

Full Length Research Paper

## The structure and function of trichomes in the leaf of *Salvia repens* Burch. Ex Benth

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The anatomical investigation using scanning and transmission electron microscopy revealed that *Salvia repens* is characterized by both non-glandular and glandular trichomes. It has been demonstrated that both trichomes appeared to originate from epidermal cells of the leaf, stem and vegetative area through a series of periclinal and anticlinal division. Non-glandular trichomes were composed of uniseriate cells. The terminal cells of the non-glandular trichomes were the first cells to mature. A progressive maturity resulted in continuous death of the uniseriate cells progressing towards the basipetal position. The dead cells resembled fibrous like clothing threads which were covering the epidermis. Structurally, these fibrous ends were similar in composition to the components of the suberized cell wall. The glandular trichomes were multicellular and uniseriated. Their shape ranged from oval to club, and they were composed of a basal cell, stalk cell and a three to four sided glandular head. The orientation of these glandular trichomes was not uniform, however, a different orientation of glandular trichomes was observed in the leaves and stem. The micrograph of these glandular trichomes showed that they started as outgrowth of the epidermis cells, and subsequent periclinal division followed by anticlinal division, giving rise to a trichome with a basal epidermal cell, stalk cell and three to four celled secretory head. At maturity the glandular trichome gland cells contained a distended cuticular sac due to the accumulation of the essential oil.

**Key words:** Non-glandular, glandular trichomes in *Salvia repens*.

### INTRODUCTION

The family Lamiaceae consists of 200 genera and 3000 - 3200 species with a cosmopolitan distribution of many taxa in the Mediterranean region (Valant-Vetschera et al., 2003; Ascensão et al., 1995; Bayrak and Akgül, 1987). According to the flower structure the Lamiaceae family is regarded as one of the highly ranked plant family (Wink, 2003; Koschier and Sedy, 2003; Lima et al., 2004). It is regarded as the largest family of aromatic plants, herbs, folk medicines and fragrant in a wider plant family spectrum. This family constitutes one of the largest and economic important groups where most plant products are extracted. The largest genus is *Salvia* with 500 species followed by *Hyptis* with 350 species (Werker et al., 1985a.b; Senatore et al., 1997).

*Salvia* is one of the wide-spread members of the

Lamiaceae family that features prominently in the pharmacopoeias of many countries throughout the world (Moujir et al., 1993). Most of the species within this genus are well documented and extensively studied (Werker et al., 1985a.b; Tan et al., 2002; Valant-Vetschera et al., 2003; Basio et al., 1999). *Salvia dominica*, L., *S. fruticosa* Mill, *S. sclarea* L. and *S. officinalis* were some of the species of these genera that composed of different types of glandular hairs than any other genera in the Lamiaceae family (Werker et al., 1985a.b; Serrato-Valenti et al., 1997; Croteau et al., 1981).

*S. repens* Burch. ex Benth which is also a member of this family is an odorous herb, widely distributed in parts of the Eastern Cape through Lesotho, Northern Cape and Limpopo of South Africa (Van Wyk et al., 1997; Germishuizen and Meyer, 2003; Goldblatt and Manning, 2000). It is a short, erect herb with long narrow deeply lobed leaves.

The leaves of this plant are very aromatic and warty. This plant may grow up to 1 m tall and its roots are modi-

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fied to form runners. The flowering period is between October to January when conditions are favourable and the characteristic flowers are small and blue.

The indigenous people of the Eastern Cape use this plant as tea. The tea is sipped to heal colic, heartburn and chronic indigestion (van der Walt, 2003). The tea is also used to wash sores, infected bites and scratches. Sometimes the leaves of this plant are collected, dried and burned to repel insects. The general use of *S. repens* by indigenous people is for remedy of mental and emotional calming effects. It is claimed to ease mental cramps, sores and a decoction of the roots has been used for both humans and cattle for treating stomachache and diarrhea (van der Walt, 2003). It is also believed to assist in swallowing. However, all of these statements are not scientifically proven. It is this challenge that experimental investigation needs to be done to prove beyond reasonable doubt that this plant is capable of healing.

Furthermore, there is no documented literature which deals with an understanding of structure in relation to physiology of the plant, let alone the pharmaceutical significance of this species. It is on this paradigm that this study attempts to address anatomical and morphological structure in relation to a physiological adaptation of this species to its habitat.

## MATERIALS AND METHODS

The plant material was collected from a site at Double Drift Nature Reserve between Alice and Peddie in the Eastern Cape Province of South Africa. The plant was identified at the Schonland Herbarium at Rhodes University, Grahamstown, and a voucher specimen (Maye-kiso 8) was deposited in the Giffen Herbarium at the University of Fort Hare in Alice.

### Scanning electron microscopy

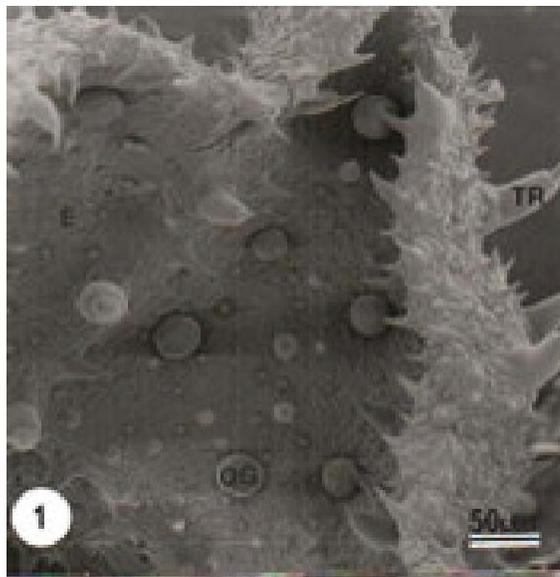
Sections of leaves (0.1 x 0.5 mm thick) were collected randomly and immediately fixed in 6% glutaraldehyde in 0.05 mM Sodium cacodylate buffer (pH 7.3), washed in 0.05 mM Sodium cacodylate for 12 h. Sections were then dehydrated in an ethanol series. The leaves were dried in a Hitachi HCP-2 critical point dryer, coated with gold using a sputter coater and viewed at 15 KV with a Hitachi S-450 Scanning Electron Microscope.

### Transmission electron microscopy/light microscopy

Leaves of *S. repens* were randomly selected from the natural environment. The leaf portions were cut into small segments approximately 2 - 3 x 5 mm in cold 50 mM Sodium cacodylate buffer, (pH 7.3). The plant segments were fixed in a buffered 6% glutaraldehyde (50 mM Na-cacodylate, pH 7) and stored overnight in a refrigerator. After rinsing in a 50 mM Na-cacodylate buffer, the

sample were then postfixed in 2% Osmium tetroxide (OSO<sub>4</sub>) in 50 mM Na-cacodylate buffer, pH 7.3, overnight at 4°C, and infiltrated in a graded series resin (Spurr, 1969).

Thin sections (0.5 - 2.0 µm) were cut with glass knives on an LKB Ultramicrotome, stained with Uranyl acetate followed by lead



**Figure 1.** This is an electron micrograph of *S. repens* leaf at higher magnification showing the adaxial/abaxial surface with the distribution of glandular trichomes and non-glandular trichomes. E = Epidermis, TR = Trichome, OG = Oil gland, S = Stomata.

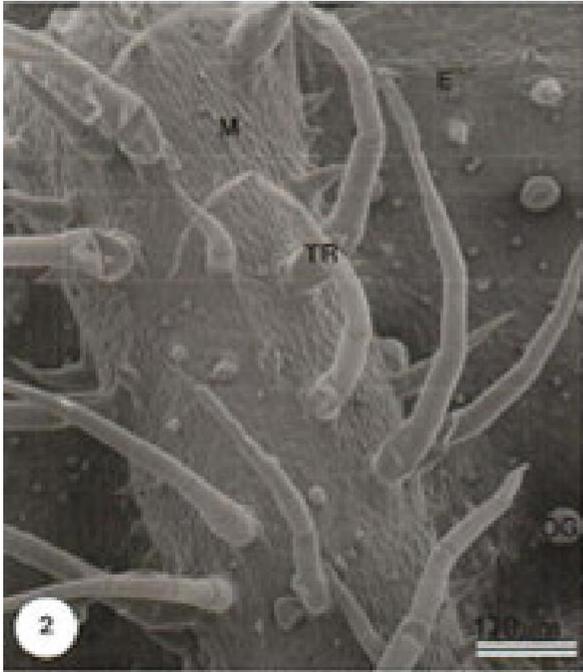
citrate and observed in a Hitachi at 75 - 100 kV. (Note: Some of the sections used in this study were obtained by cutting thin section (0.5 - 2.0 mm) using a glass knives on an LKB ultramicrotome, stained with 0.05% toluidine blue and examined with a Zeiss photomicroscope III).

## RESULTS

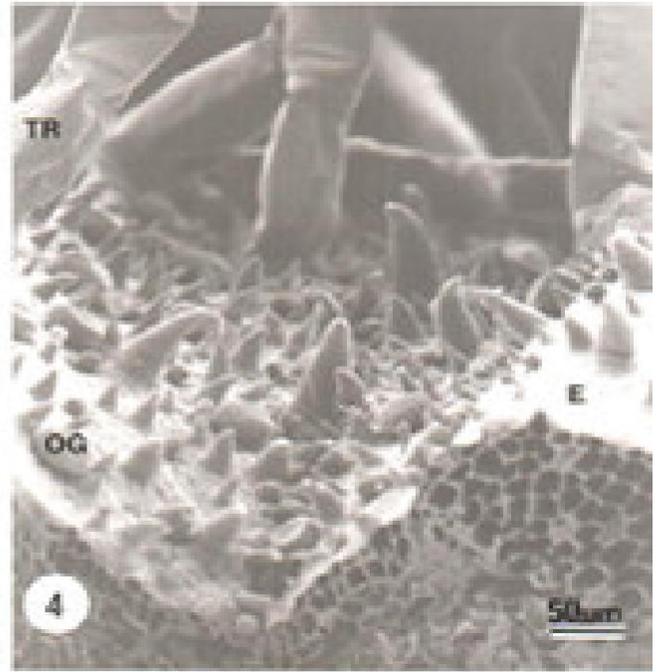
### Morphological investigation using SEM

The scanning electron microscopy (SEM) results have shown that the aerial parts of the plant bear uniseriate and multicellular non-glandular as well as glandular trichomes (Figures 1 - 5 and 7). The distribution of these non-glandular and glandular trichomes appeared to differ in different areas of the plant (Figures 1 - 4). The scanning electron microscopy has also revealed that the *S. repens* is characterized by different types of non-glandular trichomes. The first type observed was a multicellular, uniseriate, four- five celled short trichomes, with swollen basal cells, acute apices and thick warty cell wall, abundant on abaxial surfaces, over midrib and major veins (Figures 2 - 5, 7 and 8). The second type was a unicellular papillae associated with a central circumscribed area of the outer wall of the swollen basal epidermal cell, with enlarged bases and acute apices, which were frequent on the leaf and stem (Figures 1 - 5 and 8).

During their early stages of development, these non-glandular trichomes appeared to be tubular and fleshy



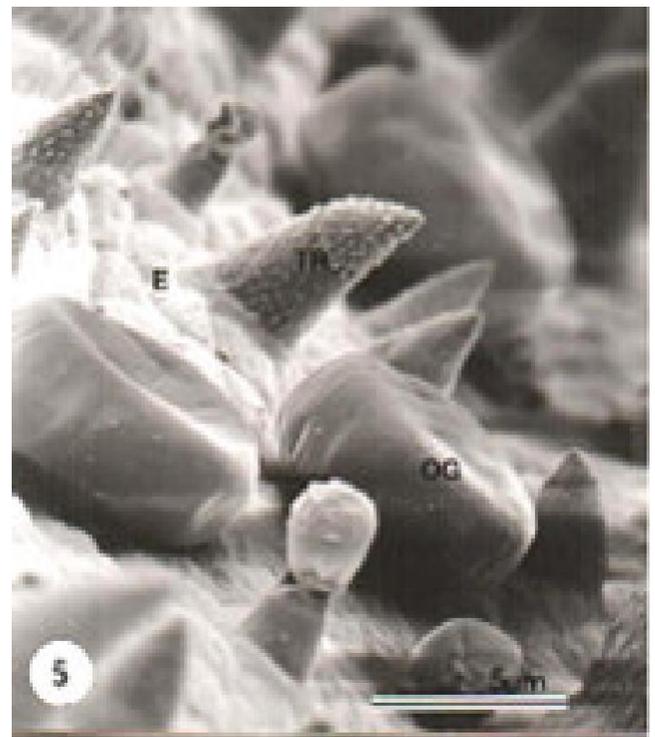
**Figure 2.** An electron micrograph of *S. repens* leaf at higher magnification showing midrib of the adaxial surface of with a high the distribution of non-glandular trichomes compare with glandular trichomes. E = Epidermis, TR = Trichome, OG = Oil gland, M = Midrib.



**Figure 4.** Electron micrograph of the stem at high magnification showing epidermal surface with a distribution of non-glandular trichomes compared with glandular trichomes. E = Epidermis, TR = Trichome, OG = Oil gland.



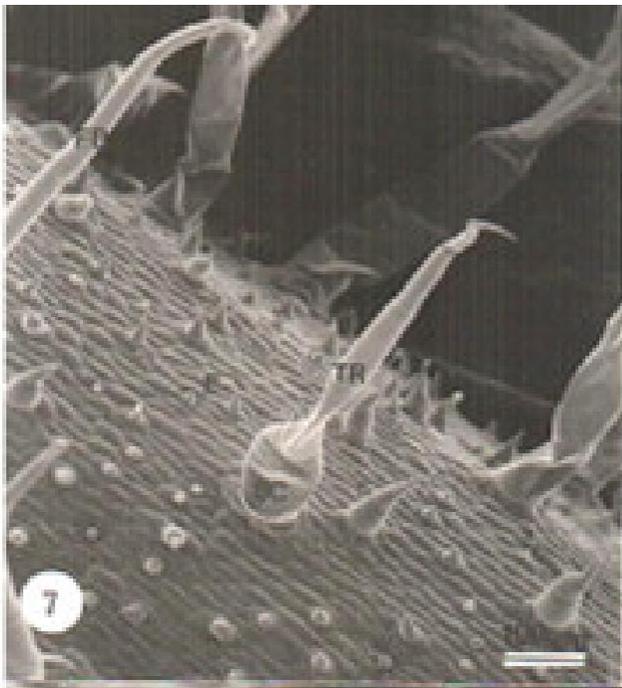
**Figure 3.** The electron micrograph of the stem at higher magnification showing epidermal surface with high distribution of non-glandular trichomes and glandular trichomes. E = Epidermis, TR = Trichome, OG = Oil gland.



**Figure 5.** Display *S. repens* leaf with grooved epidermis. Note: The full mature glandular trichome, young developing glandular uni-seriate non-glandular trichome. TR = Trichome, OG = Oil gland, E = Epidermis.



**Figure 6.** A leaves at high magnification showing a developing glandular trichome. E = Epidermis, S = stalk cell, H = Head cells.

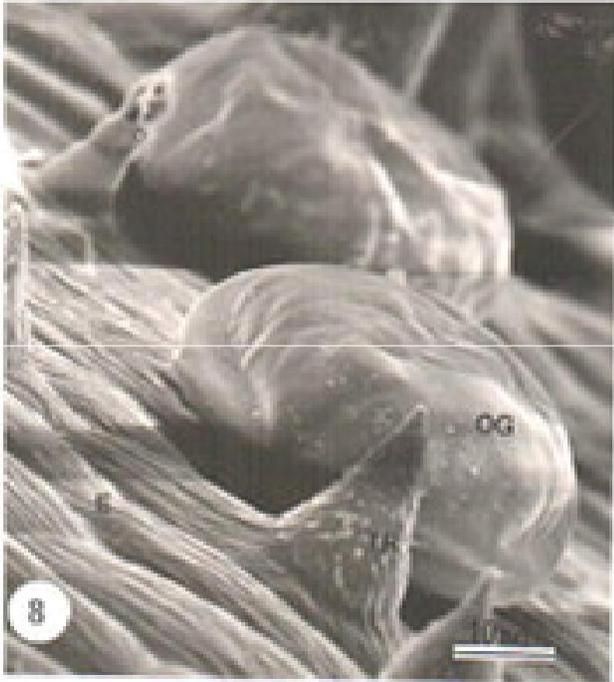


**Figure 7.** This is an electron micrograph of *S. repens* stem showing grooved epidermis.  
Note: The fibrous thread-like structure of non-glandular trichome due to progressive basipetal development. TR = Trichome, FB = Fibrous end, BC = Basal cells, E = Epidermis.

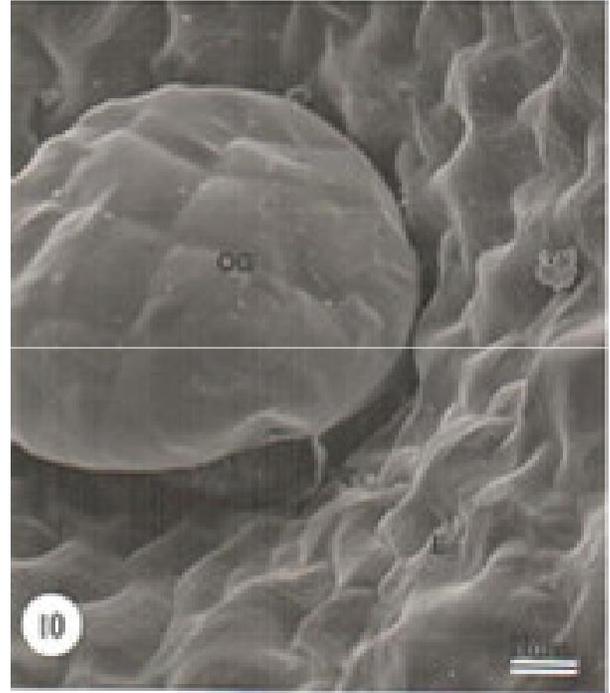
(Figures 2 - 4 and 8). At maturity these thread-like non-glandular trichomes which have long fibrous ends, appeared to be shielding and protecting the epidermal layer and glandular trichomes (Figures 2 - 5, and 7).

In contrast, the glandular trichomes resembled a sac like structures that form protrusions outside the epidermal layer and they are relatively club shaped (Figures 3 - 6 and 8). They appeared to arise from a series of anticlinal and periclinal divisions from supporting auxiliary cells and glands (Figures 5, 6 and 8) . The distribution pattern of these trichomes also varied from different organs within the same species. During the early stages of leaf, stem and shoot development, these glandular trichomes appeared to be scattered in a densely, random pattern (Figures 2 - 4). As these organs become mature, these glandular trichomes appeared to decrease their distribution. They seemed to become far apart and the number decreased progressively particularly in leaves and stem (Figures 1 and 7).

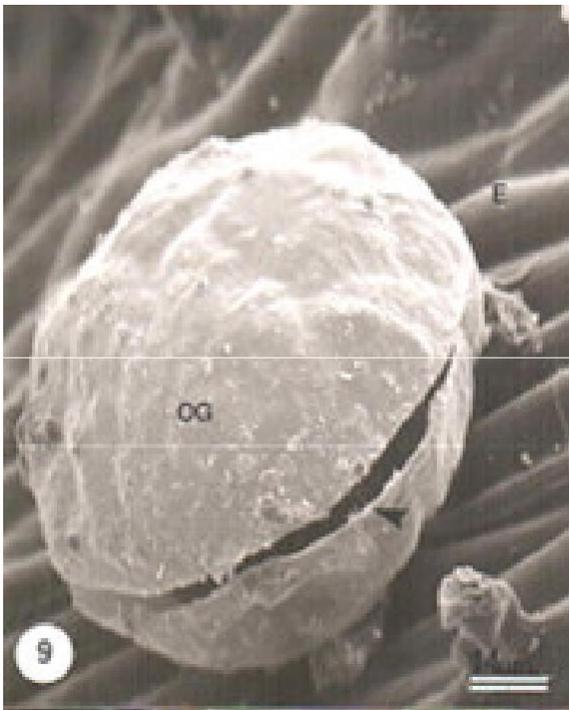
The *S. repens* is also characterized by two types of glandular trichomes: the peltate and capitate type (Figures 5, 6 and 8) . The peltate types are those long-term glandular hairs that secrete their products to the outside only when touched. This type of glandular trichome consists of a basal epidermal cell, a stalk cell and a broad head (Figures 5 and 6). The second type of glandular trichome is termed capitate glandular trichome. It is characterized by two types of glandular trichome, those glandular trichomes that are characterized by short monocellular stalk with two types of glandular trichome. Those glandular trichomes that are characterized with short monocellular stalk with two cellular head and those that are characterized by a multicellular stalk, neck cells and a small globose unicellular head. However, *S. repens* is characterized with one type which secretes the secretory materials by allowing it to accumulate in a subcuticular sac (Figures 5, 8, 9 - 12). The glandular trichome cuticular sac appeared to be intact, shriveled and hard during the early stages of the oil gland development (Figure 6). As the oil gland cells approached maturity, the surface seemed to be smooth, as these oil glands cell walls were transformed to cuticularsacs (Figures 5, 8 and 10). This subcuticular sac appeared to be formed by a thin, elevated cuticle which soon ruptures under the influence of pressure (Figures 11, 12, and 16). A further development resulted to the accumulation of the essential oil in the oil glands, and consequently to the so-formed subcuticular layer-cuticle space by increasing considerably in volume to constitute a storage pool. When touched, the thin cuticular sac ruptures releasing the secretory products over the leaf surface (Figures 11, 12 and 16). The rupturing of the cuticular sac when releasing the essential oil in this species can occur in three possible ways; one, the subsequent detachment of the cuticular sac (Figures 10 and 12) or the removal of the cuticular sac at the base of the stalk cell of the glandular hair which result in the exposure



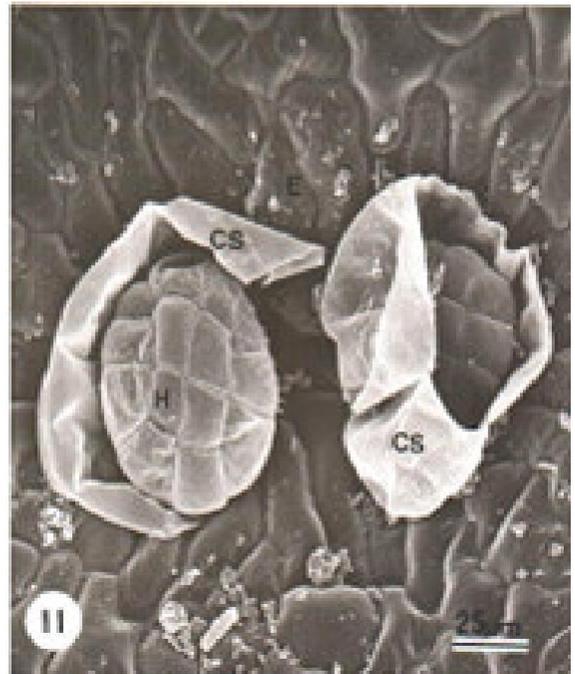
**Figure 8.** An electron micrograph of the stem showing mature glandular trichome and unicellular papillae of non-glandular type. TR = Trichome, OG = Oil gland, E = Epidermis.



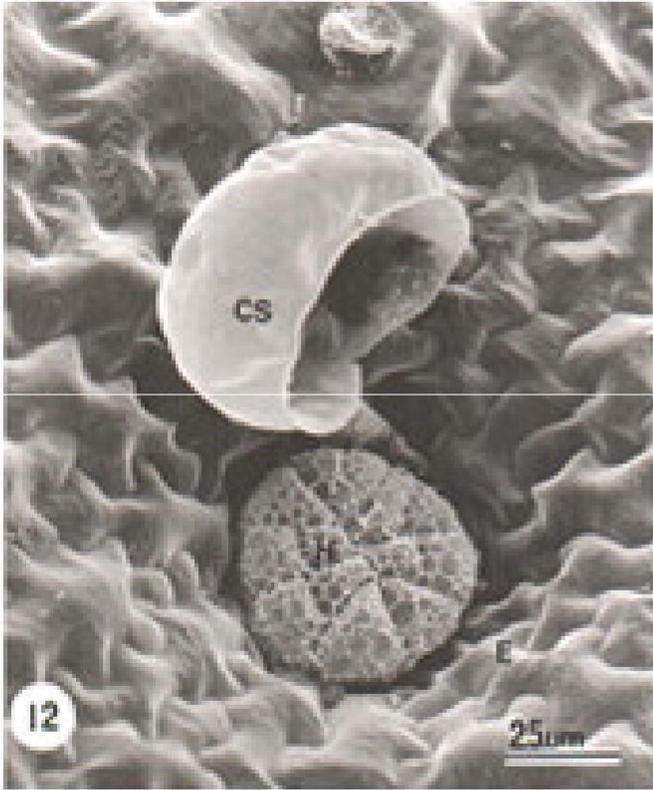
**Figure 10.** This is an electron micrograph of *S. repens* stem at maturity showing a glandular trichome with a straight line of apparent frailty is often observed in the horizontal diametrical region of the basal cell. OG = Oil gland; E = Epidermis, S = Stalk cells.



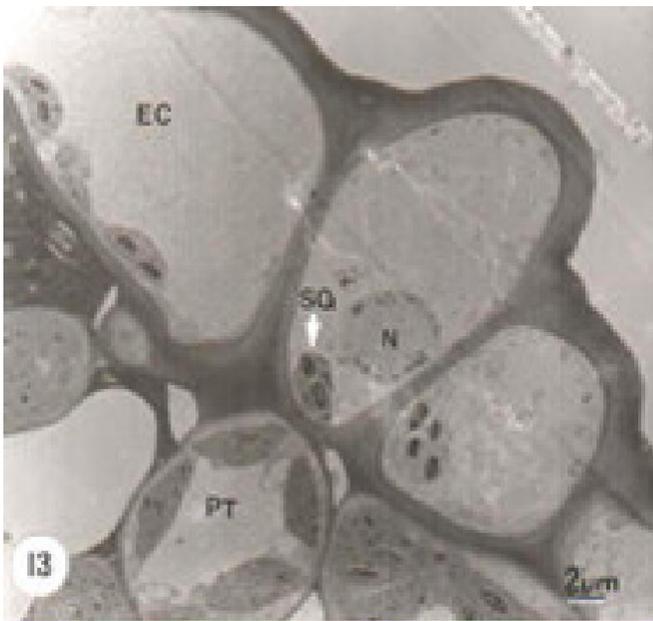
**Figure 9.** A micrograph of *S. repens* leaf at maturity showing a glandular trichome. Note a straight line of apparent frailty is often observed in the horizontal diametrical region of the head. OG = Oil gland; E = Epidermis.



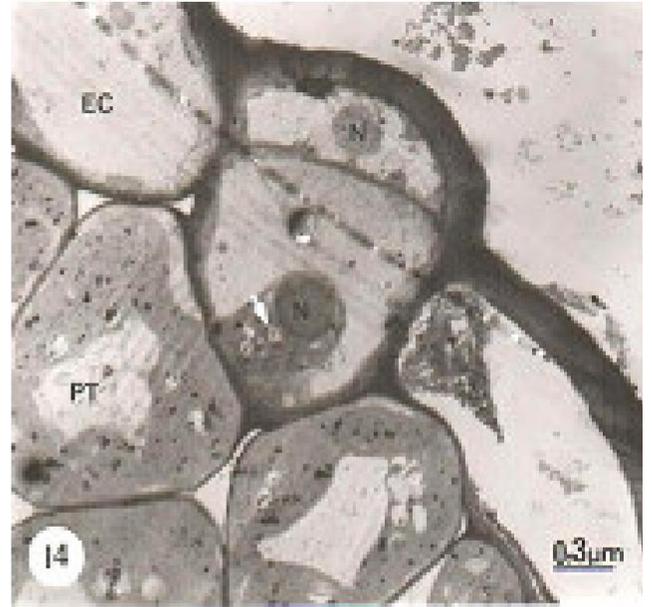
**Figure 11.** A mature leaf showing the removal of the cuticular sac disclosing some of the head cells. An arrow showing drops of secreted material. E = Epidermis, CS = Cuticular sac, H = Head cell.



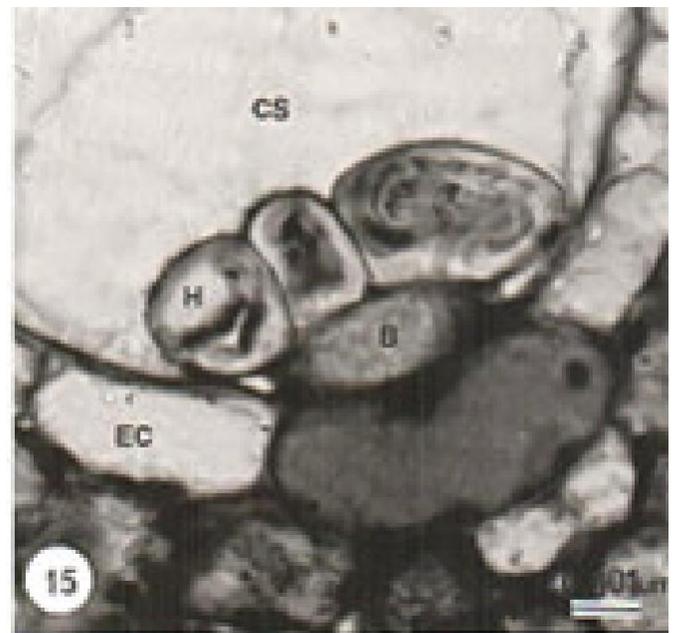
**Figure 12.** The removal of the cuticular sac disclosing some of the head cells. E = Epidermis, CS = Cuticular sac, H = Head cell.



**Figure 13.** An electron micrograph of *S. repens* leaf exhibiting a bulgy epidermal cell at an early glandular or non-glandular trichome development. EC = Epidermal cell, SG = Starch grain, PT = Parenchymatous tissue.



**Figure 14.** A dividing epidermal cell with the arrows showing the starch grains. EC = Epidermal cell, N = Nucleus, PT = Parenchyma tissue.



**Figure 15.** A transverse section of *S. repens* leaf showing a mature glandular trichome with cuticular sac, head cell and basal cell. EC = Epidermal cell, CS = Cuticular sac, H = Head cell, B = Basal cell.

of the gland cells or the failure of the cuticular sac along an equatorial line of weakness and the detachment of the sac (Figures 9 and 11).

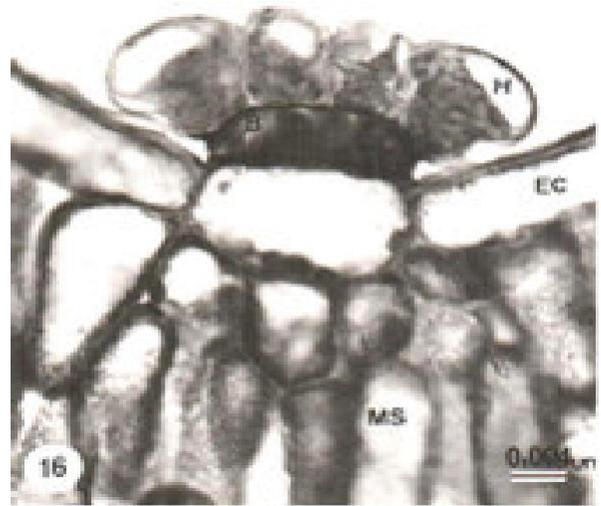
The ultrastructure and the light microscopy of the secretory tissues of *S. repens* were multicellular and biseriate, globular to oval in shape (Figures 15, 16, 19 and 20). The transmission electron microscope has shown that, at the initial stages of trichome development, the expanding epidermal cell had an electron dense cytosol, with a clearly evident large basal vacuole with nucleus associated with starch grains (Figures 13 and 14).

At this stage, it was difficult to distinguish whether the cell might be a non-glandular or a glandular trichome, because there was no clear indication of periclinal or anticlinal division with the exception of bulgeness of the epidermal cell which would form the trichome initials (Figure 13).

The glandular trichome appeared to be originating from specialized epidermal cells by periclinal division which was followed by an anticlinal division that give rise to a three or four glandular head (Figures 13 - 18). An interesting feature was the presence of plastids within the dividing basal cell or the specialized epidermal cells of the glandular trichome at early stages of development (Figures 13, 14, 17 and 18). It was also noted that there were numerous highly elongated granular structures which were considered to be modified plastids. These plastids seemed to occupy most volume of the cytoplasm of the cells above stalk cells, but below the apical cells of the developing trichome (Figures 17 and 18). When the glandular trichome was fully mature, the upper surfaces of the oil gland cells were covered with a cuticular sac (Figures 19 and 20). This cuticular sac appeared to be a modified cell wall of the glandular cell(s) which occurred on the terminal position. The subcuticular sac became distended due to the accumulation of the essential oils which were produced by the oil gland cells (Figure 15). As the progressive production of the essential oil continued, it resulted to the slow disintegration of the subcuticular sac. The essential oil was subsequently released through pores of the cuticle or more likely after the rupturing of the cuticular sac (Figures 11, 12, 16 and 19).

## DISCUSSION

*S. repens* is characterized by non-glandular and glandular trichomes. The distribution of these trichomes varies on different organs of the same plant. Non-glandular trichomes appeared to be more abundant to all plant organs than glandular trichomes. During the early stages of organ development, these non-glandular trichomes appeared to be distributed in a random fashion, whilst at maturity stage the pattern of distribution changed. For example, in the leaf, the non-glandular trichomes appeared to be more concentrated around the leaf veins. This observation supports the argument that the epidermal trichomes in the epidermal layer of young developing organs are associated with defensive strategy to protect



**Figure 16.** A transverse section of a mature glandular trichome after realizing essential oil. EC = Epidermal cell, H = Head cell, B = Basal cell, MS = Mesophyll cell.



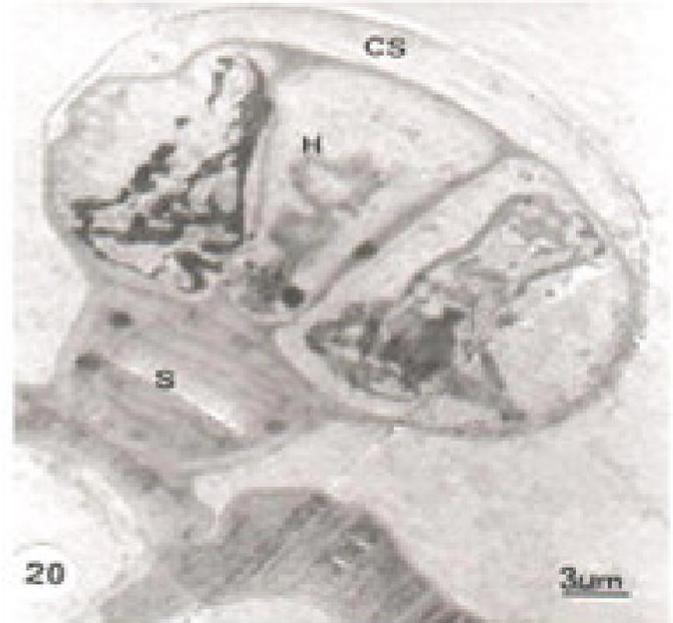
**Figure 17.** A young developing glandular trichome with a periclinal division of a gland head. EC = Epidermal cell, S = Stalk cell, H = Head cell.

these organs against the insects, herbivores and also to changing environment (Nguefack et al., 2004; Auge et al., 2003; Tan et al., 2002; Basio et al., 1999).

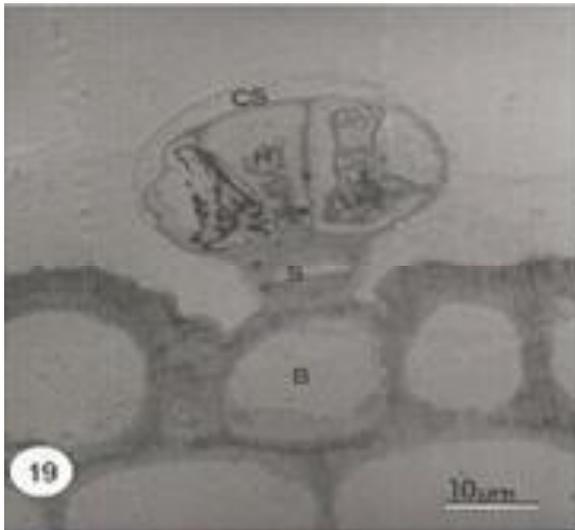
The resistance to changing temperature appeared to be depended solely on tissue morphology and on mechanical properties of cell wall (Wagner 1991; Fahh, 1986; Uphof and Hummel, 1962). Such proposition is supported by the characteristic nature of the anatomy, leaf morphology and highly specialized mechanical properties of the



**Figure 18.** A developing glandular trichome at high magnification. EC = Epidermal cell, S = Stalk cell, H = Head cell.



**Figure 20.** An enlarged electron micrograph of *S. repens* leaf at high magnification. S = Stalk cell, H = Head cell, CS = Cuticular sac.



**Figure 19.** This is an electron micrograph of *S. repens* leaf at maturity showing well organized three celled head of a glandular trichome. BC = Basal cell, S = Stalk cell, H = Head cell, CS = Cuticular sac.

epidermal cell walls. This same pattern of the leaf morphology and cell dimension has been demonstrated by various workers (Basio et al., 1999; Benayoun and Fahn 1979; Fahn and Shimony, 1998; Antunes and Sevinatè-Pinto, 1991; Dudai et al., 1988), and these characteristic features appeared to be applicable to *S. repens*.

Therefore, the overall function of these non-glandular trichomes appeared to have a protective function in these areas of development, by covering the layer of epidermis in response to these environmental changes. This distribution pattern might also be regarded as an adaptation associated with the environmental pattern and habitats of specific plants within the same species (Afolayan and Meyer 1995; Fahn 1988; 1986). These non-glandular trichomes were also assumed to have an effect on transpiration by influencing the water diffusion boundary layer of the transpiring leaf. Although, studies of the effect of plant trichome upon loss of water have not produced uniform results, it is believed that these non-glandular trichomes insulate the mesophyll cells from excessive heat. Sometimes these fibrous ends of the non-glandular trichome which are also present in *S. repens* seem to have a cooling effect to the leaves. In addition, they might also indirectly influence the water economy of the leaf or stem at early stages of development through temperature. This might also occur either through reduction energy dissipation by non-glandular trichomes, which have high reflectance properties (Gates, 1968). These non-glandular trichomes are considered to shield the stoma and oil glands from intensive heat during the dry and hot season as they had shown high distribution and surround the stoma. Such basic phenomena involved in characterization of the boundary layer resistance have also been shown in xeromorphic species (Fahn 1986; Johnson 1975; Chafe and Wardrop, 1972; Gates, 1968). Such relationship between the stomatal and boundary layer resistance was also of great significance in

attempting to assign an adaptive role to the indumentum layer shown by *S. repens*.

At maturity, some of the organs of the plants that have undergone secondary development are more likely to have less number of the non-glandular trichome than glandular trichome. In most cases where there was a change in distribution of the trichome, the layer of the primary epidermis was replaced by a periderm which was a secondary tissue and was harder than the epidermis. It was also noted that, in plant parts where secondary development has not yet occurred, the plant retained the similar arrangement of high distribution of non-glandular and glandular trichomes mechanical strategy (Ascensão et al., 1995; Wagner, 1991). For example, since leaves do not undergo secondary development, the epidermal layer was highly populated with these non-glandular and glandular trichomes on the abaxial surface of the leaf. In addition to these features, they appeared to serve as a protective barrier between the inner layers of epidermal cells to the outside environment. Since the leaf is the most delicate organ of the plant, there is a continuous growth of non-glandular and glandular trichomes. Consequently the plant had to withstand the environmental challenges such as pest, herbivores and mechanical damages. Hence this is a continuous cycle of production of non-glandular and glandular structures for the continuous existence and well-being of the plant during its active life cycle (Harbone, 1990).

The glandular trichomes of *S. repens* were multicellular and uniseriated. Their shape ranged from oval to club, and they were composed of a basal cell, stalk cell and a three to four sided glandular head. This was a characteristic feature of glandular trichome of a peltate type of glandular trichome.

The orientation of these glandular trichomes was not uniform, however, a different orientation of glandular trichomes was observed in the leaves. The distribution of these glandular trichomes appeared to be high during the vegetative growth prior to flowering period. Some of these glandular trichomes appeared in mature stems even where primary growth has been substituted by secondary development. However the distribution frequency was much low in the stems than in the mature leaves.

The mode of glandular trichome development and differentiation appeared to be different from that of the stem. It appeared that, these types of glandular trichomes exist throughout the life of the leaf, however, most of the glandular trichomes in the stem appeared to be disintegrated as the plant undergoes secondary development. In the case of the leaf, this distribution of these glandular trichomes changed, at early stages of plant development they occur on both side of the leaf. However, at maturity stage, the glandular trichomes are more on the lower surface of the leaf than on the upper surface. What is still not yet known in literature, is whether the existence of the new glandular trichome is through

regeneration from the old glandular trichome, but there is a strong evidence that, after the essential oil have been released due to the rupturing of the cuticular sac, there is a regeneration or formation of the new glandular trichome which would replace the old one. Studies on ultra-structure, chemical composition of the essential oil, microbial activity of the crude extract are on the way.

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