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Histological and Histochemical Investigations of *Myristica fragrans* Houtt. (Myristicaceae)

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Myristica fragrans Houtt. of Myristicaceae yields the nut-mug and mace which are credited with high flavours and fragrance. They are used in all parts of the world for both as food flavours and for medicinal uses. The present study provides comprehensive microscopic details of the fruit and histochemical localization of the aroma compounds. The study fills the lacuma found in the account of previous investigations

Keyword: *Myristica fragrans*, Nutmug, Mace, Microscopical and Histochemical Studies.

1. Introduction

Myristica fragrans Houtt. is an evergreen tree, native of the E. Moluccas and cultivated throughout Malaya. It is found only as a specimen tree in Botanical gardens. The seed of the plant is known as "nutmeg" and the arillus of the seed is called "mace". Both **nutmeg** and **mace** contain many volatile oils. These oil constituents have a variety of individual pharmacological effects, some of which oppose others (*Jellin et al; 2005*). The fruit contains ethereal oil-cells often with phenolic and myristicin; the seed and the aril are used for flavouring food.

Of the 72 species of *Myristica* of global level distribution, five species are reported in S. India (Gamble, 1921). *M. dactyloides* Gaert. is reported in the W.Ghats of Tamil Nadu (*Mathew, 1999; Henry et al;1987*). The S. Indian species of *Myristica* are identified on the basis of inflorescence, leaf - venation, fruit shape and colour of the aril. The main objective of the present study is to propose protocol of anatomical profile and histochemical localisation of the fragrant compounds in the fruit including seed and aril. Fragmentary account of the anatomical studies are available in the pharmacognosy books (*Wallis, 1985; Trease and Evans, 1978; Claus, 1956; Kolammal, 1979*) which give a superficial description of the fruit of *Myristica*. In the present investigation it is proposed to provide detailed cellular organization and histochemical aspect of the pericarp, seed and aril of the fruit.

2. Materials and methods

Fresh fruits of *M. fragrans* were procured from trees cultivated in Courtallum, Tamil Nadu. Dry market samples of the seed and aril were also obtained from local raw- drug dealers. Fresh samples were trimmed in different planes and fixed in the mixture Formalin- Acetic acid- 70%Ethyl alcohol (FAA) in the ratio of 5:5: 90

ml. After 24 hrs. of fixation the samples were dehydrated and paraffin infiltrated by customary method (Sass, 1940). Sections of 10µm thick were prepared with rotary microtome; sections were stained with *Toluidine - blue* (0.1% as per O'brien *et al;* 1964). For localization of aroma compounds, fresh hand sections with razor blade were prepared and stained with *Neutral red*. *Neutral red* has been reported to be a cationic dye that penetrates the cell membrane by nonionic diffusion and it accumulates in the intracellular spaces. It is said to be quick and selective staining of intact tissue of aroma storing cells (*Vogel, 1962; Effmert et al; 2005*). Microphotographs were prepared with Nikon Trinocular microscope and Nikon digital camera.

3. Observation

The fruit of *M. fragrans* is a dehiscent drupe with thick fleshy pericarp (Fig.1) and hard coated seed enclosed within much lactinated and brightly coloured aril. (Fig.3).

3.1 Pericarp

The pericarp is 7mm thick. It is differentiated into dermal tissue system, comprising outer epidermal layer an inner hypodermal layer and a vascularized middle mesocarp. The outer epidermis which is the epicarp is unistratose and consists of narrowly oblong thick walled cells with prominent cuticle. Inner to the epicarp is a narrow zone of tangentially stretched parenchyma cells. The parenchyma zone is followed by a thin discontinuous layer of **brachysclereids**. The remaining inner tissue constitutes the bulk of mesocarp of parenchymatous thin walled compact polyhedral cells. Diffusely distributed in the parenchymatous mesocarp are small vascular strands. The vascular strands are more in the middle and inner portions of the mesocarp. The vascular strands are either solitary or in clusters of three (Fig.4). The strands are collateral and consist of narrow, angular, thick walled xylem elements and prominent masses of phloem elements (Fig.4). The innermost part of the pericarp includes smaller, elliptical compressed cells and with thin inner epidermal layer of small thin walled cells.

3.2 Seed

The seed is ovoid or ellipsoidal, 3 to 3.5 cm long and 2 cm thick. The seed is enclosed within ribbon like lacinated aril which is attached at the base of the seed. The seed has shallow longitudinal furrow which represents the **raphe** (Fig.2).

3.3 Structure of the seed

The seed consists of thick seed coat measuring 140 µm in thickness along the ridges. The seed coat comprises epicarp (epidermis) which includes small, squarish cells with prominent cuticle. The inner epidermis of the seed coat includes radially elongated palisadelike compact, thin walled cells. Tannin occurs in some of the parenchyma cells which are in continuous sheath (Fig.7). Prominent vascular bundles are seen diffusely distributed within the parenchymatous ground tissue of the seed coat.

3.4 Perisperm

The perisperm is heterogeneous in cell composition. It consists of wide zone, measuring 450 - 500µm thickness. The surface layer of the outer perisperm includes a thin layer of small, angular parenchyma cells followed by compact radial files of 6 squarish cells in each row. These cells seem to be derived from a meristamatic zone found in the outer part of the perisperm (Fig.7). The inner part of the perisperm includes cells of variable size and shape. Most of the cells consist of dark, amorphous inclusions.

In the inner zone of the perisperm are seen several elongated plates which extend into the interior of the seed (Fig.7). These ingrowths are cylindrical, lobed and vermiform in appearance. The ingrowths are up to 2mm long and 450µm thick. The ingrowths have outer zone of spongy parenchyma and inner layer of small compact thick walled cells. The central core of the ingrowths have large parenchyma cells. Some of the cells in the median portion of the ingrowths are crushed into dark streak. The ingrowths have well developed vascular strands, which are collateral with distinct xylem and phloem elements.

3.5 Endosperm

In young seed the endosperm occupies the entire interior of the seed. Later, the endosperm becomes cleaved into several, radial lobes due to intrusion of the perisperm. These endosperm lobes are called **ruminate endosperm** (Fig.2.a). It is the major portion of the seed comprising soft, thin walled parenchyma cells. The endosperm cells do not stain deeply as compared to perisperm cells. Calcium oxalate prismatic crystals are sparsely seen in the outer part of the perisperm. In the mature seeds the ruminate endosperm becomes disintegrated by losing the cell contents and thinning of cell walls. The ingrowths of the perisperm assume more prominance.

3.6 Aril

The aril forms the commercial mace. The aril is flat, ribbon like repeatedly lobed creamy when young and dark red when matured. The aril is fused into wide bowl at the base of attachment to the seed and becomes free above (Fig.1.b). The aril is loosely adhering on the seed and it is easily separable.

3.7 Structure of the aril

In transectional view the aril is flat and isobilateral (Fig.11). The epidermal layers have shallow folds and have small squarish thin walled cells with prominent cuticle. The aril is 400µm thick in the middle and 150µm thick at the ends. The epidermal cells are 10µm thick. The ground tissue of the aril is in admixture of small and larger cells. The larger cells are oil bearing **idioblasts**. They are angular in outline and fairly thick walled. The idioblasts are 40µm wide, inbetween the idioblasts are smaller, angular or spindle shaped parenchyma cells (Fig.11). In the median part of the aril there are prominent discrete vascular bundles arranged in a plate. The vascular bundles have small, strands of phloem and xylem elements.

3.8 Histochemical localisation of aroma compounds

For localization of aroma compounds, fairly thin hand sections were prepared with razor blade and the sections were immersed in 0.1% *Neutral red* solution for a short time. The sections after rinsed in tap water and observed under the microscope.

All the parts of the fruit were found to possess fragrance compounds, but in varying degrees. In the seed coat dark red coloured deposits were found in the cells of the **sarcotesta** (middle parenchyma cells) (Fig.9). Those cells which possess aroma compounds occur in tangential plates alternating with empty parenchyma cells. In the pericarp of the fruit, the ground parenchyma cells exhibit aroma compounds, the cells being sparse and diffuse in distribution (Fig.5). There are also seen long, narrow, branched non-septate secretory canals in which dark stained reddish substance is seen (Fig.6). The inner zone of the perisperm is next major tissue having most of the cells filled with the darkly stained aroma compounds. When the perisperm tissue is stained with *Nile blue*, the cells in the outer zone have dark blue contents (Fig.7).

The ingrowths of the perisperm possess prominent irregular masses of darkly stained regions. These masses are inter cellular and intrude into the narrow passages of the parenchyma cells (Fig 10).

3.9 Aril (Mace) is another major source of aroma compounds of the fruit. In the flattened aril, there are wide, circular secretory cavities surrounded by epithelial cells. These secretory cavities do not possess any obvious oil content. However, the cell walls stain darker and some of the ground parenchyma cells also stain darker (Fig 11). The dark cell inclusions are obviously localization of aromatic compounds.

4. Discussion

Myristica fragrans is one of the aromatic plants that are endowed with alluring properties of fragrance and flavours. The plant produces odoriferous secondary metabolites in their fruits. The nut – meg and mace of the fruits have been popular for several hundreds of years. However the structure and organization of the aromatic parts and the specific cells or tissues which possess the fragrant compound have not been

studied fully. The seed of *M.fragrans* is so complex that it is worthwhile to make detailed study of the fruit.

The fleshy pericarp, though comparatively more voluminous than other parts contain less aromatic substances. Only some of the isolated ground parenchyma cells have darkly stained contents (Fig 5). There are also long thick branched secretory canals permeating the ground tissue of the pericarp (Fig 6). Such secretory canals with dense aroma contents are not seen in other parts of the fruit. The seed coat exhibit inclusions of the aroma compounds in the diffusely distributed cells of the **sarcotesta** (Fig 9).

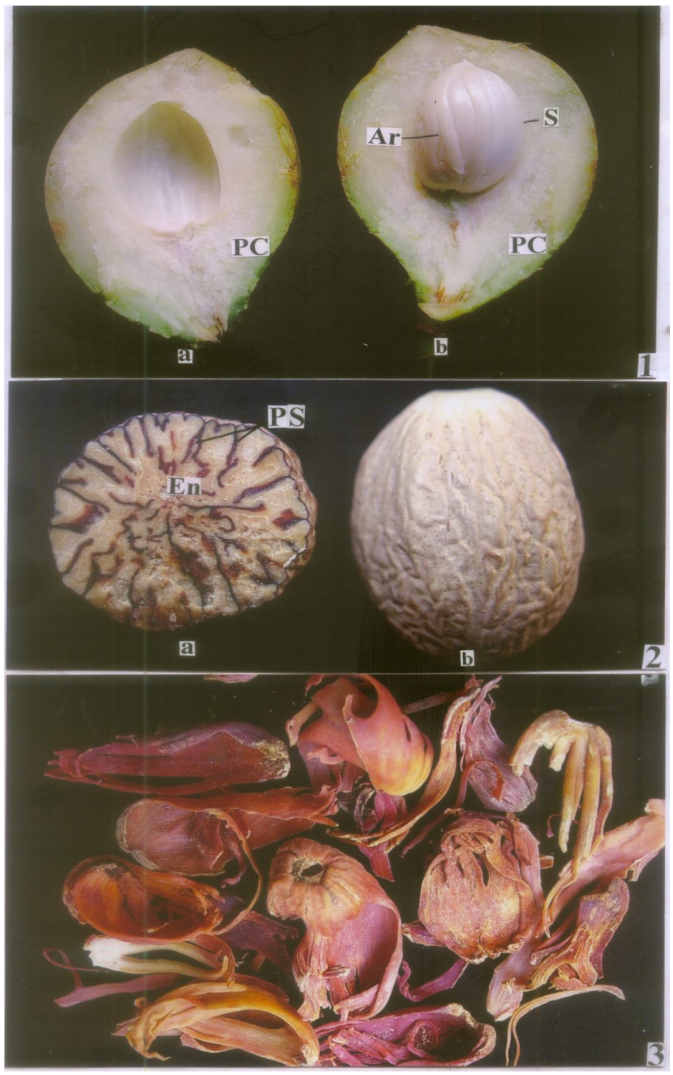

Fig.1. A fruit cut longitudinally exposing the arillate seed and seed cavity.
2.A mature seed cut across (left) and an entire seed; dark, radial and wavy lines are the ingrowths of the perisperm.
3. Dried market sample of the Aril (Mace).
(Ar - Aril, En - Endosperm, Pc - Pericarp, PS - Perisperm, S - seed).

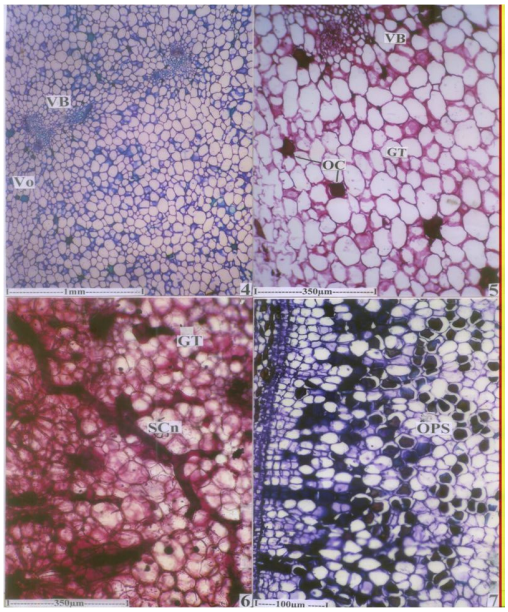

Fig.4. T.S of fruit – A sector of pericarp.
5. Ground parenchyma cells containing oil content (Stained with ***Neutral Red***).
6. Hand section of the pericarp showing anastomosing non articulate secretory canal – darkly stained (Stained with ***Neutral Red***).
7. Outer zone perisperm with dark cell inclusions (Stained with ***Nile Blue***).
(GT – Ground Tissue, OC – Oil Cavity, OPS – Outer Perisperm, SCn – Secretory Canal, VB – Vascular Bundle, Vo – Volatile oil content).

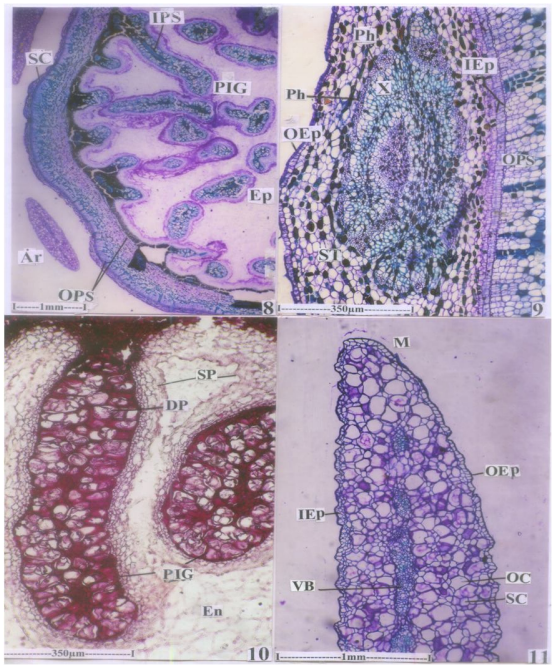

Fig.8. T.S of seed with seed coat and perisperm – ingrowths; endosperm not preserved in the section.
9. T.S of seed coat with complex vascular system.
10. Perisperm ingrowths with darkly stained cells (Stained with Neutral Red).
11. T.S of Aril – Marginal part.
(Ar - Aril, DP - Dilated Parenchyma, Ep - Epicarp, En - Endosperm, IPS - Inner Perisperm, M - Margin, OC - Oil Cavity, OEp - Outer Epidermis, OPS - Outer Perisperm, Ph – Phloem, PIG - Perisperm Ingrowth, SC -Seed Coat, SP - Spongy Parenchyma, VB - Vascular Bundle, X - Xylem).

Perisperm is a nutritive tissue which is derived from the nucellus. Maturation of the seed is followed by development of narrow radial fingerlike intrusions called perisperm ingrowths (Fig 8). Extensive development of perisperm ingrowths cleave the ground tissue of the endosperm into several wide ruminations. Later, the endosperm tissue becomes colourless and gradually disintegrates. So, the endosperm does not contribute to the aroma properties of the seed. However, the perisperm ingrowths share a major role in rendering the seed highly aromatic. The perisperm bodies have, when stained with *neutral red*, exhibits large, lobed irregular reddish masses which are intercellular in position said to originate (Fig 10).

The aril or the mace that encloses the seed was said to originate from the expanding **pachy chalaza** (Periasamy, 1961). The ruminations are perisperm in nature. According to Corner (1976) the aril is both funicular and exostomal in origin. With reference to the aroma localization, the aril consists of wide secretory ducts, surrounded by epithelial cells. These ducts possess dense accumulation amorphous substances, which are evidently the aromatic compounds.

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