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Ultra structural studies of Osmophores in the petals and pollen grains of *Jasminum grandiforum, Jasminum sambac* and *Polianthes tuberosa*

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ABSTRACT

Organelles involved in biosynthesis and especially in secretion are poorly known. The fragrance of flowers is commonly produced by volatile substances-mainly essential oils-distributed throughout the epidermis of perianth parts. The color of petals is caused by the presence of chromoplasts or pigments in the cell sap. The pigment color is usually modified by acids and other components of the cell sap. The Ultra structural studies carried out by SEM and results were obtained and revealed that most of the plant species studies in our present study Fragrance was produced by Osmophores, located in the lobes and on the adaxial surface of the experimental plant flower buds. The Osmophores of the control plants of *Jasminum grandiforum, Jasminum sambac* and *Polianthes tuberosa* were shrunken and smaller in size and surface ornamentation are poorly absorbed. The SEM study focusing on GM plants given the results of larger pollen grains. This work is described here together with anatomical studies based on flower sectioning and staining methods. The anatomical works suggests the significance different for all control plants with GM plants. The flower petals lobes of the control plants are deeply stained, and in some cases the petal lobes stained deeper than the middle portions also it shows the small circular spots. The corolla tube appears slightly stained. The petal lobes and the corolla tube of the GM plants appears slightly stained, fairly dark lobes and some black thick vertical bands on the corolla tube. These three plant species are therefore good candidates for further oil extraction and molecular studies in order to understand and manipulate the process that are responsible for high essential oil production as well as synthesis of odor.

Key words: Osmophores, corolla tube, flower petal lobes, essential oil, scents.

INTRODUCTION

The fragrance of flowers is commonly produced by volatile substances-mainly essential oils-distributed throughout the epidermis of perianth parts (Weichsel, 1956). In some plants, however, the fragrance originates in special glands named osmophores by Vogel (1962), a term derived from the Greek words scent and bearer. Various floral parts may be differentiated as osmophores and they may assume the form of flaps, cilia, or brushes. The prolongation of the spadix of the Araceae and the insect-attracting tissue in the flowers of Orchidaceae are osmophores. It is possible to identify the osmophores by staining them with neutral red in whole flowers dipped a solution of the dye. The osmophores have a secretory tissue usually several layers in depth. The emission of the volatile secretions is of short duration and is associated with a utilization of large amounts of storage products. The tissue may be compact and vascularized or it may be permeated by intercellular spaces. The oil usually vaporizes immediately but it also may appear in droplets.

The color of petals is caused by the presence of chromoplasts or pigments in the cell sap (Paech, 1955). The pigment color is usually modified by acids and other components of the cell sap. Starch is often formed in young petals. Volatile oils imparting the characteristic fragrance to the flowers commonly occur in the epidermal cells of the petals, sometimes in parts of flowers differentiated as osmophores.

Caissard *et al*., (2004) were studied aromatic plants always confused with medicinal because they secrete chemicals which sometimes have pharmacological effects. They include all the plants which produce odoriferous secondary metabolites or medicinal active compounds. Some of them are sometimes used as herbs because of their culinary properties. From a horticultural point of view, aromatic plants are often considered as plants which secrete odors by, at least, one vegetative tissue, often leaves but also roots or stems. However, in an ecological perspective, plants which produce scents by flowers can included in aromatic plants because both produce volatile organic compounds (VOCs). These compounds are generally used as attractants for species-specific pollinators or to protect plants by repulsing herbivores and pathogens. VOCs are organic molecules that have a high vapor pressure or a high volatility, i.e. they form vapors at normal pressure and temperature. In aromatic and scented plants, they originate from three categories of chemicals: phenolic compounds, fatty acid derivatives and isoprenoids. Phenolic compounds, also named aromatic compounds or benzenoids, come from tryptophan, phenylalanine and tyrosine. Examples of these include: methylbenzoate, methylsalicylate, benzylacetate, methyleugenol, benzylbenzoate. The second category of VOCs, fatty acid derivatives, is often associated with the green leaf odor emitted immediately following the breakdown and lipoxygenation of lipid membranes (e.g. linolenic acid) after mechanical damage.

However, these green leaf volatiles are sometimes also produced by flowers. The most famous among them is the phytohormone jasmonic acid involved itself in induction of other VOCs after insect or fungi injuries. The third category of VOCs, isoprenoids, also termed terpenes, comes from the mevalonic acid pathway or from the 2-C-methyl-Derythritol-4-phosphate pathway. These two pathways synthesize isopentenyl diphosphate, but the first one is located in the cytosol and the second one in plastids. Only carotenoid metabolites, monoterpenes and some other terpene derivatives are volatile e.g. limonene, menthol, linalool, caryophyllene, damascenone, and beta-ionone etc. Such benzenoids, fatty acids derivatives and terpenes can be found in flowers or leaves of different species leading to the hypothesis that the cellular secretion pathway(s) could be the same in both these types of organ. According to evolutionary theories of secondary metabolite biosynthesis and plant-insect interactions, flower scents and leaf odors could have evolved from the same metabolite pathways by co-adaptation. In plants, the main role of scented VOCs is clearly an ecological interaction with insects or other animals, even if some other roles are known. The present investigation is followed by the material methods given below to study the ultra structural variations of the petals of the experimental flowers and the volatile organic compounds analytical analysis

MATERIALS AND METHODS

Plant material

For considering the economic importance of the flower oil *J. grandiflorum*, *J. sambac,* and *P. tuberosa* were under taken in our study. In our present experiment we have used the *Agrobacterium tumefaciens* was used for this present study in the above mentioned plants. The culture was obtained from Institute Center for Advanced studies (CAS) in Botany, University of Madras, Tamil Nadu, India. Induction formations in the experimental plants tumor formation through Agrobacterium tumefaciens mediated transformation were carried out. The present investigation is the continuation work in our laboratory. Control flower buds and genetically modified flower buds of equal age (8 months) were studied for their morphological, ultra structural, anatomical, histochemical, parameters and essential oils analysis.

Scanning electron microscopic studies of flower buds

The flower buds of both control and genetically modified were preserved in FAA (Formalin + Acetic acid + Ethanol) and stored 70% alcohol. After dehydration in an ethanol-acetone (1:1) series followed by air-point drying. The flower petals and anthers were ESEM (Environmental Secondary Electron Micrograph) mode of imaging done on samples of no conduction coating made and observed with a FEI QUANTA 200 FEG HR-SEM model operated at 30kv in SAIF, IITM, Chennai.

Anatomical studies of petals

The flowers buds of both control and genetically modified were fixed in FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethanol-90ml) for two hours. The materials were washed in distilled water and dehydrated through graded series of Tertiary butyl alcohol (Sass, 1940). Following dehydration, the materials were infiltrated with paraffin wax controlled temperature (55°C). After infiltration, the specimens were cast into paraffin blocks and the blocks were stored in Refrigerator for sectioning.

Serial sections to the thickness of 6-8µm were prepared with the help of Rotary Microtome. The sections were dewaxed and stained with 0.05% Toluidine blue O **(**O'Brien *et al.,* 1964) (dissolved in water) for general anatomical studies. Since it is a Meta chromatic dye, it gave good results for studying gross anatomical features of the floral parts. For localization of the aromatic compounds in the flowers, sections were stained with 0.5% Neutral Red which was found to be aromatic compounds (Vogel, 1962). Entire flower buds were immersed in dilute Neutral Red for 10-15 minutes and washed in tap-water. This procedure indicated various regions of the flowers which stained red indicating the location of the odor emanating cells in the flower.

Both external and microtome sections were photographed with NIKON Coolpix-8400 Digital camera and NIKON Labphoto-2 microscopes. Magnifications of the micrographs are shown by the scale-bars. Descriptions of the structure of the flower were as per the standard Anatomy books (Esau, 1964). Anatomical sections of flower buds were undertaken in Prof. Jayaraman's lab, Plant Anatomy Research Center (PARC), West Tambaram, Chennai.

RESULTS

Ultrastructural studies of the osmophores and pollen grains of *Jasminum grandiflorum*, *Jasminum sambac* and *Polianthes tuberosa*

Scanning electron micrograph (SEM) of the petals and pollen grains of both control and genetically modified plants of *Jasminum grandiflorum*, *Jasminum sambac* and *Polianthes tuberosa* was observed and the results were recorded (Fig. 1A-F). Osmophores in control plants of the all above mention plants were shrinken and smaller in size. Surface ornamentation also poorly absorbed in the control flowers in the genetically modified plant osmophores and pollen grains shape and size of the osmophores were larger and good. The surface ornamentation of the petals, osmophores and pollen grains of the control plants were poorly developed.

So the genetically modification enhanced there osmophores in size and shape ornamentation in all our above mention experimental plants. Results were recorded and photographs were taken in various magnifications.

Anatomy of the flowers
***Jasminum grandiflorum* L.**
Control flowers

The flowers have five free petal lobes and narrow long, cylindrical corolla tube. The free petal lobes are pearly white; the corolla tube is pale green.

Flowers immersed in Neutral Red, show the entire petal lobes were deeply stained; the corolla tube was slightly stained.

Structure of the petals

The middle portion of the petal is 330µm thick. It has wide and darkly stained inner epidermis, slightly narrow outer epidermis and fairly well preserved mesophyll tissue the inner epidermal cells are modified into osmophores; the cells are vertically oblong and palisade-like, with dark cell inclusions. The cells are 30µm in height and 15µm wide. The outer epidermis has wide, flat squarish cells and no cell inclusions are evident. The cells are 30×20µm in size. The mesophyll tissue consists of outer zone of lobed, loosely arranged cells and inner zone of large compact parenchyma cells. The vascular strands have small cluster of xylem elements and a group of wide phloem elements.

Petal margin

The marginal part of the petal is conical and blunt with well preserved epidermal cells and mesophyll tissue. The marginal portion is 60µm thick. The inner epidermis of the petal margin is modified into distinct, papillate osmophores. They are 20ìm in height. The outer epidermal cells are flat and rectangular and are 10-15µm thick. The mesophyll has two or three layers of compact, wide, parenchyma cells. The cuticle of the inner epidermis is slightly echinate.

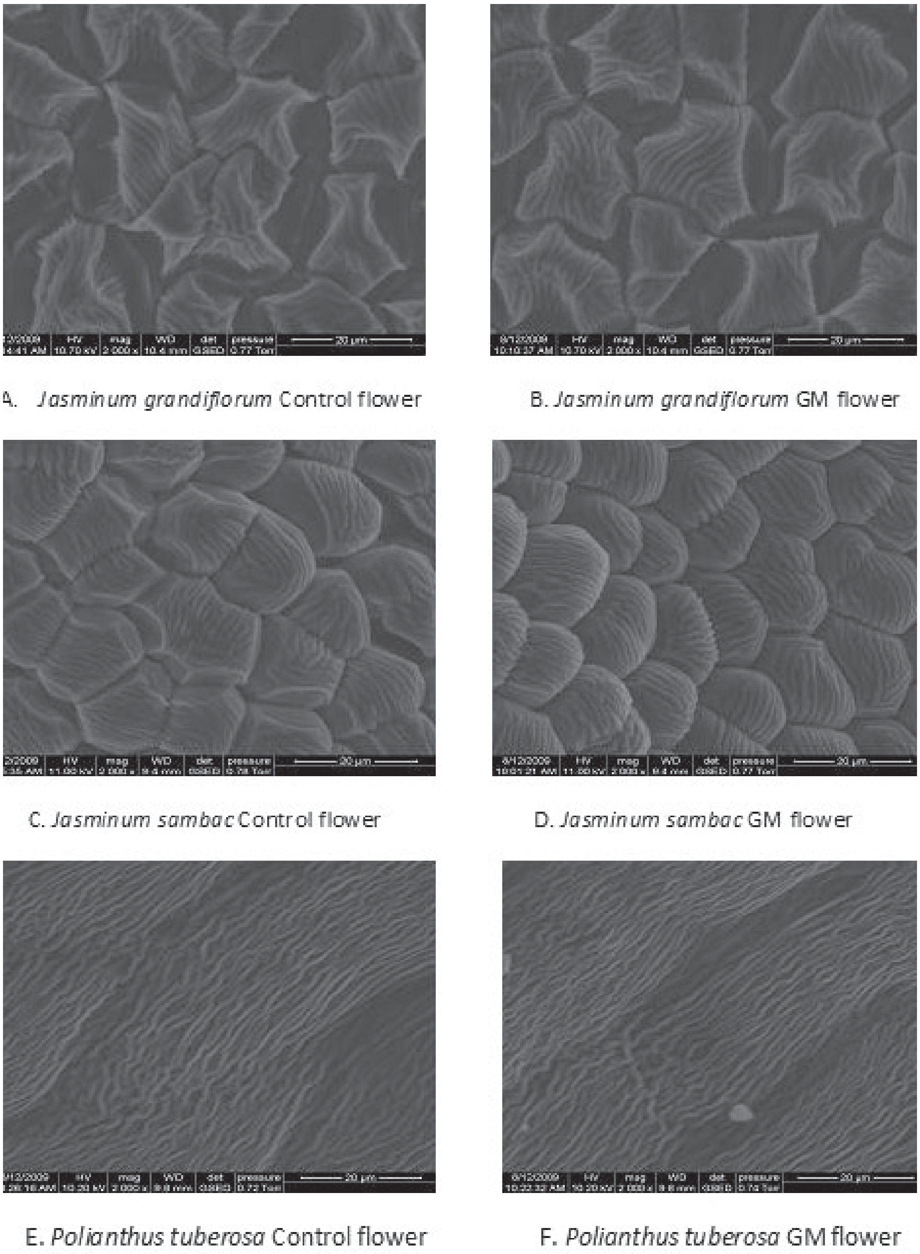
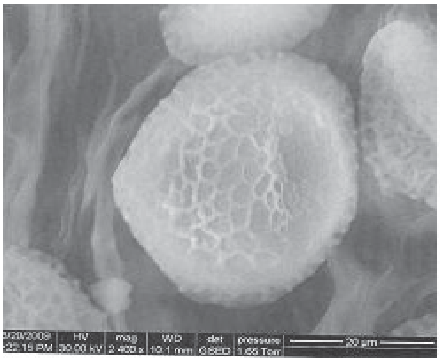


Fig. 1: Scanning electron micrograph of the osmophores in the petals of experimental plants

Sections stained with Neutral Red showed localization of the aromatic compounds. The dense contents were found to be more in the inner epidermal osmophores of middle portion of the petals comparatively less cell contents were seen in the outer epidermis and in the epidermis of the marginal portion No aromatic compounds were observed in the mesophyll tissue.

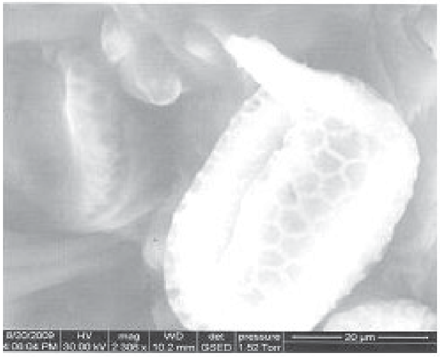
Jasminum grandiflorum **L.**
Genetically modified flowers

The flowers have bright white petals and dark brown or maroon colored corolla tube. The flowers immersed in Neutral Red showed fairly dark stains in the petal lobes. The corolla tube became pale green or white.

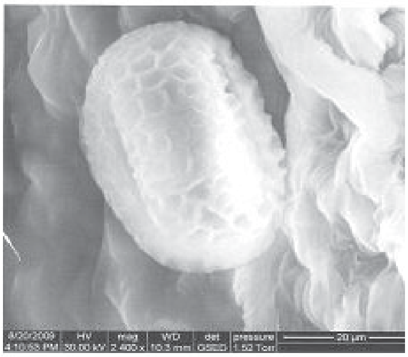


Jasminum grandiflorum Control flower

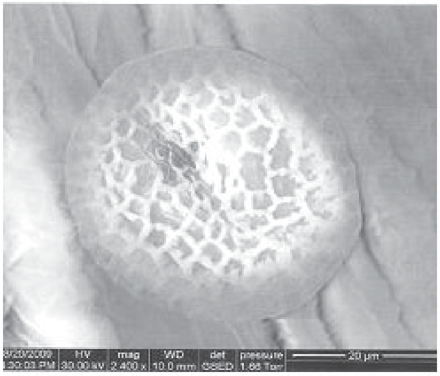
Jasminum grandiflorum GM flower



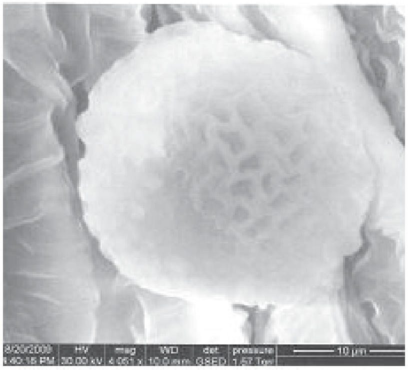
Jasminum sambac Control Flower



Jasminum sambac GM flower



Polianthus tuberosa Control Flower



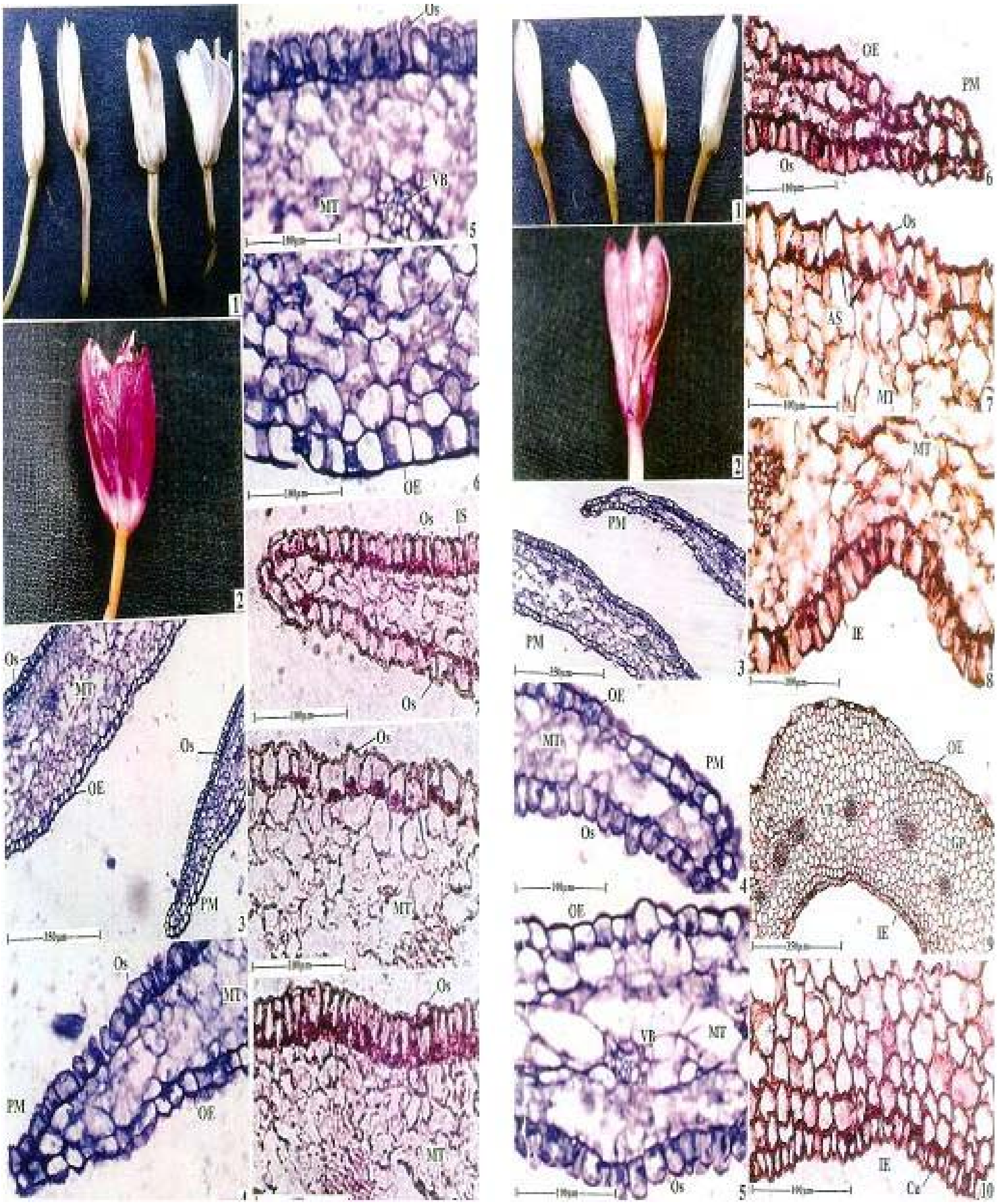
Polianthus tuberosa GM Flower

Fig. 2: Scanning electron microscope of the pollen grain

Structure of the petals

The middle and marginal portions of the petal lobes have well developed osmophores on the inner sides. The middle part of the petal is 160ìm thick; the marginal part is 70µm thick. The inner epidermal cells both along the margins and middle portions are modified into prominent osmophores.

The osmophores are either conical or bluntly papillate. The osmophores of the marginal part of the petal are 15×25µm in size. Those on the middle part of the petal are 20×25µm. The outer epidermal cells on the marginal and middle portions of the petal are flat and rectangular or squarish in shape. The osmophores have denser cell contents when compared to outer non - osmophore cells.

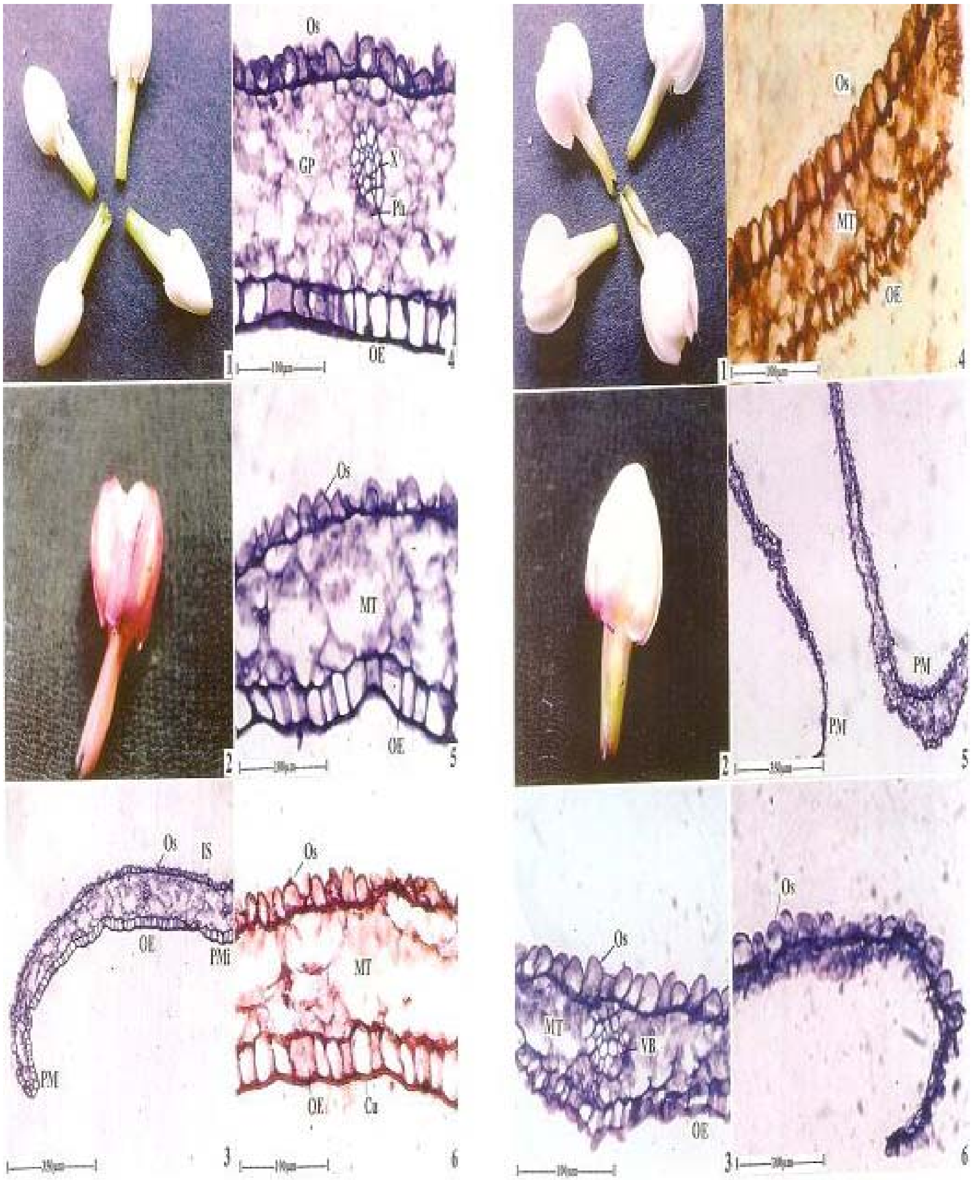


***Jasminum grandiflorum* Control flower** ***Jasminum grandiflorum* GM flower**

Fig. 3: Anatomical studies of *Jasminum grandiflorum*

In the sections stained with Neutral Red the osmophores of the inner portion of the petal margin exhibit denser and darker cell contents than the outer epidermal cells. The inner osmophores of the middle part of the petal have prominent nuclei and dense cell contents. The outer epidermal cells of the corresponding portion of the petal also show accumulation of the aromatic substance to limited extent.

Transverse sections passing through the tubular part of the corolla show osmophores along the inner surface and ordinary cells on the outer surface. The osmophores have conical shape and echinate cuticular outgrowths. The outer epidermal cells have flat smooth cell walls. The ground tissue has well preserved parenchyma cells and a ring of vascular bundles.



Jasminum sambac **Control flower** ***Jasminum sambac*** **GM flower**

Fig. 4: Anatomical studies of *Jasminum sambac*

***Jasminum sambac* (L.) Ait.**

Control flowers

The flower buds have many petal lobes which are closely imbricated forming an ovoid-conical body. The lower corolla tube is pale-green.

When the flower buds were immersed in 0.1% aqueous Neutral Red solution for five to ten minutes, different parts of the flower bud absorbed the stain in different densities. The margins of the petal lobes stained deeper than the middle portions. The basal portion of the petal lobes was also deeply stained. The tubular portion of the corolla was less stained.

Structure of the petals

The marginal part of the petal is 80µm thick; the middle portion is 120µm thick. The epidermal cells of the inner side of the petal have developed into osmophores. The outer epidermal cells remain normal. The osmophores of the marginal part of the petal have broad basal part and conical outer part. The osmophores of the marginal part of the petal are 20µm in height; the basal part is also 20µm wide. The outer epidermal cells of the marginal part are 25µm in height and 15-20µm wide.

The osmophores in the middle part is 15µm in height and 10µm wide. The outer epidermal cells are 20×20µm in size.

***Jasminum sambac* (L.) Ait**

Genetically modified flowers

When compared to *Jasminum sambac* (L.) Ait. Control flower sample, the genetically modified flower sample flowers are large; especially the free petals form prominently spherical body. The corolla tube is thin and narrows. Both the petals and corolla tube are white.

Flowers, when immersed in Neutral Red, only the basal narrow zone of the petal lobes stained slightly. Other regions do not show positive reactions.

Structure of the petals

The middle portion of the petal has prominent osmophores on the inner surface; the outer surface has normal flat epidermal cells. The osmophores are 20×20µm in size. The petal thickness is 60ìm. The vascular strands are well preserved and consist of wide, angular thick walled xylem elements, measuring 10µm in diameter. The mesophyll tissue is disintegrated partially. Neutral Red stained sections exhibit accumulation of darkly stained substances along the basal part of the osmophores. The mesophyll cells and outer epidermis show only partial response to the Neutral Red.

The marginal portion of the petals, especially the lower ends of the petals, possesses prominent osmophores with dense cell contents. The osmophores are hemispherical, measuring 20µm wide and 15µm thick. The petal has narrow compressed mesophyll tissue measuring 30µm thick.

***Polianthes tuberosa* L.**

Control flowers

The flower has long, cylindrical, bent or straight basal tubular part and six triangular petal lobes. Both the tube and petal lobes are pearly white.

The flowers immersed in Neutral Red solution showed diffuse patches of less prominent colored regions. There are small circular dark spots in the petal lobes as well as on the corolla tube. Sections passing through these colored regions show well developed osmophores both on the inner and outer surfaces. The osmophores on the inner surface have conical outer walls and prominent cuticular echinate outgrowths. The cells also possess dense masses of aroma compounds. The osmophores of the outer surface of the petals have darkly staining walls and no cuticular spines are evident. In the sections stained with Neutral Red, the outer echinate walls and the spiny outgrowths appear deeply stained. The outer osmophores are 20-30µm in height and 20µm wide. The inner osmophores are 20-50µm in height and 25µm in wide.

The tubular part of the corolla has narrow smooth inner and outer epidermal layers, excepting the narrow darkly stained spots. In these spots, there are prominent osmophores similar to those of the petal lobes. In other regions, there is no evidence of osmophores. The mesophyll tissue is not well preserved; the vascular strand has intact thick walled angular or circular xylem elements.

***Polianthes tuberosa* L**

Genetically modified flowers

The flowers have gamopetalous perianth lobes with six free petals and long, narrow, slightly lent corolla tube. The petal lobes and the corolla tube are milky white. Flowers immersed Neutral Red show small isolated small circular colored spots on the petal lobes and fairly thick vertical bands of dark colored regions on the corolla tube.

Structure of the petals

The petal lobes are thick in the middle and conical and pointed along the margins.The middle part are 160µm thick; the marginal part is 50µm thick. Osmophores are well developed both along the petal margins and in the middle portion. The outer surface of the petal has smooth walls and rectangular cells.

The osmophores on the inner surface of the middle as well as marginal parts of the petal have broad basal part and narrow, conical upper part with nipple likes ends. The osmophores in the middle part are more prominent than those of the marginal part. In the middle part, the osmophores are up to 50µm in height. In the marginal part they are up to 30µm in height. In sections stained with Neutral Red, the walls of the osmophores as well as the echinate cuticular outgrowths stain deeper. The osmophores of the marginal portion also stain darkly.

DISCUSSION

Genetically modified plants were more efficient in producing odour scent and an essential oil etc, because it possess the well grow behavior of osmophores and pollen grains than the control plants. As this *J. grandiflorum*, *J. sambac,* and *P. tuberosa* are more and more genetically treated this alternative approach will certainly lead to fruitful discoveries. The Osmophores of the control plants of *J. grandiforum, J. sambac* and *P. tuberosa* were shrunken and smaller in size and surface ornamentation are poorly absorbed. The SEM study focusing on GM plants given the results of larger pollen grains. This work is described here together with anatomical studies based on flower sectioning and staining methods. The anatomical works suggests the significance different for all control plants with GM plants. The flower petals lobes of the control plants are deeply stained, and in some cases the petal lobes stained deeper than the middle portions also it shows the small circular spots. The corolla tube appears slightly stained. The petal lobes and the corolla tube of the GM plants appear slightly stained, fairly dark lobes and some black thick vertical bands on the corolla tube. These three plant species are therefore good candidates for further oil extraction and molecular studies in order to understand and manipulate the process that are responsible for high essential oil production as well as synthesis of odor.

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