Seaweed Compost on Lycopersicum esculentum with Special Reference to Trichomes Using Scanning Electron Microscopy with Energy Dispersive Spectroscopic Analysis

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Abstract - The diversification of chemical production in glandular trichomes is important in the development of resistance against pathogens and pests. In the present study attempt was made on marine macro algae (Sargassum wightii) compost (organic manure) was treated with Lycopersicum esculentum and intensive inventerization was observed on trichomes using scanning electron microscopy, it reveals that the epidermal cells grow out in the form of hairs at trichomes. They are unicellular or multicellular and occur in various forms. They vary from small protuberances of the epidermal cells to complex branches or stellate multicellular structures. Trichomes range in size from a few microns to several centimeters and they exhibit a tremendous species, specific diversity in shape and therefore, they are often used as diagnostic characteristics for the identification of plant species, trichomes are mainly found on leaves and stems, sepals, petioles, peduncles and trichome may also complement the chemical defense of a plant by a possessing glands which exclude terpenes, phenolics, alkaloids, or other substances which are olfactory or gustatory repellents. In essence glandular trichomes afford an outer line of chemical defense by advertising the presence of "noxious" compound trichomes are abundant in tomato plants, in the present study two types of trichomes were observed multicellular hairs and glandular trichomes. The glandular trichomes, were responsible for secreting a yellow substances that gives off that characteristics tomato plant smell. Further tomato leaf of was subjected to SEM with energy dispersive spectroscopic analysis it reveal that following chemical elements were observed Si>Na>Mg>Ca>K>Cl>Zr>Mo and > Zn were noticed.
Keywords: Seaweed compost, Tomato, Scanning electron microscopy, elemental composition

I. INTRODUCTION

Virtually all plant species possess some kind of hair-like epidermal structures. When these structures are present on the aerial parts of a plant, they are commonly referred to as trichomes. The trichomes originate from single epidermal cells, which first undergo a periodinal division. A continuous series of divisions lead to the formation of the different trichomes. Trichomes range in size from a few microns to several centimeters and they exhibit a tremendous species-specific diversity shape and therefore, they are often used as diagnostic characteristics for the identifications of plant species, e.g., trichomes are mainly found on leaves and stems, but they can also occur, depending on the species, on petals, petioles, peduncles and seeds. Trichomes can be single-celled or multicellular, but the criterion that is mostly used to classify them is whether they are glandular or not. Non-glandular trichomes are present on most angiosperms, but also on some gymnosperms and bryophytes. On the model plant Arabidopsis, only non-glandular trichomes can be found, which are unicellular and can be either unbranches, or have two to five branches. These trichomes are polyploid and have been extensively studied with respect to their development, e.g. In contrast, and apical cells and can be found on approximately 30% of all vascular plants. Glandular trichomes have in common the capacity to produce, store and secrete large amounts of different classes of secondary metabolites. Many of the specialized metabolites that can be found in glandular trichomes have become commercially important as natural pesticides, but also have found use as food additives or pharmaceuticals. For instance, plants of the Laminaceae, comprising species such as mint (Mentha x piperita), basil (Ocimum basilicum), lavender (Lavandula spica), oregano (Origanum vulgare) and thyme (Thymus vulgaris), are cultivated for their glandular trichome-produced essential oils. Moreover, artemisinin, a sesquiterpene lactone that is produced in the glandular trichomes of annual wormwood (Artemisia annua), is used for the treatment of malaria. In addition, gossypol and related compounds, which are dimeric disequiterpenes produced by cotton (Gossypium hirsutum) trichomes, have strong antifungal activity and are potential natural pesticides. It is for these kinds of specialized metabolic properties, and for the opportunities to modify these properties via genetic engineering, e.g., that trichomes have received increased attention over the past year. In the present study scanning electron microscopy with energy dispersive spectroscopic analysis of L. esculentum trichomes with characteristics of chemical elements were made. Energy dispersive X-ray micro-analysis (EDAX) provides an unique approach for obtaining quantitative compositional analysis of individual cell and intracellular compartments. Elemental quantification of ultrathin sections with Electron probe X-ray Micro Analysis (EPMA) is generally based on the linear relationship between elemental concentration and the ratio of number of characteristics/continuum X-ray photons (Shuman et al., 1976; Silverberg, 1976; Kitazawa et al., 1983). The amount of different chemical elements present in the cell-wall, plastid and floridean starch of the carrageenophyte Hypnea musciformis was determined (Sivakumar and Rengasamy, 1999).

1.1 Principle of the energy dispersive X-ray microanalysis (EDAX)

The EDAX system is under control of sophisticated software that, at a simple command from the operator, automatically collects an energy-dispersive X-ray spectrum, identifies the elements present, obtains X-ray count data, and presents all of the pertinent data in an appropriate display format. The spectrum can be stored in disk memory and recalled as desired. Flexibility is central to the performance of the EDAX software. It easily adapts to fit virtually any user's needs. While peaks are identified and labeled automatically a manual mode is available to allow the operator to identify peaks by elemental symbol, atomic number, or cursor position. This may be done exclusively by the operator or in conjunction with automatic mode. The operator may also modify search parameters to fine tune the search for a particular type of analysis. Elements that are not relevant to the analysis may be excluded from the search.

Energy dispersive X-ray microanalysis system works in the principles of detection and characterization of characteristics X-ray generated in Electron Microscope. Whenever electron beam interacts with any elements or metals, X-ray are detected by a solid state detector. They are digitized, amplified and fed to a multichannel analyzer, which sorts the X-ray signals depending upon their energy. The data generated were compared with existing data stored in computer programme and the computer provides the possibility of the presence of various elements present in the samples.

II. MATERIALS AND METHODS

The natural habitat of tomato plant (Lycopersicum esculentum) were given in Fig. 1a,b.

Fig. 1. (a)Trichome density of the most abundant types on equivalent stem internodes of *L. esculentum* (b) Leaflets and internode of trichomes density level of *L. esculentum* at 40 days

2.1 Light microscopy

Leaves were collected and fixed in ethanol: glacial acidic acid (3:1 = v/v) for at least 24 h. After fixation the plant material was hydrolysed in 1 M HCl for 10 min at 60°C and stained in freshly prepared Schiff reagent for 60 min modified according to After the preparation steps the epidermis from the leaf surface was drawn off. The investigation were conducted under a Zeiss microscope. The images were obtained through Zeiss 40x, Zeiss 60x dry objectives and Zeiss 10x oil immension objective was used. The number of trichomes per mm^2 surface area was counted directly on LM images at 40x magnification.

2.2 Scanning electron microscopy

The seaweed compost organic manure S. wightii treated Lycopersicum esculentum leaves were fixed in 3% glutaraldehyde in 0.1 □ M phosphate buffer (pH 6.8) for 24 h at 4°C. After fixation, the leaves were subsequently dehydrated in a graded acetone series, critical point dried with CO_2 and the specimens were Sputter coated with a thin layer. The fixed sample were given 3 washes thoroughly in 0.1 □ M phosphate buffer (pH 6.8) they were dehydrated through a graded series of alcohol 10-15 minutes interval at 4°C upto 70%. Then 90% and 100% alcohol were kept in room temperature at 2-3 h. interval. Dehydrated samples treated with critical point drier (CPD) were on a stub and the specimens were examined with Joel JSM-56010 with INSA-EDS and electron micrograph were taken selectively from the computer screen.

III. RESULTS AND DISCUSSION

SEM-micrograph of tomato Trichome leaf showing the morphology of overview about high number of trichomes density on the leaf surface, short stalked trichome type with a three celled region. Distributions of trichomes types in the section of L. esculentum typically, eight different types are distinguished of which (i.e., type I, IV, VI and VII), glandular capitate trichomes and four (i.e., type II, III, V and VIII) are non-glandular. The glandular trichome types differ in number of stale and secretory cells as well as in their chemical contents (Fig. 2a-h).

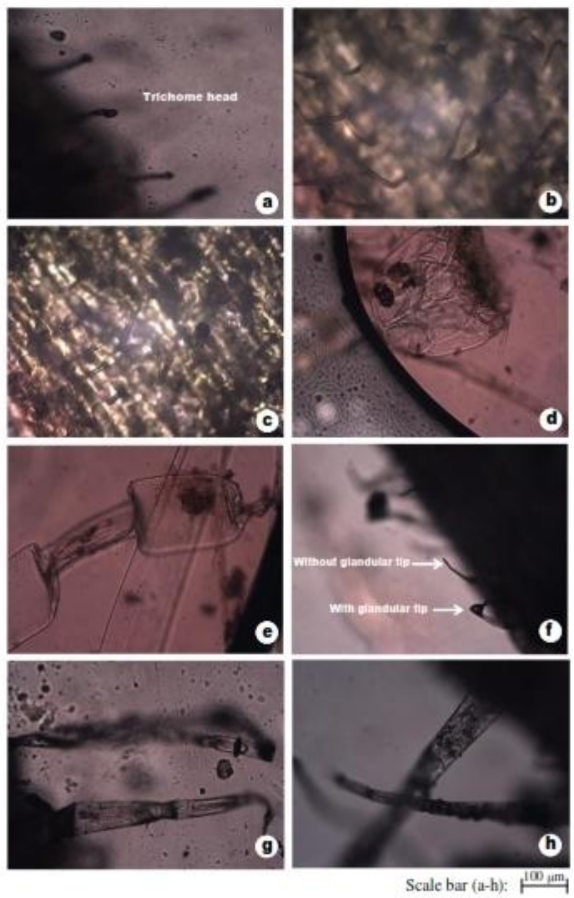

Fig. 2. Light microscopic pictures of *L. esculentum* trichomes (a) Trichome head (b) Trichome distribution on leaves of abaxial side (c&d) Well development of trichomes on stem (e) Intermediate cell (f) trochome without a glandular tip (g) the cellular component move around the cell in stands of cytoplasm with stalk glandular trichomes (h) Non-glandular trichomes

The second figures shows that glandular trichomes thick and short glandular trichomes composed of two stalk cells and a head made up of 4 secretory cells (Type VI-Triochome).

The glandular trichomes found on the surface of the leaves and stems of cultivated tomato (L. esculentum) were shown to contain mixture of volatile terpenoids, consistings mostly of monoterpene and sesquiterpenes. The glandular types of trichomes are found as predominant irrespective to treatments and control. But the size and mean density of the trichomes was higher on abaxial side than adaxial side of the leaf (Table 1).

Table 1. Mean densities number cm of glandular trichomes and their amplitudes (between control and organic manure S. wightii treated)

Tomato populations	No. of plant	Abaxial surface (per leaf area)	Adaxial surface (per leaf area)	Mean density

	evaluated	Trichomes length	Total glandular trichome (IV-VI) (1 cm)	Type IV trichomes	Type VI trichomes	Total glandular trichmes	abaxial + adaxial glandular trichomes
Control	n=5	1 trichome (0.2 – 1.0 mm)	45-50 trichomes	64 (IV) trichomes	60-65 (VI) trichomes	124.0 trichome	6.45 (Density)
Organic manure (S. wightii)	n=5	1 trichome (0.2 – 0.4 mm)	50-60 trichomes	70-8 trichomes	80-85 trichomes	165.5 trichomes	8.25 (Density)

This glandular trichomes consisting of 6-10 cells and 2-3 mm long. Globular and multicellular bar, with a small and round glandular cell in the trichome tip (Type I – trichome). Type VIII non-glandular trichomes compared of one basal and thick cell with a learning cell in the tip. Similar to the non glandular trichomes and shorter (0.2-1.0 mm). Globular and multicellular base (Type II trichomes). The maximum number of trichomes was recorded in the second compost treated L. esculentum (Fig. 1 a-b).

Acaminate-digit trichoma type consisting of three cells in the stalk and multicellular basement, amongest the trichomes are found on the leaf, stem, flower of the tomato plant. Trichomes are occur in the epidermis it is the main function of trichomes are polarization filters they are quite striking. Among the most interesting are multicelular glandular trichomes which often contain crystals. Tomato glandular trichomes of this type have been found to contain crystals composed of calcium oxalate and cadmium oxalate.

Essential oils are often stored in the bulbous regions of these structures as well giving the plant it characteristic odor and stick texture. Both crystals and essential oils may serve as defense against herbivory. This peel shows the surface view of the tomato note the four subsidiary cells, two guard cells and the aperture of in the center. Tomato plant have the tetracytic stomato its means have four subsidiary cells (Fig. 3).

The energy dispersive spectroscopic (EDS) analysis of leaf trichomes of L. esculentum treated with seaweed compost organic manure S. wightii at 40th day of control and second compost treated manure (values are % in weight) (Fig. 4). Initiation and developmental stages of glandular trichomes from Lycopersicum esculentum drawing freely from sections visualized by light microscopy (Fig. 5). Plant have developed various mechanisms of defense against phytophagus insects. Two defensive morphological features are trichomes and glands. Trichomes may be hair like on glandular. Plant hairs act as physical barriers keeping smaller insects away from the leaf surface. Glandular trichomes and plant glands may exude a stickly substance that entraps and immobilizes small insects on they may contain toxic constituents which spill into the surrounding tissue when the gland is ruptured, making it unplantable or toxic. These toxins are generally work are do not kill the insect directly, rather they retard insect growth and delay pupation. As a result, the insects are more vulnerable to disease, predation and the environment. The blank which has evolved between plants and insects could be seriously disrupted if secondary plant toxin analogs were synthesized and used as

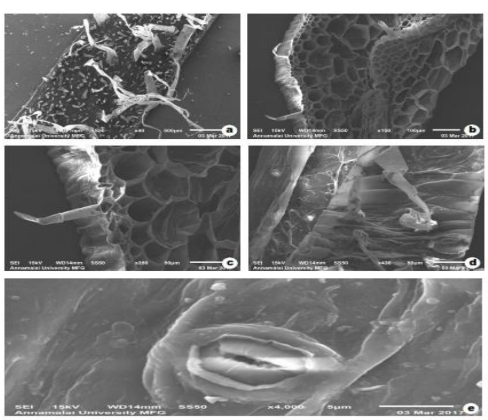

Fig. 3. (a) SEM images of tomato leaf showing the morphology of overview about high number of trichomes density on the leaf surfaces (b) Short stalked trichome type with a three celled region (c&d) Acuminate-digit trichome type consisting of three cells in the stalk and multicellular basement (e) Leaf shows the surface view of the stomato note the four subsidiary cells, two guard cells and the aperture of in the center. Tomato plant have the tetracytic stomato (four subsidiary cells)

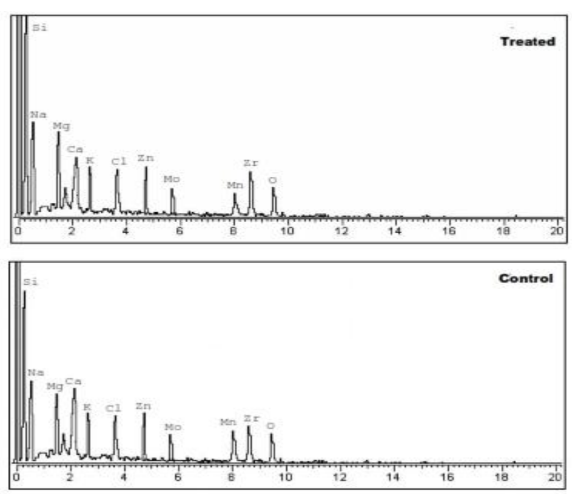

Fig. 4. *Sargassum wightii* organic manure treated with *Lycopersicum esculentum* elemental composition using SEM-EDS on trichomes

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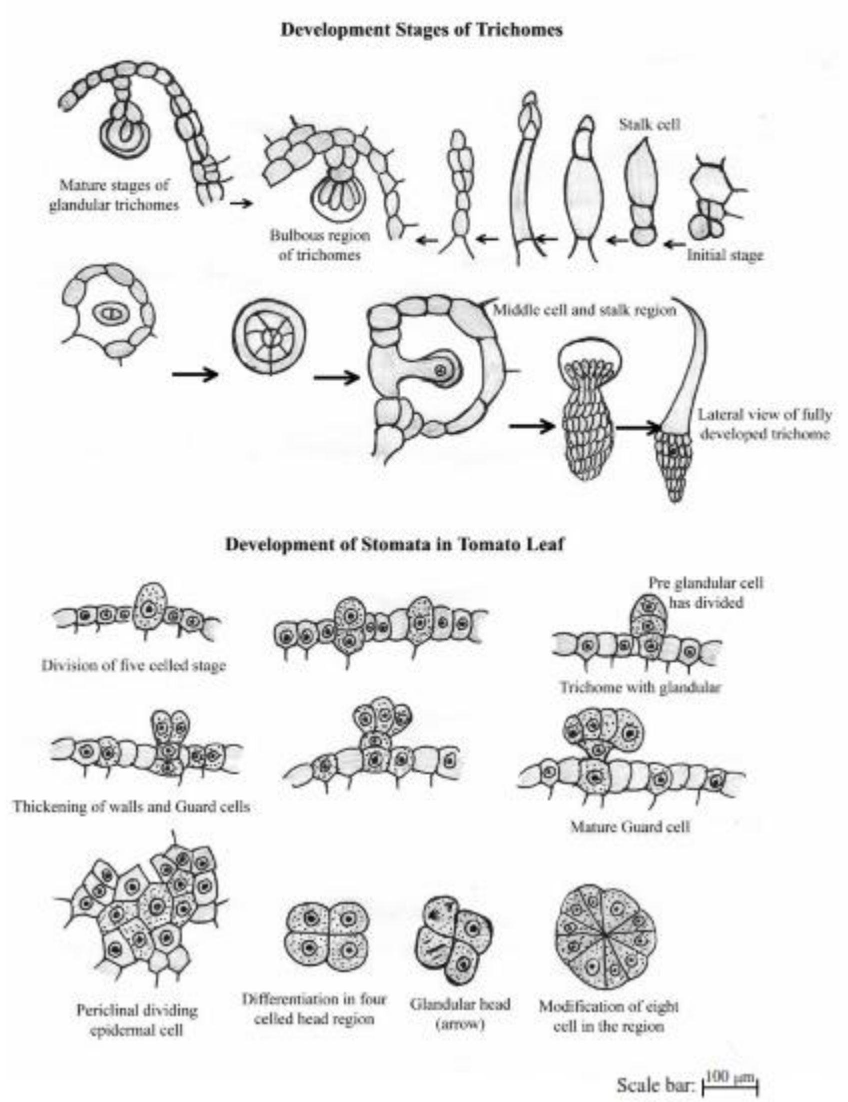

Fig. 5. Developmental stages of trichomes and development of stomato in tomato leaf

insecticides. Insects developing resistance to the analogs might develop resistance to the natural toxin. Cultivated tomato (Solanum lycopersicum) also contains glandular trichomes (Luckwill, 1943). There are two abundant types of glandular trichomes on tomato organs. In the present study our observations explain the adaptive morphologies of type VI, type IV trichomes for metabolite storage and release and provide a framework for further studies of there important metabolic cellular factories, are estimated the number of type VI glandular trichomes per leaf area on the adaxial side and also abaxial side. Type I trichomes have a multicellular stalk with a single, small gland cell at the tip. Type VI trichomes have a unicellular stalk with a four-cell glandular head, together, these glands produce a variety of compounds, including terpenes, alkyl sugars and phenylpropanoid-derived metabolites (Slocombe et al., 2008; Besser et al., 2009; Sehimiller et al., 2009; 2010). The energy dispersive X-ray microanalysis provides a unique approach for obtaining qualitative and quantitative compositional analysis of individual cell to localize distribution of chemicals elements of leaf differed not only by quality but also in quantity.

Table 2 shows the results obtained from the EDS analysis of different chemical elements present in the trichomes of seaweed compost treated L. esculentum. Totally eleven treatments namely Si, Na, Mg, Ca, K, Cl, Zn, Mo, Mn, Zr and O were observed. The results of seaweed compost organic manure treated L. esculentum showed the maximum value of Si (15.32 ± 0.33 weight) and minimum of Zn (8.46 ± 0.25) in trichomes. The cell structure and different cellular inclusions have been reported by Sivakumar and Rengasamy (1999) using X-ray microanalysis EDAX. Electron microscopic studies and X-ray microanalysis on Sargassum wightii were made by Sundari and Selvaraj

(2009). Similar works were also carried out by Clyton and Ashburnes (1990), Paul Kugreen (1983), Selvaraj et al. (2007), Cole and Sheath (1990) and Borowitzka et al. (1974).

Table 2. Sargassum wightii organic manure treated with Lycopersicum esculentum elemental composition using SEM-EDS on trichomes

	Minerals – Wewight in (%)											
	Si	Na	Mg	Ca	K	Cl	Zn	Mo	Mn	Zr	O	Total
Untreated control	11.43 ± 0.25	8.64 ± 0.25	7.90 ± 0.29	9.45 ± 0.22	9.24 ± 0.30	7.68 ± 0.23	7.26 ± 0.24	7.49 ± 0.22	8.92 ± 0.26	7.40 ± 0.22	6.49 ± 0.19	91.9 ± 9.1
Treated (S. wightii) organic manure	15.32 ± 0.33	9.67 ± 0.29	8.74 ± 0.26	11.67 ± 0.29	10.52 ± 0.31	8.87 ± 0.26	7.98 ± 0.23	9.39 ± 0.28	8.54 ± 0.25	8.46 ± 0.25	6.48 ± 0.19	99.16 ± 0.84

The silicon provides rigidity and strengthening of cell wall. It enhances the physiological availability of zinc in plants. Calcium forms the most abundant ions in plants particularly leaf (Trichomes). Magnesium is essential for plant productivity, since it goes in to the composition of the chlorophyll pigments. The presence of calcium and magnesium strengthens the plant. Molybdenum is the metal of several metalloenzymes, prominent among there are enzymes involved in nitrogen fixations.

A high level of sodium Na (9.67 ± 0.29) was recorded in the trichomes (leaf area) seaweed compost organic manure (S. wightii) treated L. esculentum, which may be due to transportation of carbondioxide (CO_2) throughout the system of many different plants which utilize. Calcium is the chemical which plays an extremely important role in producing plant tissues and enables plant to grow better it increase the plant tissue resistance and allows for more exert stress. It neutralizes organic acids, which may become poisonous to plant, and also increase the crop resistance to certain disease (Ex: tomato).

3.1 Cell wall trichomes

The cell walls of trichomes are commonly cellulose and are covered with a cuticle. They may be lignified. Plant hairs often produce thick secondary wall as, for instance, the cotton seed hairs or the chamber hairs of Hamulus. The walls or trichomes are sometimes impregnated with silica or calcium carbonate. Their contents are varied in relation to function. Cystoliths and other crystals may develop in hairs.

IV CONCLUSION

In conclusion we can summarize that on the leaf surface of tomato two types of trichomes were observed multicellular hairs and glandular trichomes, could be observed. A continuous series of periclinal and anticlinal divisions lead to the formation of the ontogenetical independent trichomes type. The storage compartment of glandular trichomes usually is located on the tip of the hair and in part of the glandular cell or cells,, which are metabolically active. Trichomes and their exudates can be harvested relatively easily and this has permitted a detailed study of their metabolites, as well as the genes and protein responsible for them. This knowledge now assists classical breeding programs, as well as targeted genetic engineering, aimed to optimize trichome density and physiology to facilitate customization of essential oil production or to tune biocide activity to enhance crop protection. Detailed histochemical and ultra structured studies may be aided to interpret their part and function on the leaf surface.

Several allelochemicals present in wild L. esculentum taxa have been associated with pest resistance. Active secretory cells of glandular trichomes have dense protoplasts and elaborate various substances, such as volatile oils, resins and mucilage’s and guns. These substances are excreted and accumulate between the walls and cuticle. Their final removal from the hair occurs rupture of the cuticle.

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