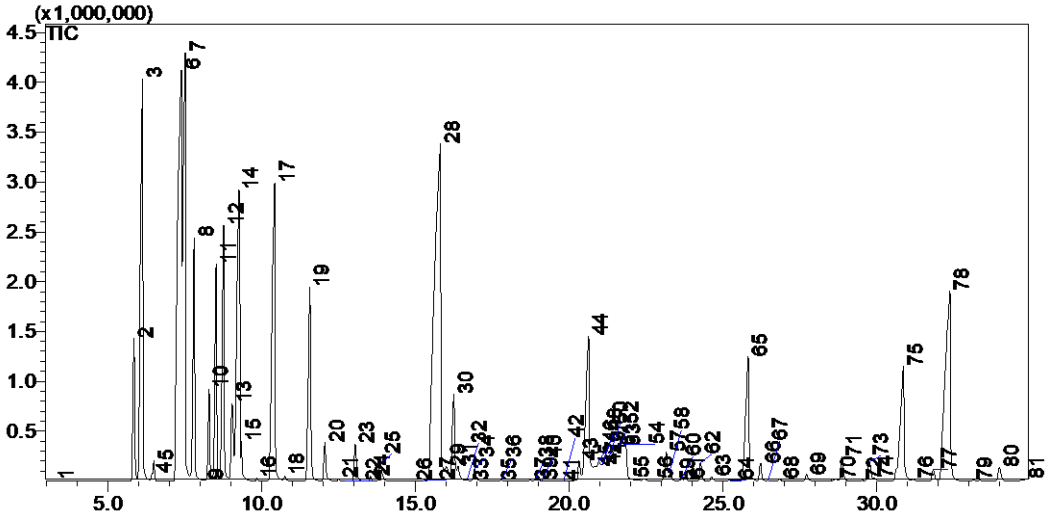


Fig. 5.11. GC-MS profile of mace volatile oil of accession 9

al. (2004) chemoprofiled sixty volatile oil samples of nutmeg and among the samples, two accessions had significantly low myristicin and high sabinene contents. Lawrence (2000) indicated high variability in the constituents of kernel and mace oil. The constituents of nutmeg and mace oils from Indian origin were studied by Mallavarapu and Ramesh (1998). They observed that both nutmeg and mace oils were dominated by monoterpenes; the mace oil containing higher quantities of oxygenated monoterpenes and phenyl propanoid ethers.

5.4.1 Changes in constituents of kernel and mace volatile oil on storage

Prolonged storage of volatile oils leads to the loss of volatile components and thereby changes the oil composition. This is due to the structural relationship of volatile compounds within the same chemical group; the volatile oil components converting into each other by oxidation, isomerization, cyclization, or dehydrogenation reactions, triggered either enzymatically or chemically. A detailed investigation has not been carried out on the chemical changes in major and minor compounds following prolonged storage of nutmeg volatiles. The present study only compared the chemical profile of fresh and one year old kernel and mace volatile oils to highlight the changes in biochemical constituents that occur during storage.

In the present study, both additions and deletions in specific volatile compounds of kernel oil and additions in specific volatile compounds of mace oil were noticed. A total of twenty nine compounds were present in the kernel oil after storage. Among the samples, Acc. 5 recorded overall increase in constituents (Fig. 5.12) and two samples (Acc. 38 and Acc. (H) 1) showed losses of constituents on storage. Myristicin and elemacin contents alone increased among the seven major compounds on storage in all the three samples (Fig. 5.13). Alpha-pinene, sabinene and beta-pinene contents decreased in all the three samples on storage. Percentage of L-4-terpineol and safrole increased in Acc. 5 and L-4-terpineol alone increased in Acc. (H) 1. A few new compounds also appeared on storage of kernel oil.

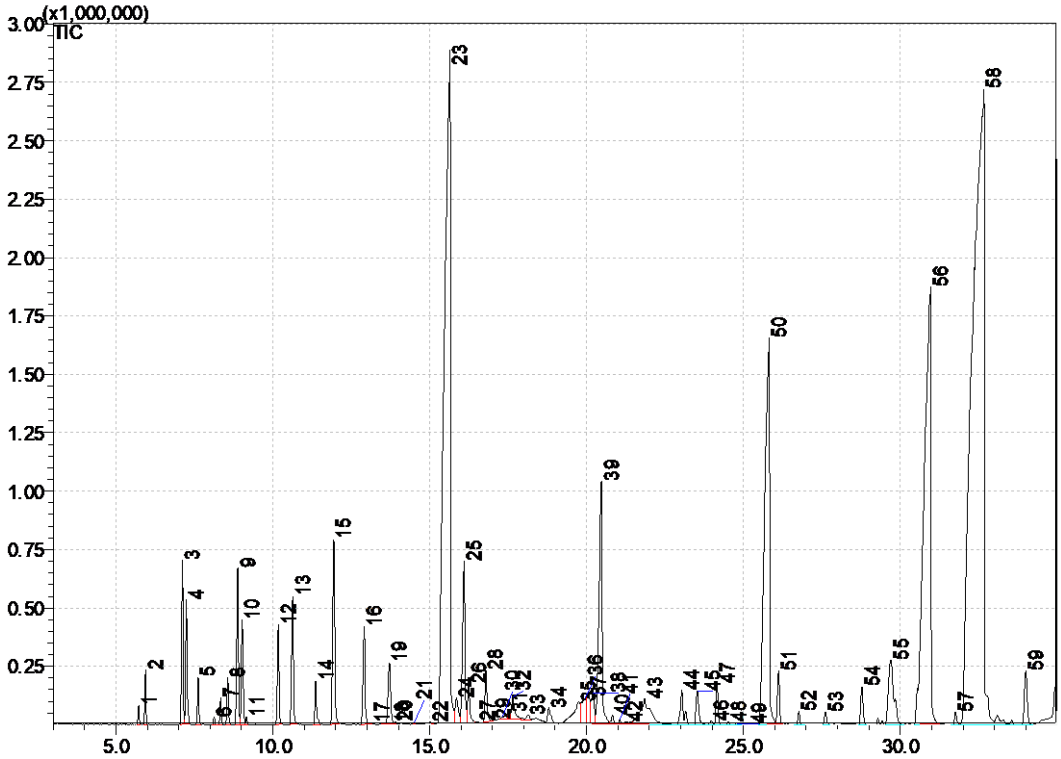


Fig. 5.12. GC-MS profile of stored kernel volatile oil of accession 5

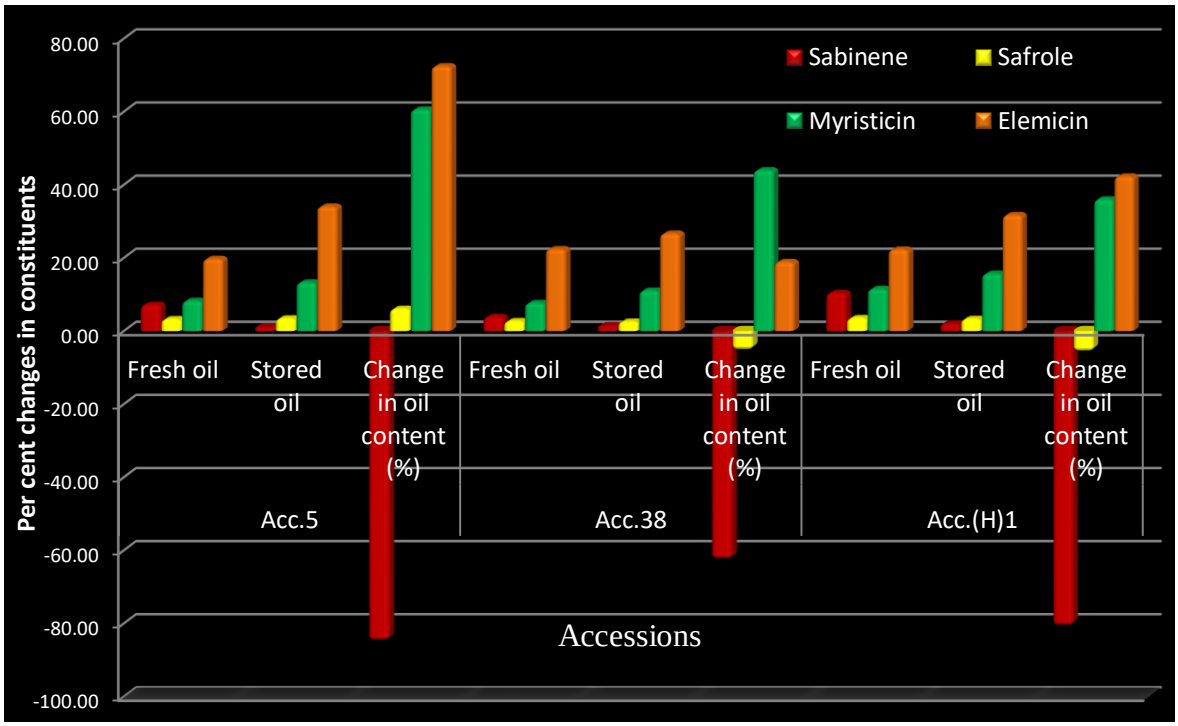


Fig. 5.13. Changes in major volatile constituents upon storage of kernel oil

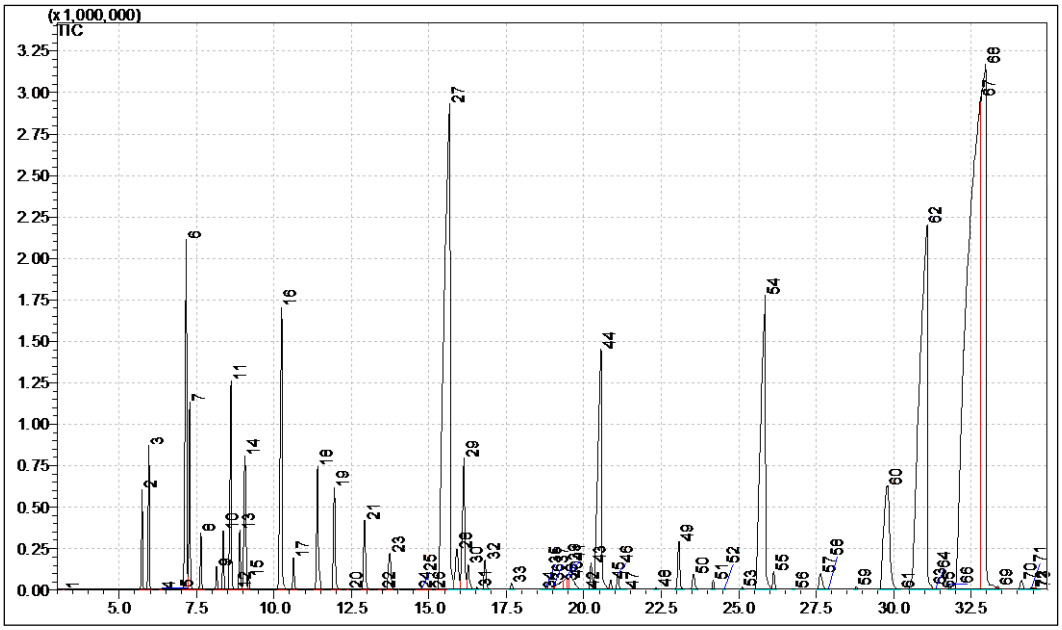


Fig. 5.14. GC-MS profile of stored mace volatile oil of accession 11

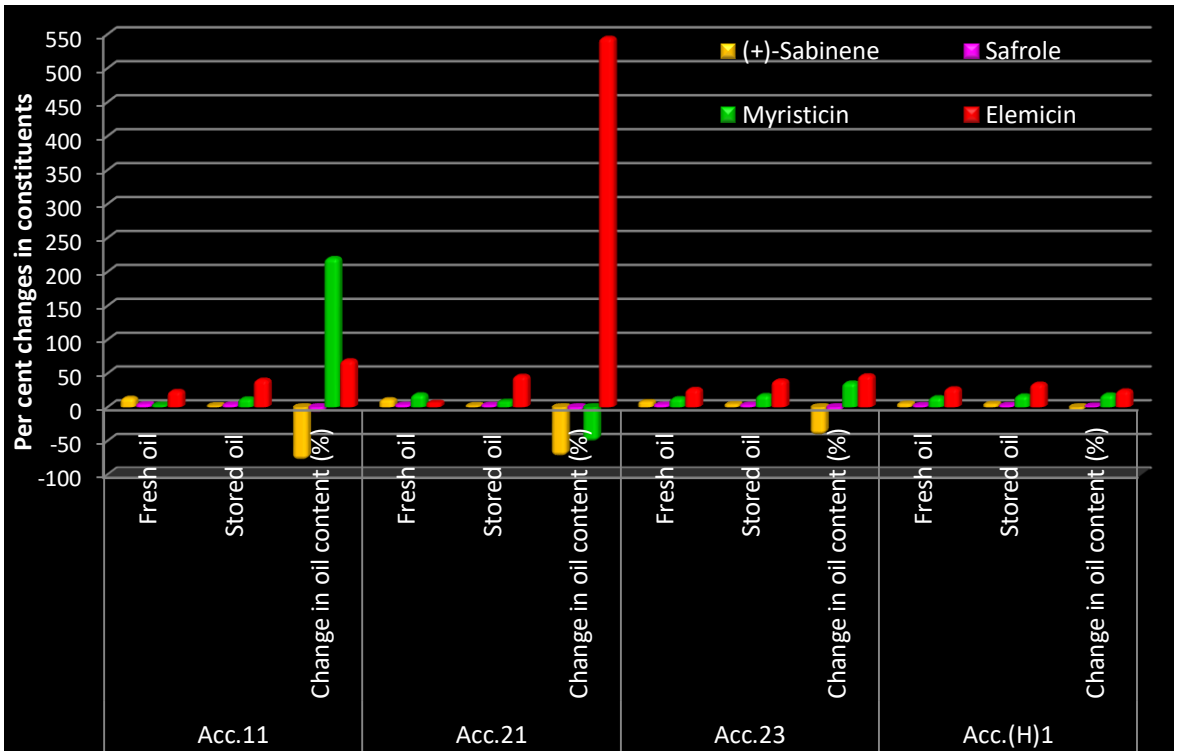


Fig. 5.15. Changes in major volatile constituents upon storage of mace oil

In the case of stored volatile oil of mace, maximum percentage change was recorded in Acc. 21 of 14.63 per cent and minimum in Acc. 11 of 1.92 per cent (Fig. 5.14). The content of elemicin alone increased in all the four samples of mace oil on storage. Myristicin content increased in all the samples except in Acc. 21 (Fig. 5.15). Alpha-pinene, sabinene and bête-pinene decreased in all the samples on storage. A few new compounds were identified in mace volatile oil on storage. In a study, Bhattacharya *et al.* (1998) reported that prolonged storage (one year storage) of lemon grass oil leads to deterioration of major compounds. The increase or decrease in the relative concentration of volatile constituents may be due to the chemical transformations during storage. Sanford and Heinz (1971) observed that the content of myristic acid might serve as an indicator of the age of ground nutmeg. Krishnamoorthy and Zachariah (2002) studied changes in volatile constituents of ground and stored nutmeg samples. The contents of alpha-pinene and sabinene increased and that of safrole, myristicin and elemacin decreased on storage.

5.4.3 Biochemical analysis of pericarp

The fresh nutmeg pericarp contributing 80 to 85 per cent of total fruit weight is widely considered as a farm waste due to its astringency and strong aroma. In Grenada, pericarp is used to prepare a jam called *Morne delice* and in Indonesia, pericarp is sliced finely, cooked and crystallized to make a fragrant candy called *Manisan pala* (Leela, 2008). In India, processing techniques for products like nutmeg rind leather, wine, pickle, syrup, jam, cake, powder and candy were standardised (Teena, 2015). These studies are oriented towards effective utilization of farm waste so as to transform them into value added products. The major interest for the processors is the biochemical composition of pericarp. Variation in biochemical composition may be due to the geographic locations, maturity conditions as well as genotypes.

Table 5.1. Ranking of select nutmeg accessions based on biochemical constituents in pericarp*

Accessions	Moisture	Acidity	Ascorbic acid	Pectin	Protein	Starch	Total phenol	Tannin	Total mineral	Crude fibre	Total score
Acc. 1	1.00	0.50	0.50	0.67	0.27	0.57	0.71	0.50	1.00	0.79	6.51
Acc. 5	0.20	0.50	0.50	0.94	0.55	0.93	0.71	1.00	1.00	0.79	7.92
Acc. 8	0.70	0.33	0.33	0.28	0.32	1.00	0.93	0.90	0.63	0.50	5.92
Acc. 9	1.00	0.33	0.33	0.11	0.27	0.79	0.71	0.65	0.75	0.14	5.09
Acc. 11	1.00	0.50	0.33	0.22	0.73	0.64	0.71	0.60	1.00	0.64	6.38
Acc. 14	1.00	0.50	0.50	0.67	0.91	0.50	0.50	0.30	0.25	0.50	5.63
Acc. 18	1.00	0.50	0.33	0.33	1.00	0.50	0.14	0.70	0.88	0.79	6.17
Acc. 21	0.70	0.50	0.83	0.33	0.59	0.50	1.00	0.80	1.00	0.86	7.11
Acc. 23	0.80	0.50	0.33	0.94	0.09	0.14	0.36	0.35	0.25	0.14	3.91
Acc. 24	1.00	0.50	0.83	1.00	0.45	0.21	0.36	0.10	0.38	1.00	5.83
Acc. 30	0.20	0.33	0.50	0.56	0.55	0.50	0.64	0.85	0.88	1.00	6.00
Acc. 35	0.40	0.50	0.50	0.83	0.59	0.36	0.71	0.50	1.00	1.00	6.40
Acc. 36	0.60	0.50	0.50	0.83	0.18	0.64	0.86	0.20	1.00	1.00	6.32
Acc. 37	1.00	0.50	0.50	0.44	0.68	1.00	1.00	0.85	1.00	0.64	7.62
Acc. 38	1.00	0.50	0.33	0.78	0.82	0.57	0.21	0.45	1.00	0.29	5.95
Acc. (H)1	0.80	0.50	1.00	0.83	0.41	0.50	0.71	0.45	0.25	0.36	5.81
Acc. (H)4	0.40	1.00	0.33	0.17	0.59	0.64	0.93	0.60	1.00	0.79	6.45

* Ranking based on DMRT

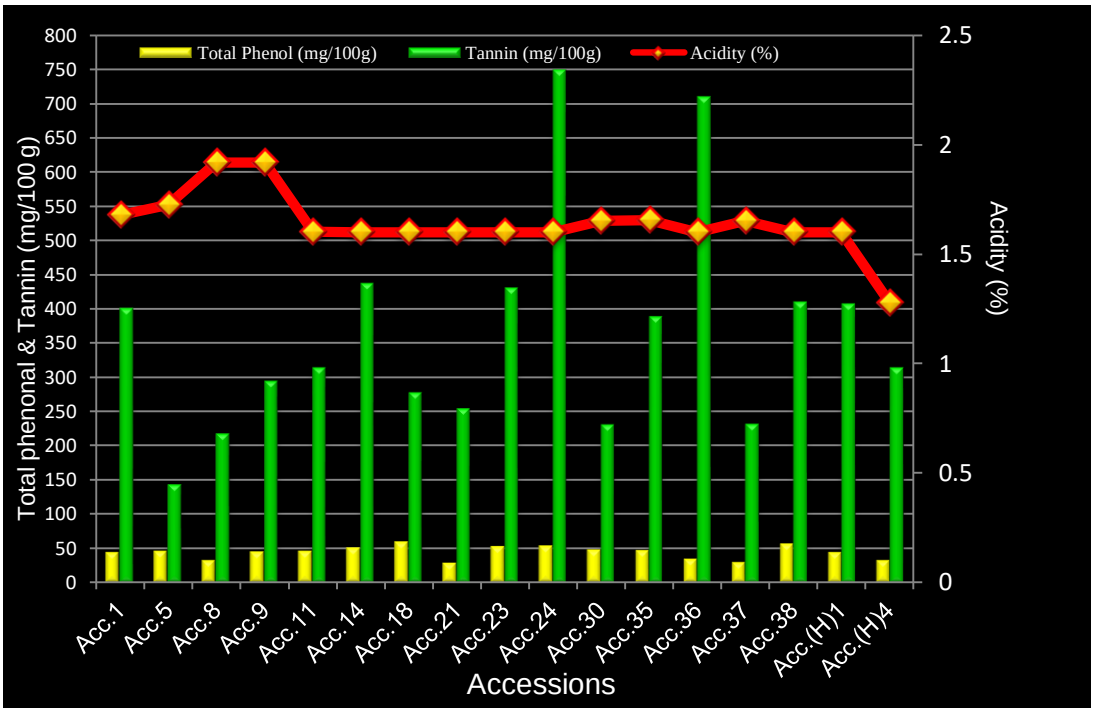


Fig. 5.16. Pericarp constituents in select nutmeg accessions

The biochemical constituents of all the accessions were statistically analysed and subgroups of the accessions were formed using DMRT. Since varied overlapping sub groups were obtained constituents wise, the method to make decisions jointly on a number of dependent characters as proposed by Arunachalan and Bandyopadhyay (1984) was co-opted. The final score is an indicator of the relative superiority of the accessions in terms of the biochemical constituents of pericarp (Table 5.1).

Nutmeg rind is thick and fleshy, contains 86 per cent moisture (Jayashree and Zachariah, 2014). Teena (2015) observed the average moisture content of nutmeg pericarp as 89.17 per cent. Accessions varied significantly for biochemical constituents of pericarp. Average moisture content recorded in the study was 88.01 per cent. The titrable acidity is an important physicochemical parameter which affects the product quality and also acts as a protectant against the development of microorganisms to a large extent. In nutmeg rind, acidity

content ranged from 1.28 to 1.92 per cent (Fig. 5.16). Gopalakrishnan (1992) reported higher acidity of nutmeg rind compared to that of that of kernel and mace. Ascorbic acid content in the nutmeg pericarp varied from 4.54 to 9.52 (mg/100g). Highest ascorbic content was recorded in Acc. 9 and minimum was in Acc (H) 1. In an earlier study, Teena (2015) reported 13.32 (mg/100 g) of ascorbic acid content in nutmeg rind. Presence of vitamin C adds nutritive value to the produce.

Nutmeg pericarp showed very low range of protein, ranging from 0.21 to 1.85 (g/mg). Accessions 23 and 36 registered higher protein content. Phenolics are naturally occurring compounds widely distributed in the plant kingdom and are beneficial components of daily human diet. They are important constituents of plants with multiple functions and as dietary phytochemicals for humans, they display a broad range of functional and; biological activities (Le *et al*., 2007). Total phenol content differed significantly and ranged from 27.77 mg/ 100 mg (Acc. 21) to 57.55 mg/100 mg (Acc. 18). Teena (2015) reported 0.44 mg/g of total phenol in nutmeg rind. Tannin content recorded highest value in Acc. 24 (750 mg/100g) and lowest in Acc. 5 (143.30 mg/100 g). Tannins are responsible for the astringent taste of nutmeg rind and low concentration of this constituent could be useful in making value added products from rind. Proximate analysis of nutmeg rind collected from central parts Kerala, recorded 0.33 mg/g of tannins (Teena, 2015). Starch is the most vital carbohydrate in the human diet and is the major constituent of staple foods such as potato, rice, wheat, cassava, and corn. In nutmeg rind, starch content ranged from 0.30 to 1.23 (g/100g).

Crude fibre content was maximum in Acc. 9 (3.65%) closely followed by Acc. 23 (3.48%). In an earlier work, Teena (2015) obtained 3.75 per cent crude fibre in nutmeg rind. Varghese (2000) reported that kernel possessed the highest crude fibre content of 11.70 per cent and that of mace was 3.93 per cent. Nutmeg rind powder is a rich source of minerals like iron and some other micro and macro nutrients. In this study, highest total mineral content was observed in Acc. (H) 1 (2.57%), followed by accessions, Acc. 14 (2.50%), Acc. 23 (2.50%) and Acc. 24

(2.46%). Pectin occurs in varying amounts in fruit cell walls and contains nutritional and technological properties, because of its ability to form gels (Westerlund *et al*., 1991). Pectin is used in the manufacture of many value added products like jams, jellies, marmalades, preserves and also used as a thickening agent in sauces, ketchups and flavoured syrups. Besides, it finds many uses in pharmaceutical preparations such as pastes, cosmetics etc. A valuable by-product that can be obtained from fruit wastes is pectin. In the present study, pectin content of fresh pericarp ranged from 0.21 to 1.08 (% Calcium pectate). Acc. 9 recorded a significantly higher pectin content in the fresh pericarp followed by accessions Acc. (H) 4 and Acc. 11. Teena (2015) had reported a pectin content of 1.03 per cent in nutmeg rind.

5.4.4 Isozyme profiling

Isozyme genetic markers are efficient tools to study genetic variations within and between populations of less known wild species as well as for studies on spatial distribution of genetic variation (Rajora, 1989).

Therefore, in the present study, variation in select distinct nutmeg accessions were assessed using isozyme profiling. Total of four isoforms were exhibited in the accessions screened for peroxidase enzyme. Maximum isoforms were recorded in Acc. 21. In case of polyphenol oxidase, Acc. 5 exhibited three bands. Clustering pattern based on isozyme study was different from the clustering pattern of morphological characterization. From the dendrogram, eight clusters were made at 85 per cent similarity. The result indicated that variation in nutmeg accessions based on isozyme profiling was very low. In a similar study, Latha (2010) studied the isozyme profiling of peroxidase and polyphenol oxidase of *Vigna* spp. and they obtained low variations among the populations of *Vigna* in both the isozyme markers. Parthasarathy *et al.* (2010) studied peroxidase, polyphenol oxidase, esterase and superoxide dismutase in *Garcinia gummi-gutta* populations collected from Western Ghats of Kerala and Karnataka. The extent of

polymorphism was 52.5 per cent and clustering was according to the geographic location of populations.

5.4.5 Enzyme activity

Peroxidase is phenol oxidizing enzyme which oxidizes phenols to form quinones and also produce hydrogen peroxide. It is a key enzyme in the biosynthesis of lignin (Brisson *et al.*, 1994). In the present study, peroxidase enzyme activity ranged from 0.128 to 1.727. Highest peroxidase activity was observed in Acc. 30 followed by Acc. 5. Polyphenol oxidase activity in nutmeg accessions noticed was low, ranging from 0.098 (Acc. 36) to 0.329 (Acc. 9). Enhanced activity of peroxidase and polyphenol oxidase enzymes are directly correlated to the defence reaction in the plants. Similar observations were made by Nazeem *et al*. (2008) in black pepper and Kurian (2011) in cocoa.

5.4.6 Molecular characterization

Molecular markers have been proved to be a fundamental and reliable tool for fingerprinting varieties, establishing the fidelity of progenies and germplasm characterization. Molecular markers which detect variation at DNA level overcome most of the limitations of morphological and biochemical markers. Molecular markers are independent of developmental stages of the crop and are not influenced by the varying environmental conditions (Agarwal *et al*., 2008). Hence, molecular markers are preferred for variability analysis than traditional morphological markers. Most of the molecular markers are developed by PCR technology. These markers amplify unique regions in the genomic DNA based on the primers designed for amplification. In the present study, PCR based markers such as RAPD and ISSR were used for molecular characterization of seventeen distinct nutmeg accessions.

5.4.6.1 RAPD analysis

In RAPD analysis, out of the forty three primers used, only twenty one primers produced clear amplification. There were total of 164 amplicons of which

63.21 per cent were polymorphic (Table 4.37). Karihaloo *et al.* (2003) reported 94.7 per cent of genetic diversity in mango using RAPD marker analysis. The number of amplicons produced ranged from five to ten. The number of markers detected by each primer depends on primer sequence and extent of variation is genotype specific (Upadhyaya *et al*. 2004). This is understandable as product amplified depends upon the sequence of random primers and their compatibility within genomic DNA. Out of the twenty one primers used for the study, only five primers *viz*., OPA 07, OPA 02, OPB 06, OPC 05 and OPP 13 gave polymorphism of more than 80 per cent. The primer OPA 07 gave highest polymorphism of 90 per cent. Simi (2006) characterized mango accessions using thirty six decamer primers which gave 157 RADPs. There were 151 polymorphic bands out of 157 reproducible.

The OPA 07 primer produced specific bands which were present only in the accessions, Acc. 23, Acc. 24, Acc. 30 and Acc. 35 (850 bp, 900 bp and 950 bp respectively), these accessions exhibiting similar qualitative characters were also grouped in the same qualitative cluster. The primer OPE 15 produced distinct bands specifically in monoecious accession, Acc. (H) 1 (450 bp and 550 bp). Decamer primer OPE 16 produced specific bands in monoecious accession, Acc. (H) 4 at 200 bp. The primer OPB 19 produced specific bands (900 bp) in accession 21 and (H) 4. Both these accessions possessed very small nut and highly dissected mace.

The RAPD analysis of seventeen distinct nutmeg accessions suggested that diversity was high. The pair wise similarity coefficient values varied between 0.77 and 0.91. Corresponding to a similarity coefficient of 0.86, all the seventeen accessions were observed to group into seven clusters. Maximum of six accessions were included in cluster II. Cluster I, III, VI and VII had one accession each. A cluster analysis based on RAPD data in mango showed two groups- the first consisting of mango cultivars form western, northern and eastern parts of India and the second group consisting of cultivars form southern parts (Ravishankar *et al.*, 2000).

5.4.6.2 ISSR analysis

The selected 12 ISSR primers used for characterization produced a total of 87 amplicons of which 69.44 per cent were polymorphic bands (Table 4.40). The number of bands resolved per amplification was primer dependent and varied from four to ten. Out of 12 primers used, three primers namely, ISSR 26, $(CT)_7AC$ and UBC 812 gave maximum (above 80 %) polymorphism. The primer $(CT)_7AC$, gave the highest polymorphism of 100 per cent and lowest being the primer UBC 864 (40 %). Aswini (2015) studied the molecular genetic diversity in jackfruit accessions using ISSR maker and obtained a total of 86 reproducible bands, with 55.01 per cent polymorphism.

Among the amplified primers, five ISSR primers in this study produced unique banding patterns that could differentiate from all other accessions. ISSR 25 produced two unique bands, one distinct band (300 bp) specific to Acc. 1 and other distinct bands (400 bp) specifically in accessions 1 and 37, these accessions were having bold fruit as well as bold nut and thick mace. ISSR 26 produced unique band of size 300 bp specifically in accessions 1, 5, 9, 21 and 24, which possess the bold fruit with thick pericarp. The primer $(TC)_7C$, produced unique band (850 bp) in accession 38 and (H) 1, which exhibited medium sized fruits. The primer $(CT)_7AC$, produced unique bands (750 bp) specific to accession 14 and 38 which are entire maced types. UBC 857 produced unique band of size 320 bp specifically in monoecious accession, Acc. (H) 4.

The ISSR analysis of seventeen distinct nutmeg accessions suggested that diversity is high. The pair wise similarity coefficient values varied between 0.75 and 0.91. Corresponding to a similarity coefficient of 0.85, all the seventeen accessions were observed to group into eleven clusters. Cluster III and VII contained maximum of three accessions each. Cluster I, II, V, VI, VIII, X and XI included single accessions each. DNA fingerprinting of Indian cashew varieties using RAPD and ISSR techniques was done by Archak *et al.* (2003). They could not establish any correlation between the relationships based on molecular data

and the pedigree of the varieties. Sulassih *et al.* (2013) carried out evaluation of *Garcinia* spp. using ISSR makers, and they obtained three clusters.

5.4.6.3 Combined RAPD and ISSR cluster analysis

The mean similarity coefficient was calculated and used for cluster analysis using UPGMA method and a dendrogram generated using software NTSys pc (Rohlf, 1992). The combined RAPD and ISSR analysis of seventeen distinct nutmeg accessions suggested that diversity was high. The pair wise similarity coefficient values varied from 0.76 to 0.90. Seventeen accessions were grouped in to nine clusters at 85 per cent of similarity level. Cluster II was the largest with four accessions. Cluster VI included three accessions. Earlier, Sheeja *et al.* (2013) have reported the combined analysis of RAPD and ISSR markers and observed the genetic relationships better than individual analysis in *Myristica* spp. Gupta *et al.* (2008) had also reported similar results in *Jatropha* sp.

5.5 INTER CLUSTER ASSOCIATION OF A QUALITATIVE CLUSTER WITH OTHER CLUSTER AGGLOMERATIONS

Clustering pattern based on qualitative characters and quantitative characters were different. The forty six accessions were grouped into eleven and ten clusters in qualitative and quantitative clustering respectively.

A comparison of the two clustering patterns was done by finding out the per cent distribution of accessions of a qualitative cluster over the different quantitative clusters. Majority of accessions in a single qualitative cluster fell in a single quantitative cluster indicating the similarity among these accessions at quantitative level also. The remaining accessions of the pre-disposed qualitative cluster even though seemed to be similar at qualitative level, were dissimilar at quantitative level.

Similarly, clustering based on biochemical characters grouped the forty six accessions into 26 clusters. The relationship between the accessions of qualitative clusters and biochemical clusters was explained by computing the per cent

distribution of accessions of a qualitative cluster over the different clusters. In the biochemical clusters, majority of the accessions remained as single entities and the intra cluster association of the clustering patterns showed highly scattered distribution.

Based on isozyme analysis, accessions were grouped into 10 clusters, while the RAPD analysis resulted in seven clusters. Based on ISSR analysis there were eleven clusters and based on combined RAPD and ISSR marker analysis accessions there were nine clusters. When a comparison was made among the qualitative clusters with isozyme and molecular clustering patterns, it was observed that the accessions which belonged to the same clusters behaved differently at isozyme and molecular levels. The distinction between accessions was much more evident with molecular rather than with isozyme markers. The clustering pattern based on isozyme and molecular analysis was not strictly in accordance with that based on morphological characters. Hence, to obtain a still more clear observation about the relationships, more accessions are to be subjected to molecular characterization.

5.5.1 Summary statistics of quantitative clusters

The mean, standard deviation and coefficient of variation were computed for twenty six quantitative characters of each of the quantitative cluster. In all the clusters, characters having more than thirty percent coefficient of variation were considered as the most variable characters (Table 4.16). These variable characters are identified as the important characters which can influence the yield in nutmeg.

5.5.2 Perceived morphological dimensions

The perceived morphological dimensions of the different qualitative clusters are given in Table 4.17. For each character a perusal of the range of variation of the values over the different clusters was made to identify the least performing clusters for major characters. The clusters served as the data base for visualization of an ideotype of nutmeg and also to develop a key for identification of an elite nutmeg.

5.6 KEY FOR IDENTIFICATION OF ELITE NUTMEG TREE

The discussions on qualitative, quantitative, biochemical and molecular characters have unravelled to a certain extent the mystery of the member of the Myristicaceae family. The variability existing in a member of the plant kingdom is usually explored to visualise the plant in terms of its desirable characteristics. Such desirable characteristics shall be easily measurable, recognizable and understandable to a person who is at least familiar with the plant. Thus, the key for identification of an elite genotype of nutmeg was developed from the perceived characteristics data base already developed. As the key has to be simple and easily recognizable, thirteen easily measurable characters having direct effect on yield as also economically important were selected for developing the key (Plate 5.4). The desirable characteristic traits for the said thirteen characters were thus enlisted.

The detailed study on characterization and evaluation of nutmeg accessions has brought forth the existing variations in nutmeg, qualitatively, quantitatively, biochemically as also at the molecular level. Inter lacing these variations accounting four different dimensions the most important easily distinguishable characters; numbering thirteen; important morphologically and economically were identified. Based on the data base of characters evolved from the survey, a statistical key was developed using the aforesaid identified characters. The statistical key is only a scale for identifying an elite nutmeg tree. The total whereabouts of this particular member of plant kingdom can never be fully unveiled and of course this is so for every other member. A true observation is always accidental. To which extent such an observation will tally with the key will be known only progressively. Identification alone is not the solution. We have to provide proper environment for the growth of the tree in terms light infiltration as also the vegetative load, especially in terms of the spread in N-S direction. The above mode of management itself will inculcate many desirable characteristics, especially floral, ultimately leading to aboveoptimal yield characteristics, the goal that a farmer always cherishes.

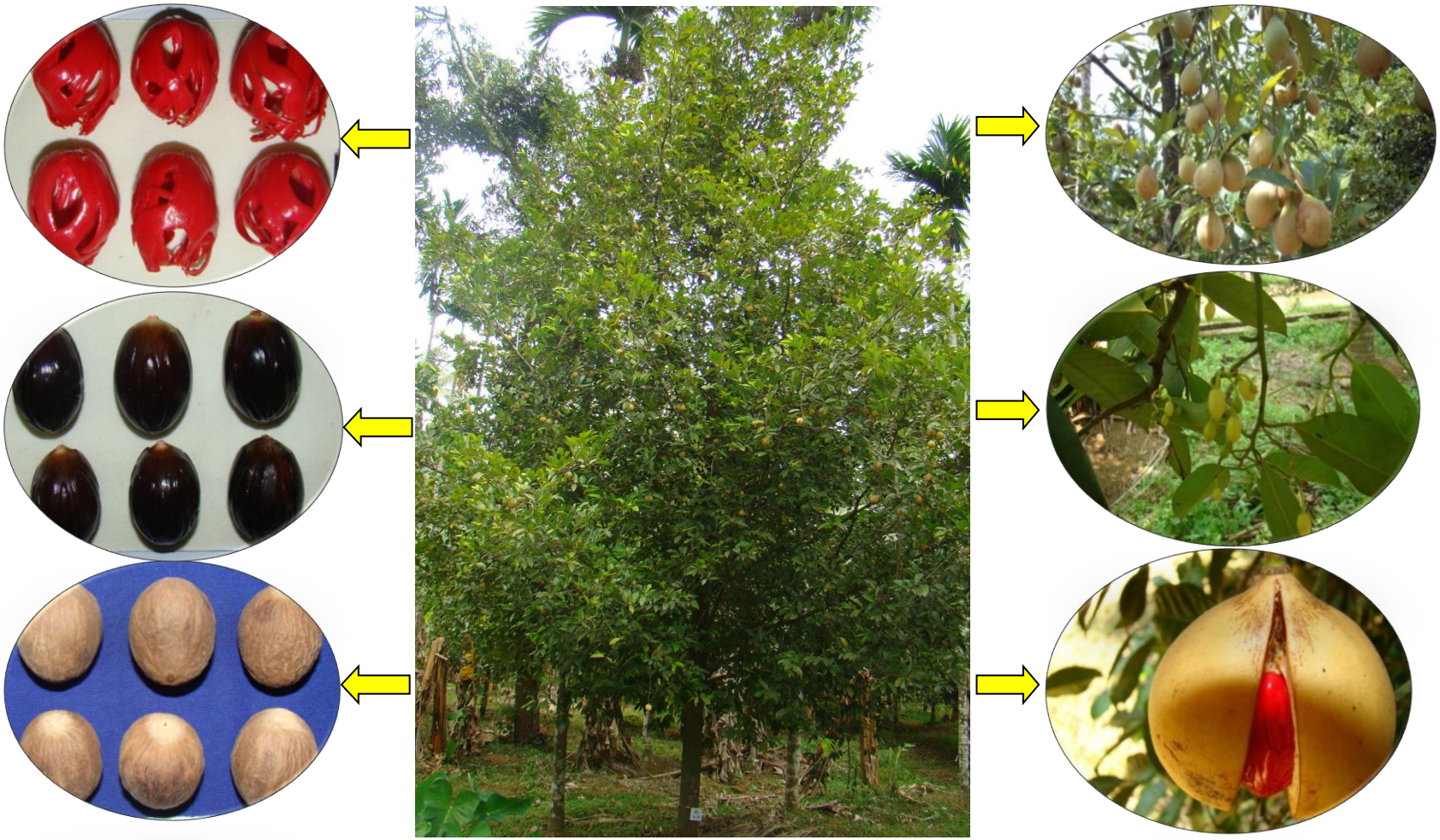


Plate 5.4. Nutmeg tree with desirable key characters at the age of fifteen years

Summary

6. SUMMARY

The present investigation entitled "Characterization and evaluation of nutmeg (*Myristica fragrans* Houtt.) accessions" was carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara, Kerala Agricultural University, Thrissur, during 2012-2015. The results of the study are summarized hereunder:

Fifty accessions of nutmeg collected from diverse locations of Kerala; planted in Chalakudy river basin and belonging to the same age of fifteen years, formed the material for the study. Among the select fifty accessions, forty two were females, four monoecious and four males.

I. Qualitative evaluation

Fifty one qualitative and thirty eight quantitative parameters were recorded at various phenophases of the tree and descriptor states for each of these characters have been formulated. The varying degrees of qualitative as well as quantitative characters recorded in the study formed the basis towards development of a descriptor with different descriptor states for nutmeg. This is the first attempt to develop a descriptor for *Myristica fragrans*, which in due course will serve as a stepping stone for further research; germplasm management and crop improvement studies.

Wide variability was observed among the accessions for forty seven out of fifty one qualitative characters. Four characters *viz*., leaf margin, fruit pubescence, grooves on nut and nature of fruit dehiscence were uniform in all the accessions and hence, these were not included for further analysis.

Sex forms and tree characters *viz*., stem colour, branching pattern, canopy shape, foliage density and colour of flushes varied among the accessions evaluated. There were two sex forms among the accessions evaluated; majority being dioecious in nature. Predominantly spreading branches were observed against the erect types. Four types of canopy shapes *viz*., conical, pyramidal,

oblong and globular were observed among the accessions evaluated. Three accessions exhibited conical canopy shape.

Wide variability could be observed in leaf characters. Shape of mature leaf and colour of mature leaf showed wide range of variability. As regards the leaf apex, majority of the accessions had acuminate and acute leaf apex, with a lone accession having obtuse leaf apex.

The accessions varied widely with respect to flowering and fruiting pattern. Most of the accessions exhibited seasonal flowering with three peaks. Apart from male and monoecious types, two prominent female accessions produced flowers round the year. Three flowering seasons were observed *viz*., early flowering (July), mid flowering (late July to early August) and late flowering (late August). Female accessions had axillary raceme type of inflorescence. Monoecious and male accessions had umbellate cyme inflorescence. The character, number of flowers per cluster and frequency of flower clusters varied among the accessions.

There was wide variability with regard to the fruiting pattern, nature of fruit bearing, periodicity of fruit bearing, number of fruits per cluster, frequency of fruit clusters, fruit bearing densities and bearing season. Majority were seasonal fruiting and year round fruiting was observed in a few accessions. Majority of the accessions exhibited peak bearing in the month of June-August and very few were very early bearing.

Floral characters *viz*., colour of pedicel, perianth, filament and pistil and shape of perianth also showed variation among the accessions.

The fruit characters like colour of immature and mature fruit, shape of fruit, shape of fruit base, shape of fruit apex, number of seeds per fruit, nature of fruit splitting and number of splits in split fruit showed great variation among the accessions. Majority of accessions had round shaped fruits. Pyriform fruit was produced by two accessions. Number of seeds per fruit was one in all the accessions except two monoecious accessions, which contained two seeds per

fruit. Two types of fruit splitting was observed *viz*., full fruit splitting and partial fruit splitting (Acc. 5 alone). Generally, ripened fruits split into two splits. But in one of the monoecious accessions fruit split into three splits and in another into four splits.

Significant variation for all the mace and nut characters were recorded among the accessions. Mace shape, fresh mace colour and dry mace colour varied among the accessions evaluated. Two accessions had orange-red mace. Entire mace could be observed in two accessions only. Beakness of mace was observed only in one accession and the rest were devoid of mace beakness. Attachment of mace to nut, nut shape, fresh nut colour, dry nut colour and kernel colour also varied among accessions. Light brown kernel was observed in a few. Nut as well as kernel grooves also exhibited different expressions.

Cluster analysis based on qualitative characters following the UPGMA method suggested by Sneath and Sokal (1973), resulted in eleven clusters at 66 per cent similarity level.

II. Quantitative evaluation

All the quantitative characters except shelling percentage showed significant difference among accessions. Wide variability could be observed in growth characters. Tree height varied from 3.20 m to 12.35 m. Tree girth, internodal length and number of orthotrops per tree varied significantly among the accessions. Accessions differed significantly for canopy spread in both directions. Canopy spread in E-W direction varied from 3.11 m to 9.02 m and canopy spread in N-S direction ranged from 3.11 m to 9.30 m.

The accessions varied widely with respect to leaf characters like leaf length, leaf breadth and chlorophyll content. Leaf area varied from 20.29 cm^3 to 54.63 cm^3.

Floral parameters *viz*., number of flowers per 10 cm^2, length of flower, breadth of flower, length of tepal, breadth of tepal, length of pistil and breadth of

pistil varied significantly among the accessions. Percentage of fruit set varied from 6.45 to 44.45 per cent.

Among the evaluated accessions, average fruit weight ranged from 39.33 g to 99.56 g. Marginal variations were observed for fruit length and fruit breadth among the accessions. Pericarp thickness significantly varied form 8.36 mm to 15.70 mm.

Accessions differed significantly for fresh as also dry mace weights. Fresh mace weight ranged from 0.91 g to 5.27 g. After drying, mace weight varied from 0.46 g to 2.61 g.

The highest nut weight observed was 13.67 g and the dry nut weight observed was 11.01 g. Kernel weight registered a maximum value of 8.04 g and a minimum value of 2.65 g. Accessions differed significantly for shell thickness, fruit volume, nut volume, mace volume and kernel volume. Maximum nut to mace ratio recorded was 9.69 and minimum was 2.06.

Number of fruits per m^2 varied from 4 to 32. Few accessions produced more than 3000 fruits per tree per year. The same accessions recorded significantly superior values for mace, nut and kernel yield per tree also. Considering these economic parameters accessions 9, 8 and 22 were noticed as promising nutmeg types.

Accessions showed high GCV, PCV, heritability and GG for most of the characters evaluated. Among the twenty six characters studied, number of fruits per tree, fruit set percentage, number of fruits per meter square, fresh and dry weights of mace, mace volume as well as ratio of nut to mace exhibited highest genetic gain. Hence, the selection programme based on these characters will be very effective in improving nutmeg populations.

Correlation studies revealed that growth parameters like tree height, canopy spread (both E-W and N-S); fruiting parameters such as number of fruits per m^2 and fruit set percentage and fruit parameters like fruit weight, fruit breadth,

dry mace weight, fresh nut weight, shell thickness, kernel volume and nut breadth had significant positive correlation with number of fruits per tree both at genotypic and phenotypic levels. This indicates that selection based on these parameters will result in effective improvement of the genetic stock.

The results of path analysis among nutmeg accessions revealed that greater emphasis should be given to fruit weight, mace volume, thickness of pericarp, kernel volume, dry weight of mace, fruit set percentage and number of fruits per tree as they exhibited high positive and direct effect on yield.

Clustering of the accessions using select twenty six quantitative characters following D^2 statistics developed by Mahalanobis (1936) resulted in ten clusters. Among the different clusters, maximum divergence was found among the accessions falling in cluster V and III.

III. Contents of volatile oil, oleoresin and fixed oil

The volatile oil content ranged from 1.57 to 7.67 per cent in kernel and 2.05 to 9.33 per cent in mace. A few accessions were found rich in kernel and mace oils. The oleoresin content varied from 18.59 to 36.20 per cent in kernel and 11.38 to 31.66 per cent in mace. Fixed oil content in kernel varied from 17.79 to 44.80 per cent. In the cluster analysis based on oil, oleoresin and fixed oil contents, majority of accessions remained as independent units even at 20 per cent level of similarity.

IV. Pest and disease incidence

Major diseases affecting nutmeg tree were noticed as *Phytopthora* leaf fall, *Colletotrichum* leaf spot, fruit rot, thread blight and die back. The accessions varied in their susceptibility to diseases. However, severity of all the diseases was very minute to medium only. Two accessions showed medium incidence of *Phytopthora* leaf fall and remaining accessions showed field tolerance to this emerging disease. Pest infestation was not noticed in the accessions during the period of study.

V. Chemoprofiling based on kernel and mace volatiles

GC-MS analysis of 17 distinct accessions for kernel and mace volatile oil resulted in twenty and twenty four constituents respectively. Volatile oil composition exhibited wide variability and also recorded presence of some unique compounds in the accessions. Principle compounds *viz*., myristicin, elemicin, safrole and sabinene were seen in the kernel and mace volatile oil of all the accessions and it was highest in mace volatile oil. Based on per cent content of major volatile compounds, seventeen accessions were grouped into three viz., high, medium and low.

VI. Changes in kernel and mace volatiles on storage

In the present study, both additions and deletions in specific volatile compounds of kernel oil and additions in specific volatile compound of mace oil were observed upon storage for one year. A total of twenty nine and thirty four compounds were present in the kernel and mace oils respectively after storage. A few new compounds also appeared upon storage of kernel and mace oils.

VII. Biochemical analysis of pericarp

Nutmeg rind recorded the average moisture content of 88.01 per cent. Acidity content varied significantly from 4.54 to 9.20 per cent. Nutmeg rind, a poor source of ascorbic acid, could produce the same only in the range of 4.54 to 9.52 (mg/100g). The pericarp showed protein content of 0.21 to 1.85 (g/100g) and starch content varied from 0.30 to 1.23 (g/ 100g). Total phenol content ranged from 27.7 to 57.55 (mg/100g) and tannin content from 143.30 to 750.00 (mg/100g). Total mineral content in the rind varied form 2.04 to 2.57 per cent and crude fibre content ranged from 2.15 to 3.65 per cent. The pericarp recorded pectin content of 0.21 to 1.08 (% Calcium pectate). The accessions were ranked based on the content of biochemical constituents.

VIII. Isozyme profiling

Isozyme analysis of seventeen distinct nutmeg accessions using peroxidase enzymes produced a total of four isoforms. Polyphenol oxidase exhibited three bands. Based on isozyme analysis using peroxidase and polyphenol oxidase enzymes, the distinct accessions were grouped into eight clusters at 85 per cent similarity.

IX. Enzyme activity

In the present study, peroxidase activity among the nutmeg accessions ranged from 0.128 to 1.727 and polyphenol oxidase activity noticed was negligible. It ranged from 0.098 to 0.329.

X. RAPD assay

The selected 21 primers produced clear and distinct amplification pattern with the seventeen select nutmeg accessions. There were a total of 164 amplicons of which 63.21 per cent were polymorphic. The number of amplicons produced ranged from five to ten. Among the selected RAPD primers, the unique bands produced by OPA 07 (850 bp, 900 bp and 950 bp), OPE 15 (450 bp and 550 bp), OPE 16 (200 bp) and OPB 19 (900 bp) were found specific to some accessions. The dendrogram generated using NTSys grouped the nutmeg accessions into seven clusters with a Jaccard's similarity coefficient of 0.77 to 0.91, the overall variability observed among the seventeen accessions was 63.20 per cent.

XI. ISSR assay

The selected twelve primers produced a total of 87 amplicons with an average polymorphism of 69.44 per cent. The number of bands produced per amplification varied from four to ten. Among the selected ISSR primers, ISSR 25 (300 bp and 400 bp), ISSR 26 (300 bp), $(TC)_7C$ (850 bp), $(CT)_7AC$ (750 bp) and UBC 857 (320 bp) produced unique amplicons. The dendrogram generated using NTSYS grouped the nutmeg accessions into eleven major clusters with a

Jaccard's similarity coefficient of 0.76 to 0.90. The overall variability observed among the seventeen accessions studied was 69.43 per cent.

XII. Inter cluster association of various clustering patterns

The inter cluster association of qualitative cluster with other clustering patterns were studied based on per cent distribution of the accessions belonging to each of the qualitative clusters, into various quantitative, biochemical and molecular clusters. Majority of the accessions in a single qualitative cluster fell in a single quantitative cluster indicating the similarity among these accessions at quantitative level also. The remaining accessions of the pre-disposed qualitative cluster even though seemed to be similar at qualitative level, were dissimilar at quantitative level. In the biochemical clusters, majority of the accessions remained as single entities and the intra cluster association of the clustering patterns showed highly scattered distribution. When a comparison was made among the qualitative clusters with isozyme and molecular clustering patterns, it was observed that the accessions which belonged to the same clusters behaved differently at isozyme and molecular levels.

XIII. Key for identification of elite nutmeg tree

A statistical key was developed using the thirteen key quantitative characters, which can serve as a preliminary tool for identification of an elite nutmeg tree. Accordingly an elite nutmeg tree may be characterized as having the ideal characteristics with approximate values *viz.*, tree height (8 m), canopy spread E-W (7 m), canopy spread N-S (8 m), number of flowers per 10 cm^2 (6), fruit set percentage (37), number of fruits per m^2 (19), fruit weight (81 g), thickness of pericarp (14 mm), dry mace weight (2 g), dry nut weight (10 g), kernel weight (7 g), ratio of nut to mace (6.63) and number of fruits per tree (3342).

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Appendix I: Degree and genotypic association of characters

Sl. No.	Characters	Degree and association with other characters			
		Highly positive & significant	**Highly negative & significant**	**Moderately positive & significant**	**Moderately negative & significant**
1	Tree girth (cm)	Tree height			
2	Canopy spread (E-W)	Tree height, tree girth			
3	Canopy spread (N-S)	Tree height, tree girth, canopy spread (E-W)			
4	Leaf area (cm^2)	Tree height, tree girth, canopy spread (E-W), canopy spread (N-S)			
5	No. of flowers/ $10cm^2$			canopy spread (E-W), canopy spread (N-S)	
6	Fruit set percentage			canopy spread (E-W)	No. of flowers/ $10cm^2$
7	No. of fruits/ m^2	Fruit set percentage			No. of flowers/ $10cm^2$
8	Fruit weight (g)			Tree girth, canopy spread (E-W), canopy spread (N-S), Fruit set percentage, No. of fruits/ m^2	
9	Fruit length (mm)	Fruit set percentage, fruit weight		No. of fruits/ m^2	
10	Fruit breadth (mm)	Tree girth, canopy spread (E-W), canopy spread (N-S), Fruit set percentage, No. of fruits/ m^2, fruit weight, fruit length			
11	Thickness of pericarp	Tree height, tree girth,		Fruit set percentage,	

	(mm)	canopy spread (E-W), canopy spread (N-S), leaf area, fruit weight, fruit length, fruit breadth		no. of fruits/m^2	
12	Fresh mace weight (g)	Fruit weight, fruit breadth, thickness of pericarp		canopy spread (E-W), canopy spread (N-S)	
13	Dry mace weight (g)	canopy spread (N-S), fruit breadth, thickness of pericarp, fresh mace weight		canopy spread (E-W), fruit set percentage	
14	Fresh nut weight (g)	Fruit set percentage, fruit weight, fruit length, fruit breadth, thickness of pericarp, fresh mace weight, dry mace weight		No. of fruits/m^2	Leaf area
15	Dry nut weight (g)	Fruit set percentage, fruit weight, fruit length, fruit breadth, thickness of pericarp, fresh mace weight, dry mace weight, fresh nut weight		No. of fruits/m^2	Leaf area
16	Shell thickness (mm)	Canopy spread (E-W), canopy spread (N-S), fruit weight, fruit length, fruit breadth, thickness of pericarp, fresh mace weight, dry mace weight, fresh nut weight		Dry nut weight	
17	Kernel weight (g)	Fruit set percentage, fruit weight, fruit length, fruit breadth, thickness of pericarp, dry mace weight, dry nut weight		No. of fruits/m^2, fresh mace weight	Leaf area

18	Fruit volume (cm^3)	Fruit weight, fruit length, fruit breadth, thickness of pericarp, fresh mace weight, dry mace weight, fresh nut weight, dry nut weight, shell thickness, kernel weight		Dry mace weight	
19	Nut volume (cm^3)	Fruit set percentage, number of fruits/m^2, fruit weight, fruit length, fruit breadth, thickness of pericarp, fresh mace weight, dry mace weight, fresh nut weight, dry nut weight, kernel weight, fruit volume		Shell thickness	Tree height, leaf area
20	Mace volume (cm^3)	Fruit weight, fruit length, fruit breadth, thickness of pericarp, fresh mace weight, dry mace weight, fresh nut weight, shell thickness, fruit volume, nut volume		Dry nut weight, kernel weight	
21	Kernel volume	Fruit set percentage, fruit weight, fruit length, fruit breadth, thickness of pericarp, fresh mace weight, dry mace weight, fresh nut weight, dry nut weight, kernel weight, fruit volume, nut volume, mace volume		No. of fruits/m^2	Tree height, leaf area
22	Nut length (mm)	Fruit set percentage, fruit weight, fruit length, fruit breadth, fresh nut weight,		No. of fruits/m^2, thickness of pericarp, fresh nut weight, dry	Leaf area

		dry nut weight, kernel weight, fruit volume, nut volume, mace volume, kernel volume		mace weight	
23	Nut breadth (mm)	Canopy spread (E-W), canopy spread (N-S), fruit set percentage, no. of fruits/m^2, fruit weight, fruit length, fruit breadth, thickness of pericarp, fresh mace weight, dry mace weight, fresh nut weight, dry nut weight, shell thickness, kernel weight, fruit volume, nut volume, mace volume, kernel volume, nut length			
24	Ratio of nut to mace		Fresh mace weight, dry mace weight, shell thickness, mace volume	No. of fruits/m^2, nut length	
25	Number of fruit/tree	Canopy spread (E-W), canopy spread (N-S), fruit set percentage, no. of fruits/m^2, fruit breadth, thickness of pericarp, kernel weight, ratio of nut to mace		Tree height, fruit weight, fresh mace weight, dry mace weight, kernel volume	

Appendix II: Degree and phenotypic association of characters

Sl. No.	Characters	Degree and association with other characters			
		Highly positive & significant	**Highly negative & significant**	**Moderately positive & significant**	**Moderately negative & significant**
1	Tree girth (cm)	Plant height,			
2	Canopy spread (E-W)	Plant height, Plant girth			
3	Canopy spread (N-S)	Plant height, Plant girth, Canopy spread (E-W)			
4	Leaf area (cm^2)	Plant height, Plant girth, Canopy spread (E-W), Canopy spread (N-S)			
5	Fruit set percentage			Canopy spread (E-W)	
6	No. of fruits/ m^2	Fruit set percentage			
7	Fruit weight (g)	Plant girth, Canopy spread (E-W)			
8	Fruit length (mm)	Fruit set percentage, Fruit weight		No. of fruits/ m^2	
9	Fruit breadth (mm)	Plant girth, Canopy spread (E-W), Canopy spread (N-S), Fruit set percentage, Fruit weight, Fruit length		Plant height, No. of fruits/ m^2	
10	Thickness of pericarp (mm)	Plant height, Plant girth, Canopy spread (E-W), Canopy spread (N-S), Fruit weight, Fruit length, Fruit breadth		No. of fruits/ m^2, Leaf area, Fruit set percentage	
11	Fresh mace weight (g)	Fruit weight, Fruit		Canopy spread (E-	

		breadth, Thickness of pericarp		W), Fruit length	
12	Dry mace weight (g)	Fruit weight, Fruit breadth, Thickness of pericarp, Fresh mace weight		Canopy spread (E-W), Canopy spread (N-S), Fruit set percentage	
13	Fresh nut weight (g)	Fruit set percentage, Fruit weight, , Fruit breadth, Thickness of pericarp, Fresh mace weight, Dry mace weight		No. of fruits/ m^2	
14	Dry nut weight (g)	Fruit set percentage, Fruit length, Fruit weight, , Fruit breadth, Thickness of pericarp, Dry mace weight, Fresh nut weight		Fresh mace weight, No. of fruits/ m^2	
15	Shell thickness (mm)	Fruit weight, , Fruit breadth, Thickness of pericarp, Fresh mace weight, Dry mace weight		Canopy spread (E-W), Canopy spread (N-S), Fruit length, Fresh nut weight, Dry nut weight	
16	Kernel weight (g)	Fruit set percentage, Fruit weight, Fruit length, Fruit breadth, Thickness of pericarp, Dry mace weight, Fresh nut weight, Dry nut weight		Fresh mace weight, No. of fruits/ m^2 , Shell thickness	
17	Fruit volume (cm^3)	Fruit weight, Fruit length, Fruit breadth, Thickness of pericarp,		Fresh mace weight, Kernel weight	

		Fresh nut weight, Dry nut weight, Shell thickness			
18	Nut volume (cm^3)	No. of flowers/ $10cm^2$, Fruit weight, Fruit length, Fruit breadth, Thickness of pericarp, Fresh mace weight, Dry mace weight, Fresh nut weight, Dry nut weight, Kernel weight, Fruit volume		No. of fruits/ m^2	Plant height, Leaf area
19	Mace volume (cm^3)	Fruit weight, Fruit length, Fruit breadth, Thickness of pericarp, Fresh mace weight, Dry mace weight, Fresh nut weight, Shell thickness, Fruit volume, Nut volume		Dry nut weight, Kernel weight	
20	Kernel volume	Fruit set percentage, Fruit weight, Fruit length, Fruit breadth, Thickness of pericarp, Fresh mace weight, Dry mace weight, Fresh nut weight, Dry nut weight, Kernel weight, Fruit volume, Nut volume, Mace volume			Plant height
21	Nut length (mm)	Fruit set percentage, Fruit weight, Fruit		No. of fruits/ m^2, Thickness of pericarp,	

		length, Fruit breadth, Fresh nut weight, Dry nut weight, Kernel weight, Fruit volume, Nut volume, Mace volume, Kernel volume		Fresh mace weight	
22	Nut breadth (mm)	Fruit set percentage, No. of fruits/ m^2, Thickness of pericarp, Fruit weight, Fruit length, Fruit breadth, Fresh mace weight, Dry mace weight, Fresh nut weight, Dry nut weight, Shell thickness, Fruit volume, Nut volume, Kernel weight, Mace volume, Kernel volume, Nut length		Canopy spread (E-W)	
23	Ratio of nut to mace	Fresh mace weight, Dry mace weight, Mace volume			
24	Number of fruit/tree	Canopy spread (E-W), Canopy spread (N-S), Fruit set percentage, No. of fruits/ m^2, Fruit breadth, Thickness of pericarp, Kernel weight, Nut breadth			

Appendix III: Genotypic path effects of characters

Sl. No.	Characters	High, positive & significant	High, negative & significant	Moderate, positive & significant	Moderate, negative & significant	Very high, positive & significant	Very high, negative & significant
1	Tree height (m)	*Thickness of pericarp, fresh mace weight, nut volume*		*Canopy spread (N-S)*	Plant height		
2	Tree girth (cm)	*Fruit weight, thickness of pericarp*	*Canopy spread (E-W), Fruit volume*	*Canopy spread (N-S)*	*Fruit breadth*		
3	Canopy spread (E-W)	*Canopy spread (N-S), fruit weight, thickness of pericarp*	Canopy spread (E-W), *fruit breadth, fresh mace weight, fruit volume*	*Fruit set percentage, dry mace weight, mace volume*			
4	Canopy spread (N-S)	Canopy spread (N-S), *fruit weight, thickness of pericarp*	*Canopy spread (E-W), fruit breadth, fresh mace weight, fruit volume*	*Dry mace weight, mace volume*			
5	Leaf area (cm^2)	*Thickness of pericarp, nut volume*		*Fruit weight, fresh mace weight*	*Canopy spread (E-W),* leaf area, *fruit volume, mace volume, kernel volume*		
6	No. of flowers/ $10cm^2$	No. of flowers/$10cm^2$	*Fresh mace weight*				
7	Fruit set percentage	Fruit set percentage, *fruit*	*Fresh mace weight, nut*	*Dry weight, mace volume*	*Fruit length, fruit breadth,*		

		weight, thickness of pericarp, kernel weight, Kernel volume	*volume*		*dry nut weight, fruit volume*		
8	No. of fruits/ m^2	*Fruit set percentage, fruit weight, thickness of pericarp*	*Nut volume*	*Kernel weight, kernel volume*	*Fruit breadth*		
9	Fruit weight (g)	*Dry mace weight, kernel weight, kernel volume, nut breadth*	*Fruit length, fruit breadth*	*Fruit set percentage*	*Canopy spread (E-W), dry nut weight*	Fruit weight, *thickness of pericarp*	*Fresh mace weight, fruit volume, nut volume*
10	Fruit length (mm)	*Thickness of pericarp, kernel weight, kernel volume, nut length*	Fruit length, *fruit breadth, fresh mace weight, dry nut weight*	*Fruit set percentage, nut breadth*		*Fruit weight, mace volume*	*Fruit volume, nut volume*
11	Fruit breadth (mm)	*Dry mace weight, kernel weight, kernel volume, nut breath*	*Canopy spread (E-W), fruit length,* fruit breadth	*Fruit set percentage*	*Dry nut weight*	*Fruit weight, thickness of pericarp, mace volume*	*Fresh mace weight, fruit volume, nut volume*
12	Thickness of pericarp (mm)	*Dry mace weight, kernel weight, mace volume, kernel volume, nut breadth*	*Canopy spread (E-W), fruit length, fruit breadth*	*Canopy spread (N-S)*	*Dry nut weight*	*Fruit weight,* thickness of pericarp	*Fresh mace weight, fruit volume, nut volume*
13	Fresh mace weight (g)	*Fruit weight, thickness of pericarp, dry mace weight, kernel volume*	*Fruit breadth, fruit volume, nut volume*	*Kernel weight*	*Fruit length, dry nut weight, nut breadth*	*Mace volume*	Fresh mace weight
14	Dry mace	*Fruit weight,*	*Nut weight,*		*Canopy spread*	*Mace volume*	*Fresh mace*

	weight (g)	*thickness of pericarp,* dry mace weight, *kernel weight, kernel volume*	*fruit volume, nut volume*		*(E-W), fruit breadth, nut breadth*		*weight*
15	Fresh nut weight (g)	*Fruit set percentage, thickness of pericarp, dry mace weight, kernel weight, kernel volume, nut length, nut breadth,*	*Fruit length, fruit breadth, nut weight*			*Fruit weight, mace volume*	*Fresh mace weight, fruit volume, nut volume*
16	Dry nut weight (g)	*Fruit set percentage, fruit weight, thickness of pericarp, dry mace weight, kernel weight, mace volume, kernel volume, nut breadth*	*Fruit length, fruit breadth, fresh mace weight,* dry nut weight		*Nut length*		*Nut volume*
17	Shell thickness (mm)	*Canopy spread (E-W), fruit weight, thickness of pericarp, dry mace weight,* shell thickness, *mace volume, nut breadth*	*Fruit breadth, fruit volume, nut volume*			*Fruit length*	*Mace weight*
18	Kernel weight (g)	*Fruit set percentage, fruit*	*Fruit length, fruit breadth,*		*Nut length*		*Nut volume*

		weight, thickness of pericarp, dry mace weight, kernel weight, *mace volume, kernel volume, nut breadth*	*fresh mace weight, dry nut weight, fruit volume*				
19	Fruit volume (cm^3)	*Kernel weight, mace volume,* kernel volume	*Fruit length, fruit breadth, fresh mace weight*	*Dry mace weight*	*Dry nut weight, nut weight, nut breadth*	*Fruit weight, thickness of pericarp*	*Fruit volume, nut volume*
20	Nut volume (cm^3)	*Fruit set percentage, thickness of pericarp, kernel weight, kernel volume, nut length, nut breadth*	*Fruit length, fruit breadth, dry nut weight*	*Dry mace weight*		*Fruit weight, mace volume*	*Fresh mace weight, fruit volume,* nut volume
21	Mace volume (cm^3)	*Thickness of pericarp, dry mace weight, kernel volume*	*Fruit length, fruit breadth, fruit volume*	*Kernel weight, nut length, nut breadth*		*Fruit weight,* mace volume	*Mace weight, nut volume*
22	Kernel volume	*Fruit set percentage, thickness of pericarp, dry mace weight, kernel weight, mace volume,* kernel volume, *nut breadth*	*Fruit length, fruit breadth, dry nut weight*		*Nut length*	*Fruit weight*	*Fresh mace weight, fruit volume, nut volume*
23	Nut length	*Fruit set*	*Fruit length,*	*Nut breadth*		*Mace volume*	*Nut volume*

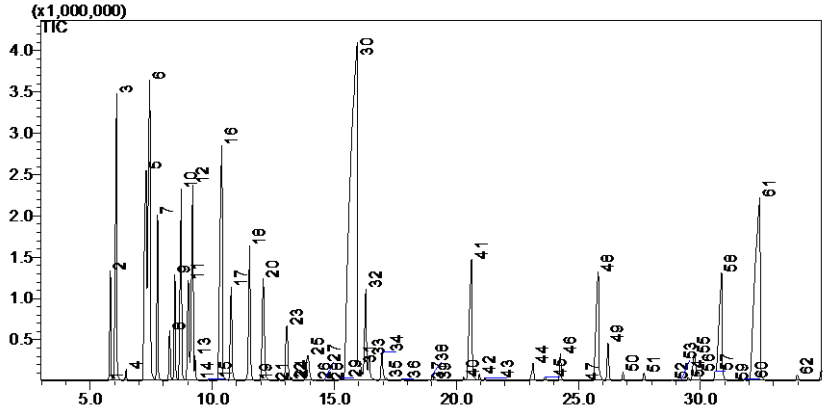
	(mm)	*percentage, fruit weight, thickness of pericarp, kernel weight, kernel volume,* nut length	*fruit breadth, fresh mace weight, dry nut weight, fruit volume*				
24	Nut breadth (mm)	*Fruit set percentage, thickness of pericarp, dry mace weight, kernel weight, mace volume,* nut breadth	*Fruit length, fruit breadth, dry nut weight*		*Canopy spread (E-W)*	*Fruit weight*	*Fresh mace weight, fruit volume, nut volume*
25	Ratio of nut to mace		*Dry mace weight, nut volume, mace volume*			*Fresh mace weight*	

*Caharcters in italics indicate their indirect effect

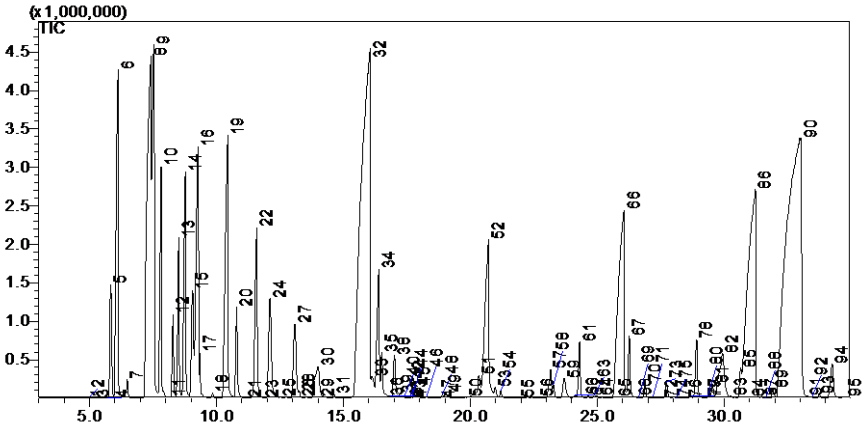
***Scales for path coefficents**

Value of direct or indirect effects	**Rate/scale**
0.00 to 0.09	Negligible
0.10 to 0.19	Low
0.20 to 0.29	Moderate
0.30 to 0.99	High
>1.00	Very high

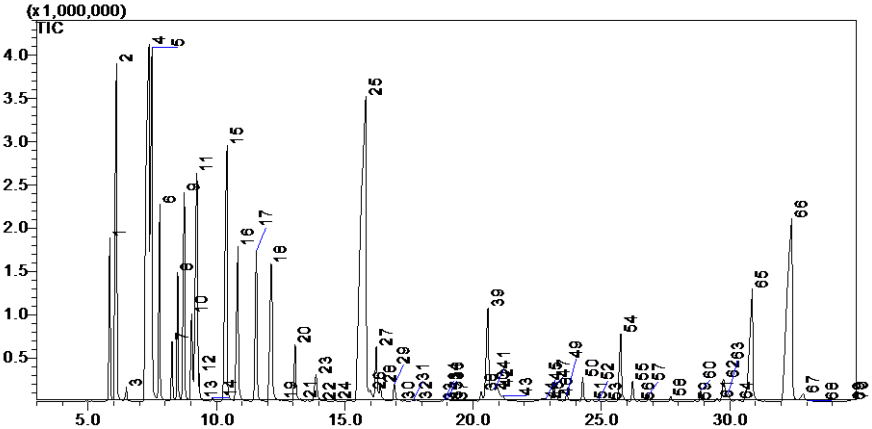
Appendix IV: GC-MS profiles of kernel volatile oil



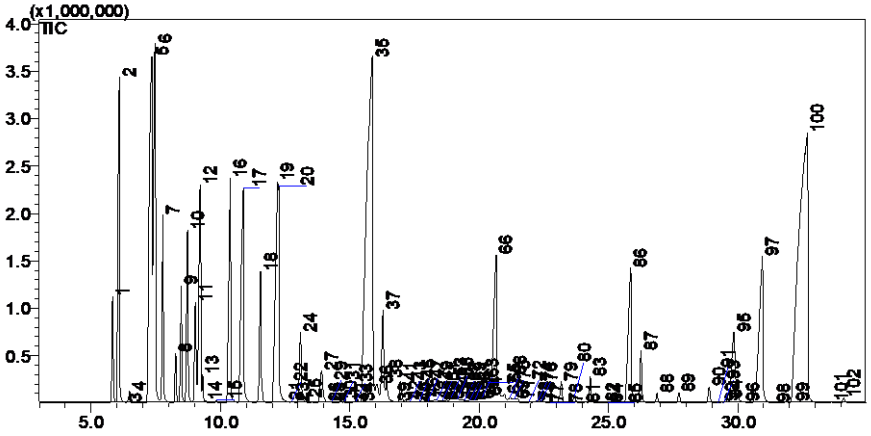
A1. GC-MS profile of kernel volatile oil of Acc. 1



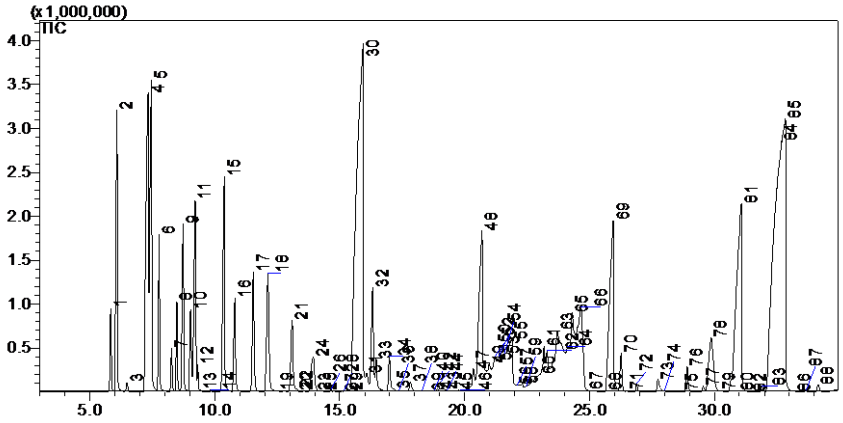
A2. GC-MS profile of kernel volatile oil of Acc. 5



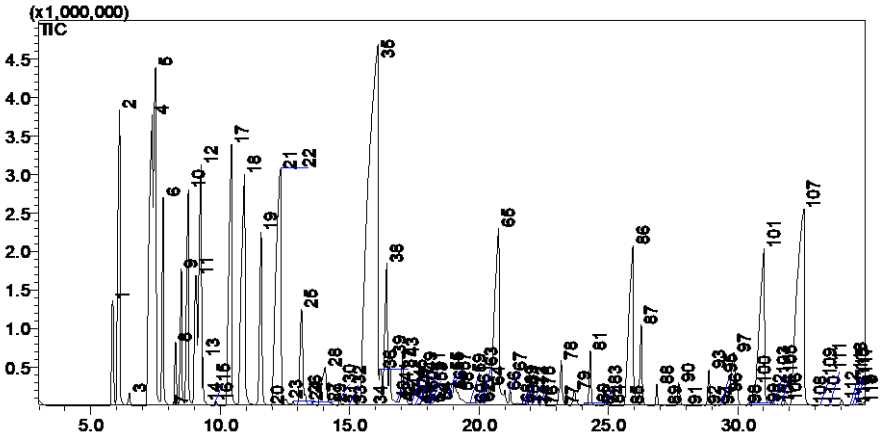
A3. GC-MS profile of kernel volatile oil of Acc. 8



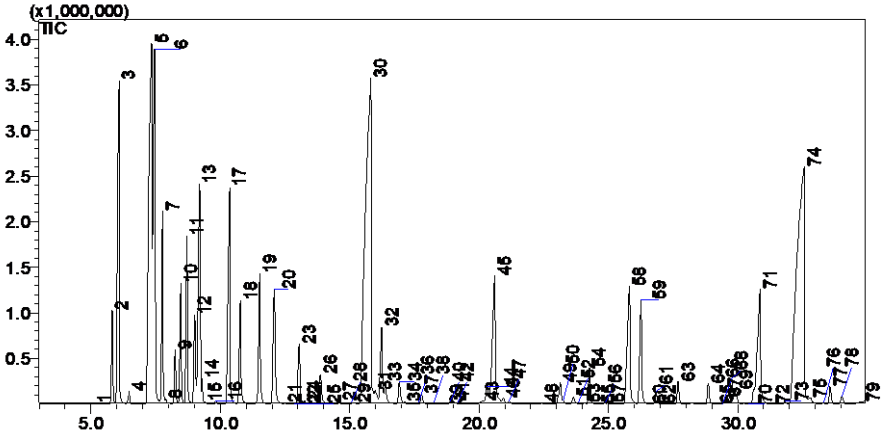
A4. GC-MS profile of kernel volatile oil of Acc. 11



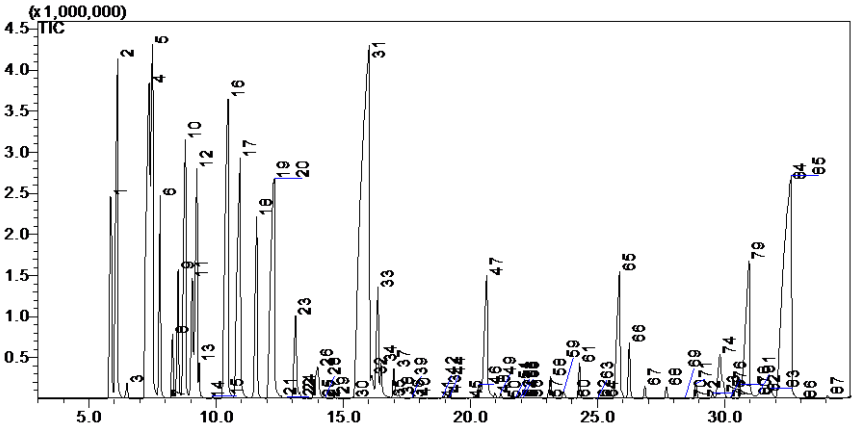
A5. GC-MS profile of kernel volatile oil of Acc. 14



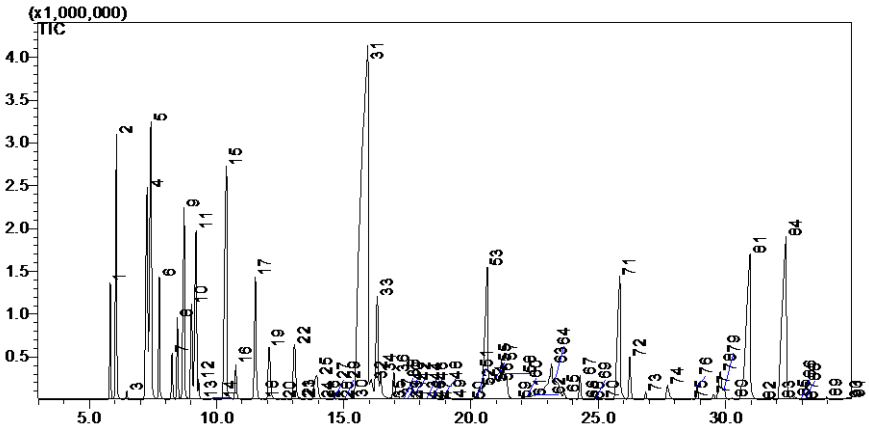
A6. GC-MS profile of kernel volatile oil of Acc. 18



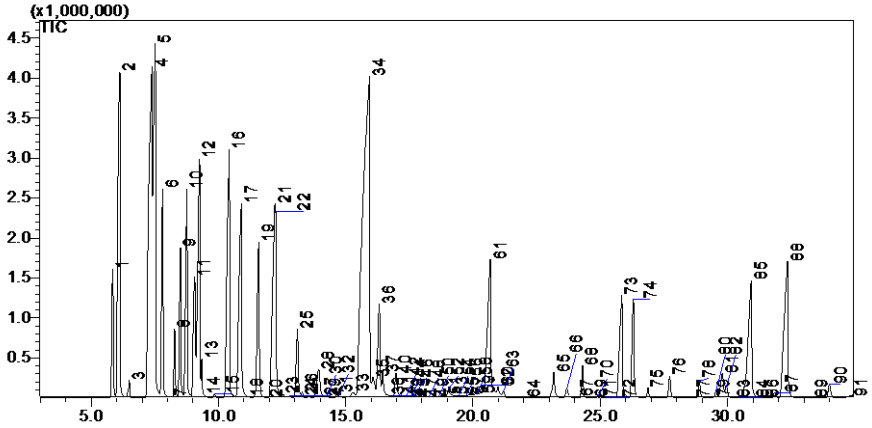
A7. GC-MS profile of kernel volatile oil of Acc. 21



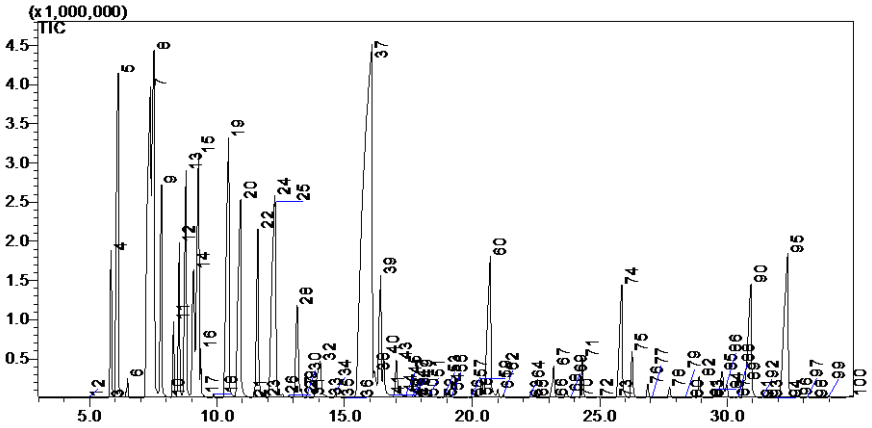
A8. GC-MS profile of kernel volatile oil of Acc. 23



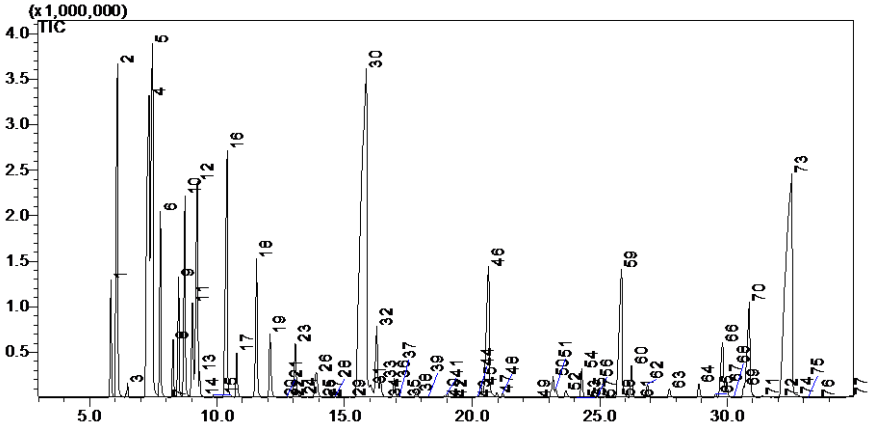
A9. GC-MS profile of kernel volatile oil of Acc. 24



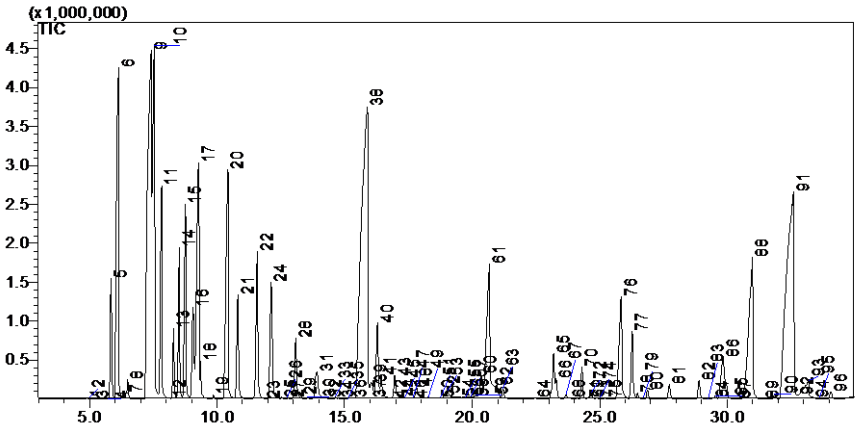
A10. GC-MS profile of kernel volatile oil of Acc. 30



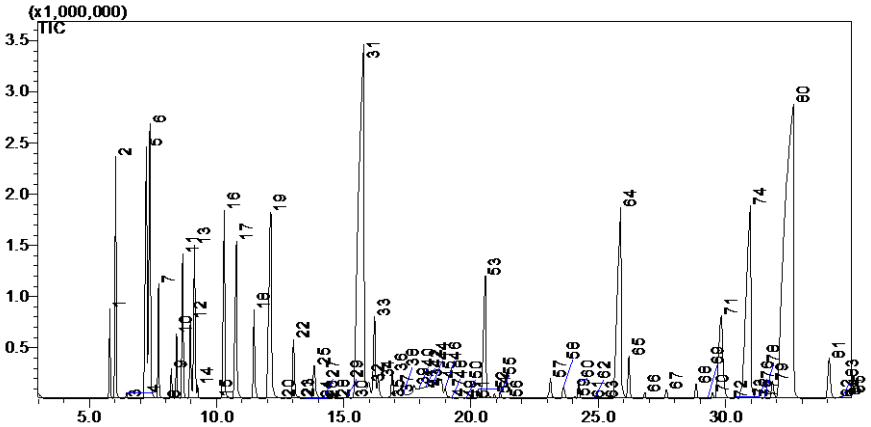
A11. GC-MS profile of kernel volatile oil of Acc. 35



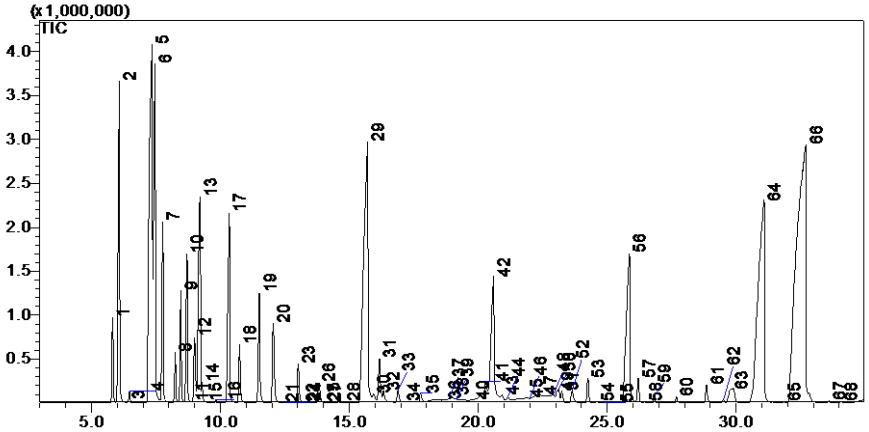
A12. GC-MS profile of kernel volatile oil of Acc. 36



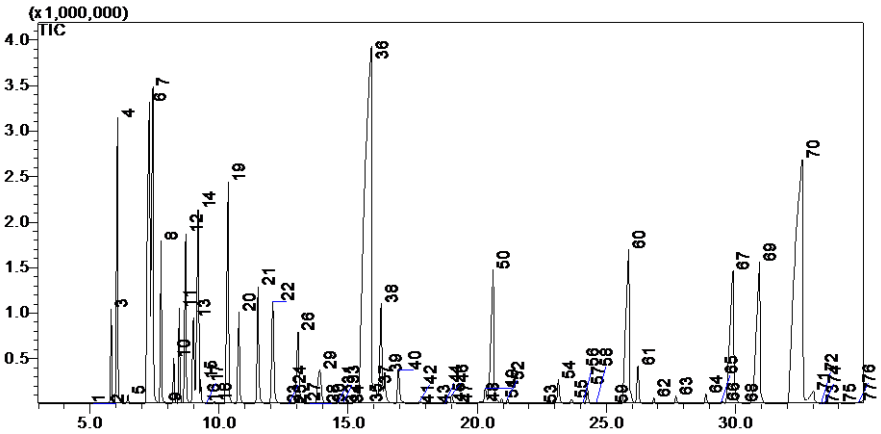
A13. GC-MS profile of kernel volatile oil of Acc. 37



A14. GC-MS profile of kernel volatile oil of Acc. 38

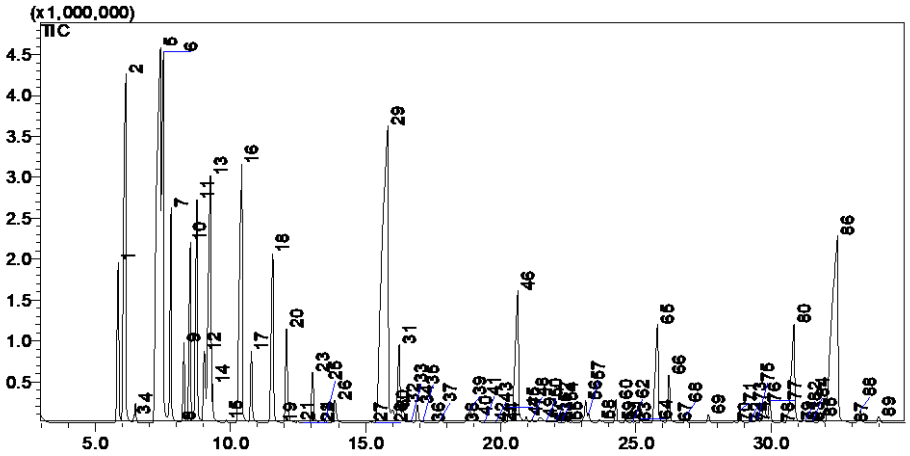


A15. GC-MS profile of kernel volatile oil of Acc. (H) 1

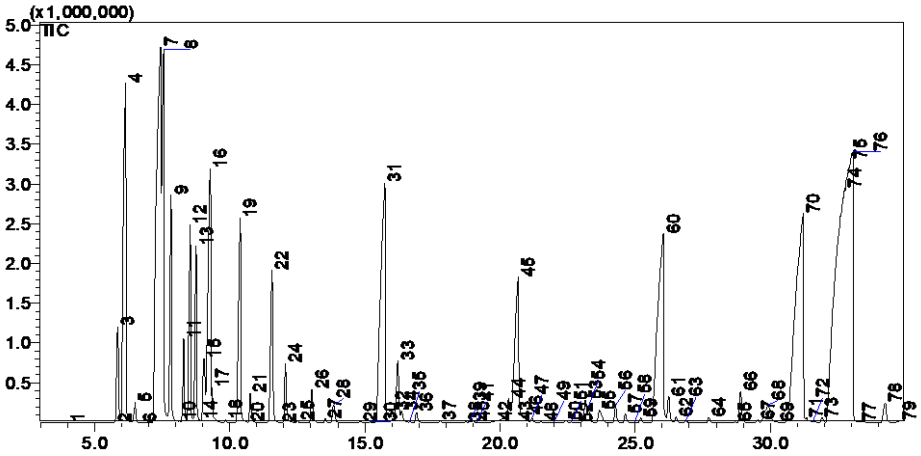


A16. GC-MS profile of kernel volatile oil of Acc. (H) 4

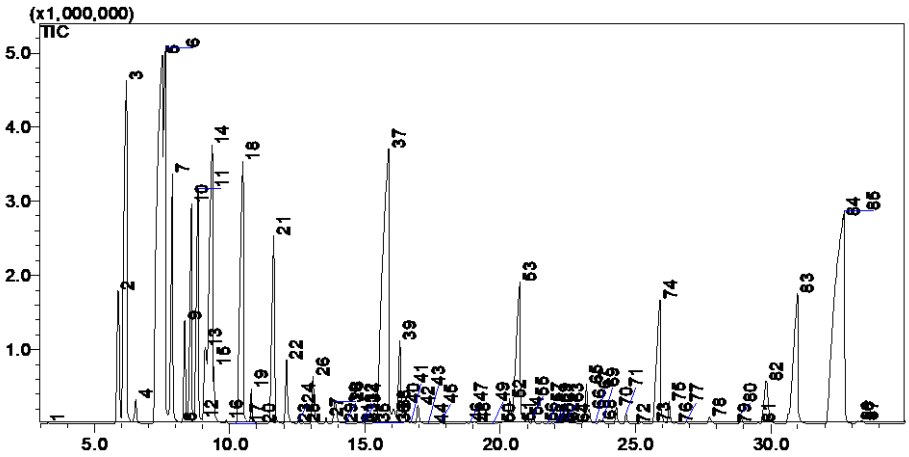
Apendix V: GC-MS profiles of mace volatile oil



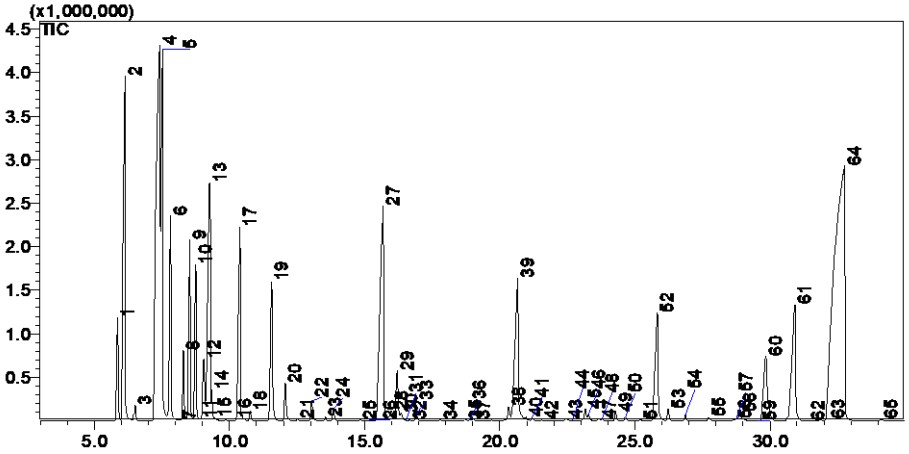
A1. GC-MS profile of mace volatile oil of Acc. 1



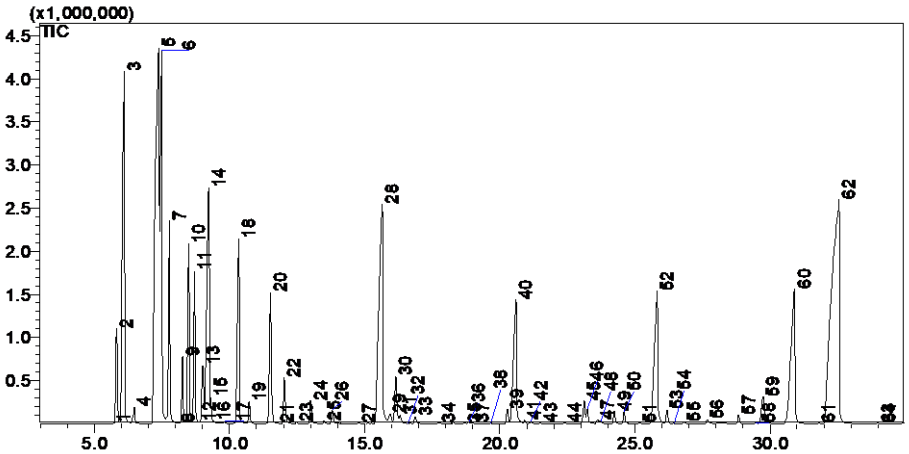
A2. GC-MS profile of mace volatile oil of Acc. 5



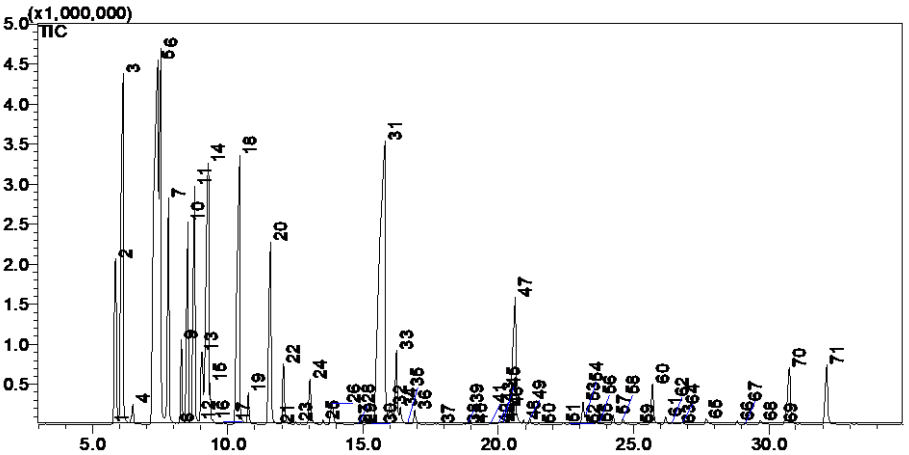
A3. GC-MS profile of mace volatile oil of Acc. 8



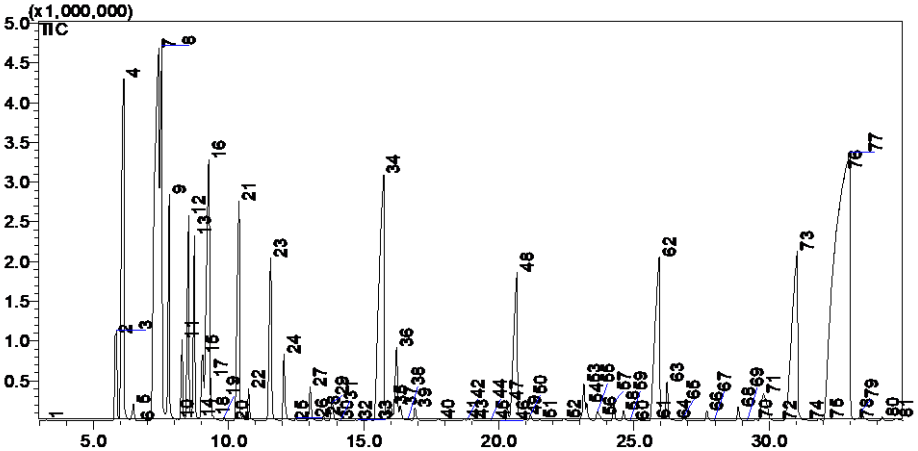
A4. GC-MS profile of mace volatile oil of Acc. 11



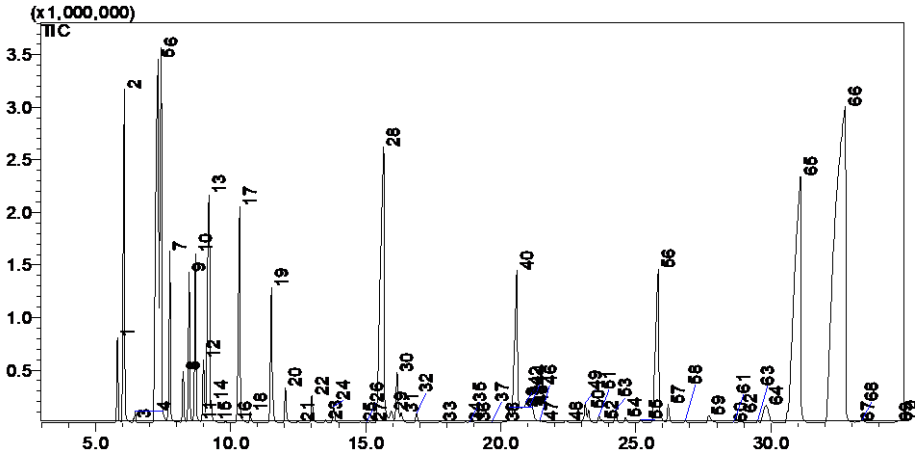
A5. GC-MS profile of mace volatile oil of Acc. 14



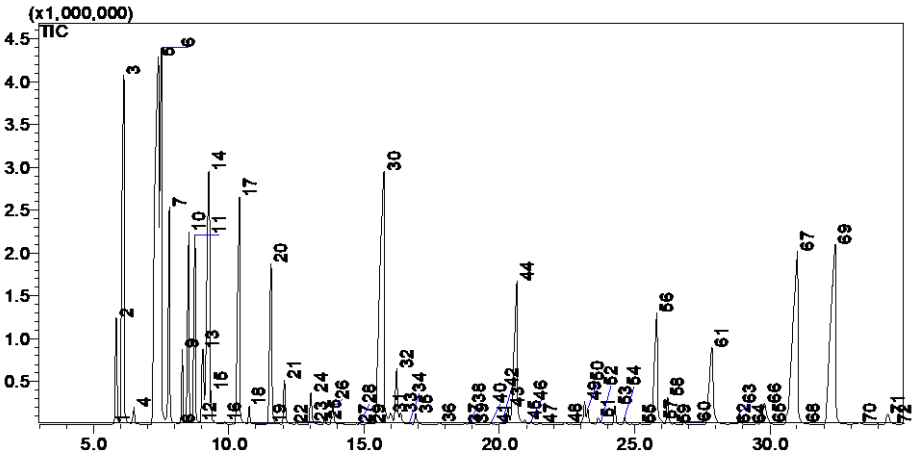
A6. GC-MS profile of mace volatile oil of Acc. 18



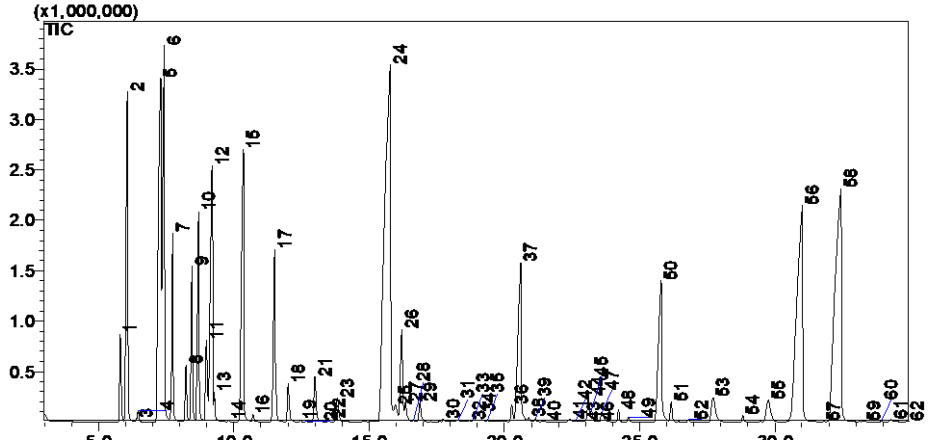
A7. GC-MS profile of mace volatile oil of Acc. 21



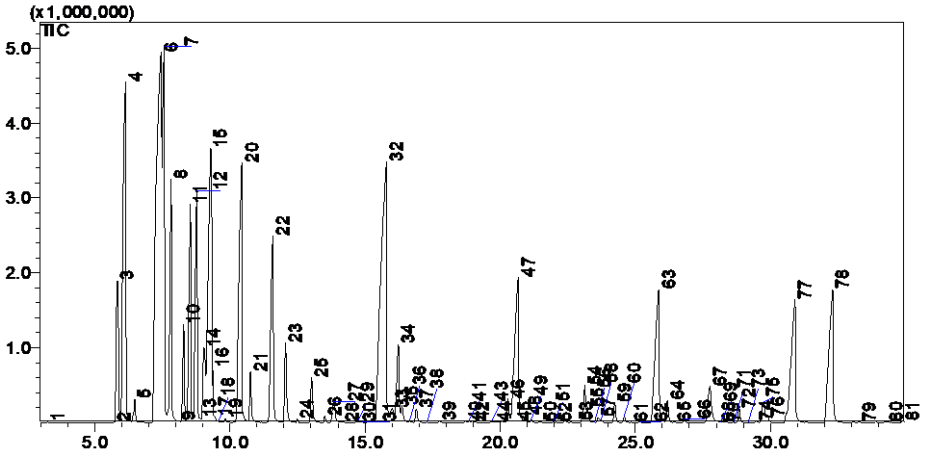
A8. GC-MS profile of mace volatile oil Acc. 23



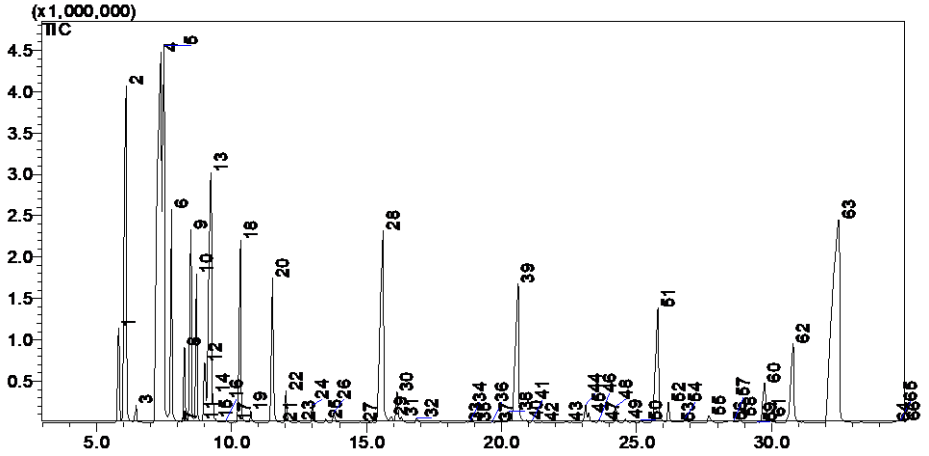
A9. GC-MS profile of mace volatile oil Acc. 24



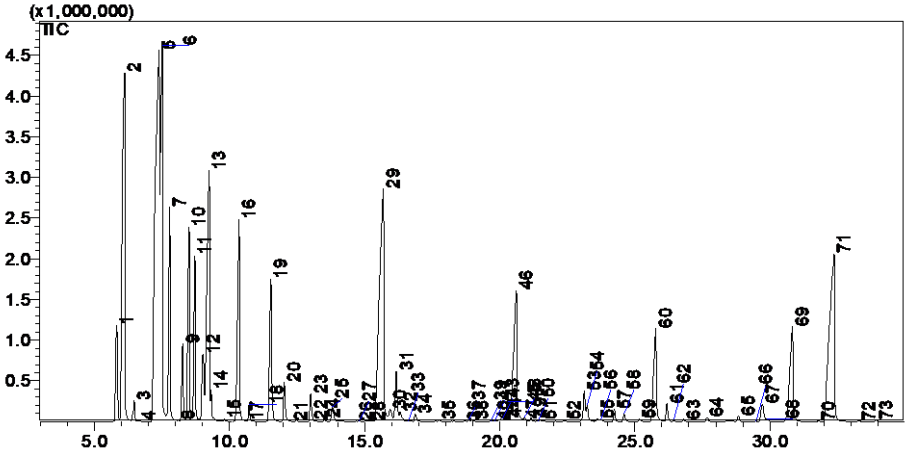
A10. GC-MS profile of mace volatile oil Acc. 30



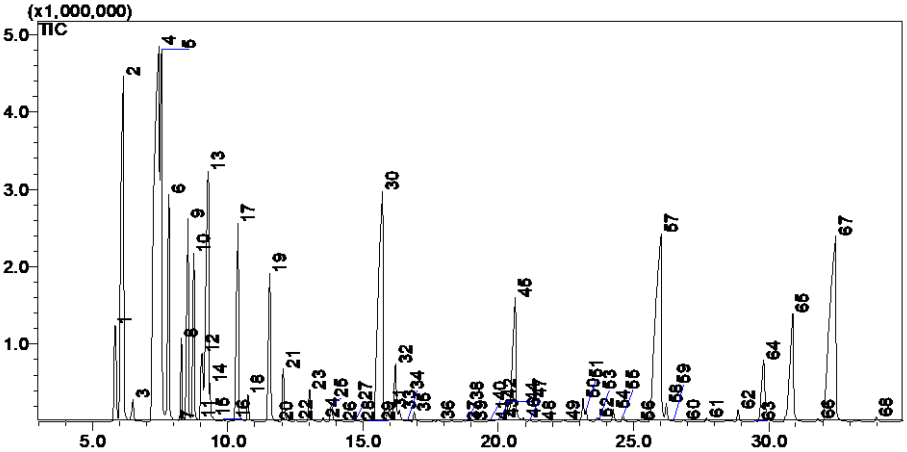
A11. GC-MS profile of mace volatile oil Acc. 35



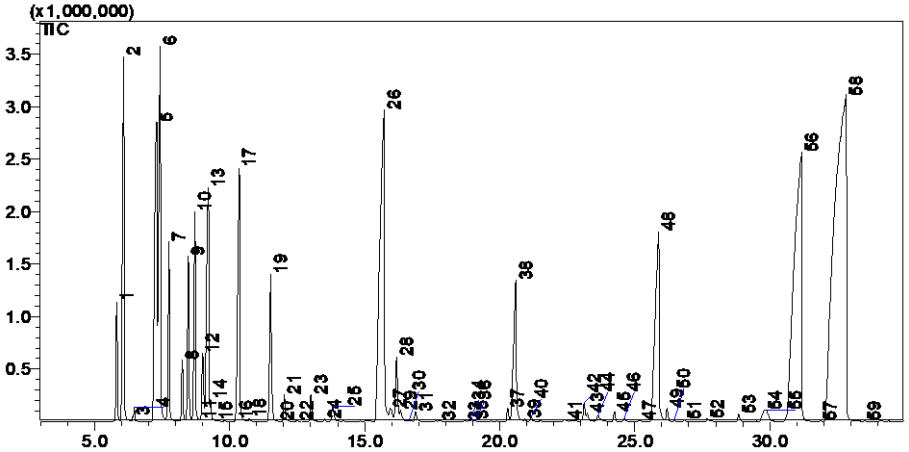
A12. GC-MS profile of mace volatile oil Acc. 36



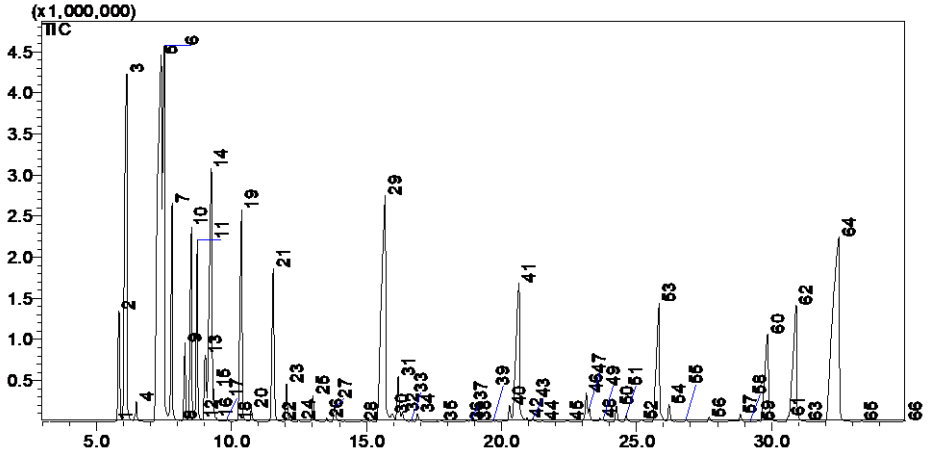
A13. GC-MS profile of mace volatile oil Acc. 37



A14. GC-MS profile of mace volatile oil Acc. 38

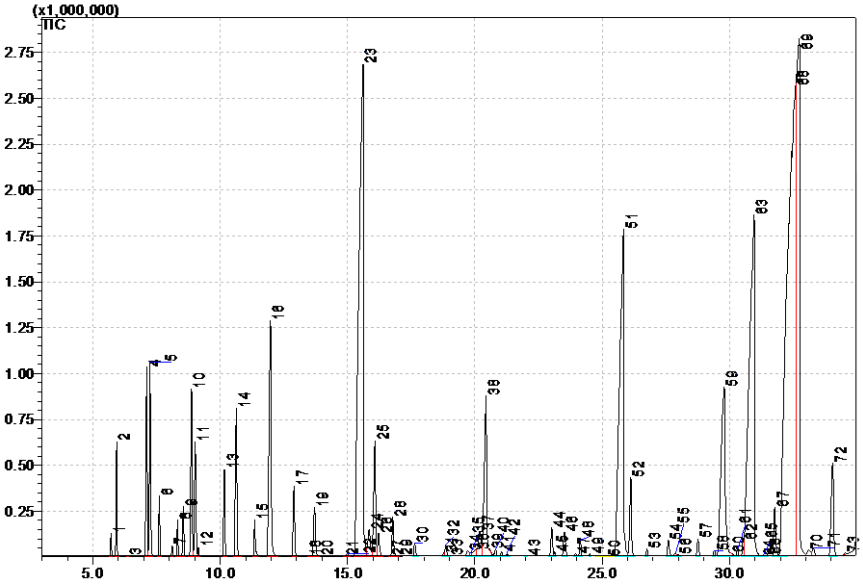


A15. GC-MS profile of mace volatile oil Acc. (H) 1

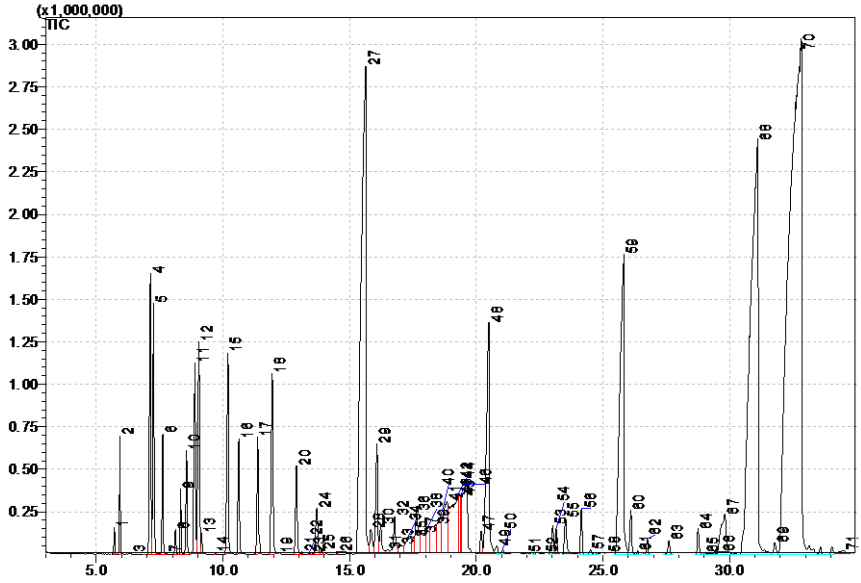


A16. GC-MS profile of mace volatile oil Acc. (H) 4

Appendix VI: GC-MS profiles of stored kernel volatile oil

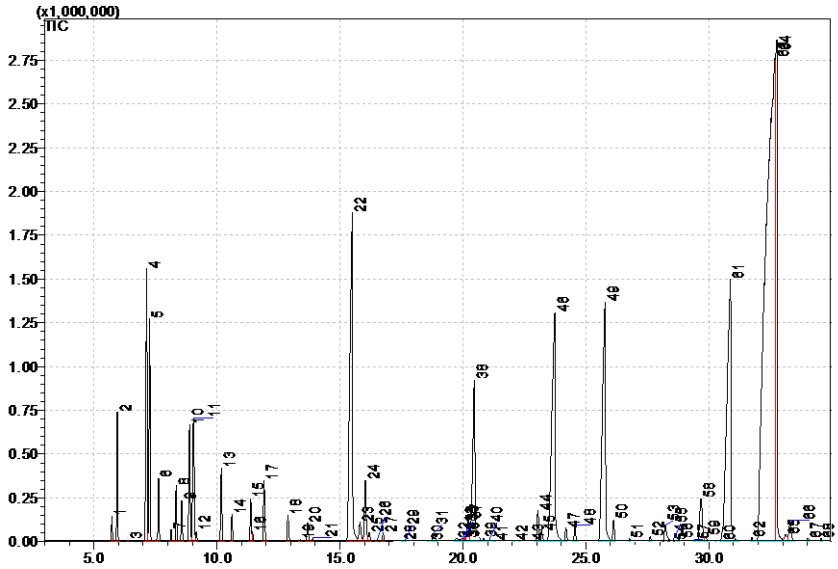


A1. GC-MS profile of stored kernel volatile oil of Acc. 38

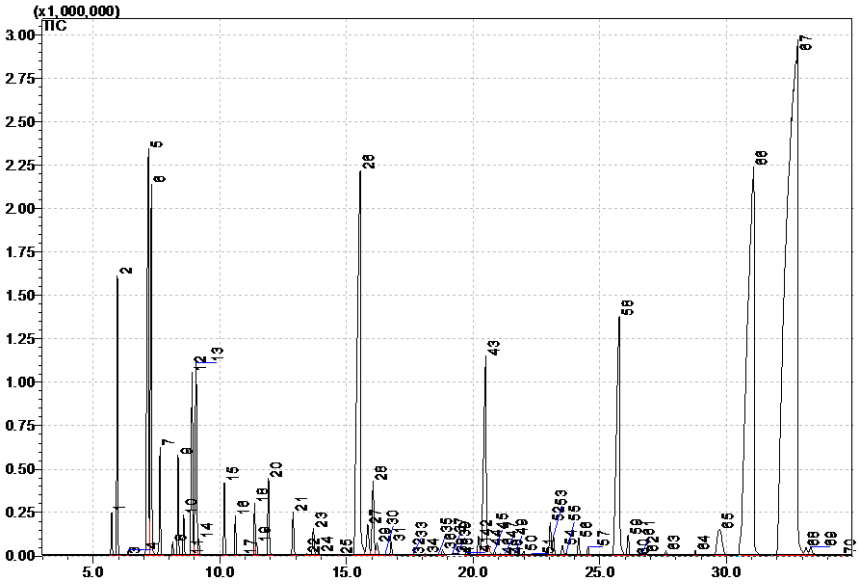


A2. GC-MS profile of stored kernel volatile oil of Acc. (H) 1

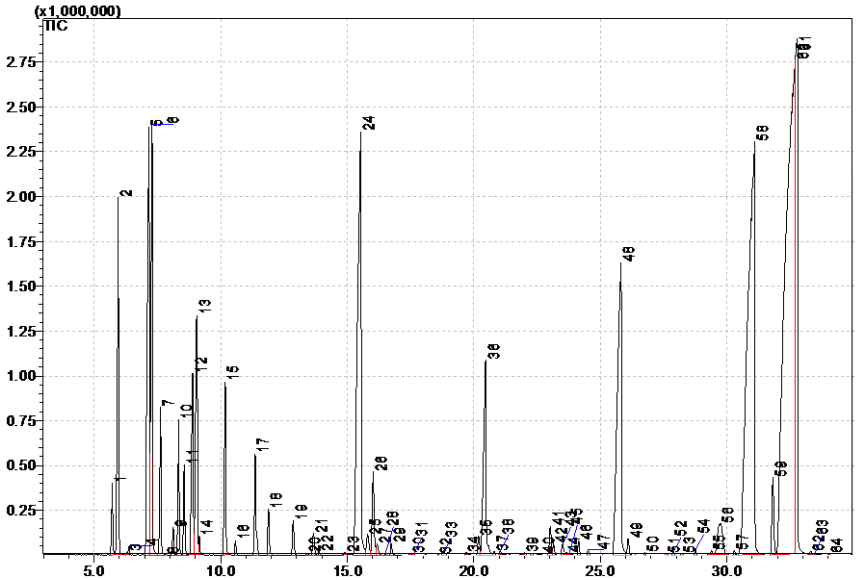
Appendix VII: GC-MS profiles of stored mace volatile oil



A1. GC-MS profile of stored mace volatile oil of Acc. 21



A2. GC-MS profile of stored mace volatile oil of Acc. 23



A3. GC-MS profile of stored mace volatile oil of Acc. (H) 1

CHARACTERIZATION AND EVALUATION OF NUTMEG (*Myristica fragrans* Houtt.) ACCESSIONS

by
Vikram H. C.
(2012-22-108)

ABSTRACT OF THE THESIS

Submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY IN HORTICULTURE
(Plantation Crops and Spices)

Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF PLANTATION CROPS AND SPICES
COLLEGE OF HORTICULTURE
KERALA AGRICULTURAL UNIVERSITY
VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA, 2016

ABSTRACT

Nutmeg (*Myristica fragrans* Houtt.) is an introduced crop to India. There exists tremendous variability in the nutmeg population in Kerala, which is the major nutmeg growing state in the country. Assessment of the existing variability is a prerequisite for taking up successful crop improvement programmes, which is very much limited in this tree spice. In this context, the present study entitled "Characterization and evaluation of nutmeg (*Myristica fragrans* Houtt.) accessions" was taken up exclusively with the specific objectives to characterize nutmeg accessions based on morphological, biochemical and molecular parameters so as to scale the variability in a multidimensional way.

Select fifty nutmeg accessions from a core germplasm collected and maintained in a private plantation in the Chalakudy river basin, belonging to age of fifteen years, formed the material for the study. Among the select fifty accessions, forty two were females, four monoecious and four males. In the morphological characterization, 51 qualitative and 38 quantitative characters were recorded from two trees per accession. Biochemical characterization was done in the select seventeen distinct accessions. GC-MS profiling was done in kernel and mace oils. Biochemical constituents of fresh pericarp were estimated. Isozyme profiling was done for peroxidase and polyphenol oxidase enzymes. The molecular characterization was attempted with 21 RAPD and 12 ISSR primers after screening. A key for identification of an elite nutmeg tree was developed.

A descriptor for nutmeg with a set of 51 qualitative and 38 quantitative parameters and descriptor states for each of these characters was developed as the first step. This is the first study of its kind to develop a minimal descriptor for nutmeg.

The descriptor developed from the present study was simultaneously utilised for morphological characterization and evaluation of the accessions. Wide variability was noticed among the accessions for 47 out of 51 qualitative characters. Four characters *viz.*, leaf margin, fruit pubescence, grooves on nut and

nature of fruit dehiscence were noted as non variable characters and hence, these were not included for further analysis. Based on the qualitative characters, accessions were classified into 11 clusters at 66 per cent similarity level. Accessions differed significantly for all the quantitative characters except shelling percentage. Performance evaluation of the accessions brought out the superiority of accession 8, 9 and 22 for yield. The accessions showed high GCV, PCV, h^2 and genetic gain for most of the characters. Number of fruits per tree, fruit set percentage, number of fruits per m^2, fresh and dry weight of mace, mace volume as well ratio of nut to mace exhibited high genetic gain. Hence, selection programme based on these characters will be very effective in improving the base populations. Based on Mahalanobis D^2 analysis, accessions were grouped into 10 clusters. Wide range of variation was observed in contents of volatile oil, oleoresin and fixed oil of kernel and mace. Based on these constituents accessions were grouped into 26 clusters, which indicated their distinct quality.

Based on the results of the morphological characterization, seventeen distinct accessions were selected for further biochemical and molecular analysis. GC-MS analysis of kernel and mace oils exhibited 20 and 24 constituents respectively. Volatile oil composition exhibited wide variability for the major constituents *viz*., myristicin, elemicin, safrole and sabinene apart from the presence of some unique compounds. Grouping of the accessions was done based on the per cent content of these important compounds. Two accessions recorded high contents of both myristicin and elemicin whereas another two accessions were in the complimentary; belonging to low myristicin group. High sabinene combined with low myristicin was the intrinsic quality attribute of one of the accessions. Change, as well as addition/deletion of specific constituents was also noticed in the volatile oils after storage for one year. Accessions exhibited wide range of variation in the biochemical constituents of pericarp, a valuable information for the value addition of pericarp. Total phenol and tannins exhibited high variation. The accessions were ranked based on the content of biochemical

constituents. Isozyme profiling using peroxidase enzyme produced four bands and that based on polyphenol oxidase exhibited three bands.

Molecular markers could assess the variability among the accessions. The selected 21 RAPD primers produced a total of 164 amplicons of which 63.21 per cent were polymorphic. The 12 ISSR primers selected produced a total of 87 amplicons of which 69.44 per cent were polymorphic. Few unique bands were detected for specific characters.

Inter cluster association of each of the qualitative clusters with other clustering patterns was worked out. The results indicated the differences as well as similarities of the qualitative clusters with other clustering patterns.

Finally, key quantitative characters were identified based on their direct and indirect effect on yield as also economic importance. The statistical key thus developed using 13 key quantitative characters will serve as a preliminary tool for identification of an elite nutmeg tree.