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Advances in Agronomy, Plant Breeding and Horticulture

Vol. 4 (1), pp.5 - 11, January, 2016

Investigation of foliar morphological features of *Salvia greggii* leaf

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Abstract

Foliar micromorphology of *Salvia greggii* A. Gray was investigated using the scanning electron microscope (SEM). The leaves were characterized by anisocytic stomata which were more abundant on the abaxial epidermal surface. Short multicellular glandular trichomes were observed on both leaf surfaces. Uniseriate and multicelullar non glandular trichomes were also found but more abundant over the mid-rib. Crystal deposits were observed on the epidermal surfaces. Energy dispersive X-ray spectroscopy-SEM of the crystals showed Na, Cl, Ca, Cd, Zn, Mo, Pb, Pi, Si, and S to be the major components. These elements are very essential for plant growth. Glandular and non glandular trichome stability can be attributed to the presence of these elements in this species.

Keywords: *Salvia greggii* A. Gray, micromorphology, glandular and non glandular trichomes.

INTRODUCTION

Salvia L., the largest genus of Lamiaceae, is composed of nearly 1000 species distributed extensively in three regions of the world: Central and South America (500 spp.), western Asia (200 spp.) and eastern Asia (100 spp.) (Walker and Sytsma, 2007). This genus name is derived from the Latin salveo meaning 'to heal or to be well and in good health' referring to the medicinal properties of some of the species (Blumenthal et al., 2000). The genus has a sub-cosmopolitan distribution, but it is largely absent in the North Amazon basin and Central and West Africa (Paton, 1991). In Southern Africa there are nearly 30 *Salvia* species that have been identified (Hedge, 1974; Codd, 1985; Paton, 1991; Kamatou et al., 2008) but species such as *S. coccinea, S. officinalis, S. reflexa, S. sclarea, S. tillifolia* are exotic to the region (Kamatou et al., 2008). Most of the South African species are confined to the Cape region (Codd, 1985).

Salvia species, commonly known as sage, are an important group of useful plants, which have not lost their importance since ancient times. *Salvia* species are used in traditional medicines all around the world, possessing antioxidant, antibacterial, antidiabetic, anti-tumer, antiplasmodial and anti-inflammatory activities (Watt and Breyer, 1962; Jalsenjak et al., 1987; Sur et al., 1991; Sivropoulou et al., 1997; Dorman and Deans, 2000; Velickovic et al., 2002; Ulubelen, 2003; Tepe et al., 2004; Seaman, 2005; Kamatou et al., 2008). Many *Salvia* species are used as herbal tea and in food cosmetics, perfumery and the pharmaceutical industry (Chalchat et al., 1998; Baylac and Racine, 2003; Mayekiso et al., 2008). Besides their medicinal value, *Salvia* sp. is grown in parks and gardens as ornamental plants (Nakipoğlu, 1993).

Salvia greggii A. Gray (autumn sage), which naturally grows in Central America, locally known as 'Isikhiki' is a medicinal plant used by the people of the Eastern Cape Province of South Africa. It is a herbaceous perennial shrub with pink flowers and has a height of 0.3 to 1.4 m, blooming throughout summer and autumn (Kamatou et al., 2008). It has leaves that are mid-green and obovate in shape and maybe smooth or lightly covered with hairs. Ethnomedical information from the indigenous people of the Eastern Cape revealed that the leaves of *S. greggii* are chewed raw or boiled and taken orally to cure throat infections.

There are a number of studies on foliar micromorphology on the genus (Corsi and Bottega, 1999; Kaya et al., 2003; Siebert, 2004; Kamatou et al., 2007; Ozkan, 2008; Kahraman et al., 2009; Celep et al., 2011). Leaves of many plants are densely covered with glandular and non-glandular trichomes, which originate from the epidermal cells (Werker, 2000). According to Franceschi and Giaquinta (1983) developmental and structural studies of trichomes can shed light on the nature of the secreted material and their functional significance.

Plant species that contain glandular trichomes that develop from epidermal cells generally produce relatively large amounts of bioactive compounds which include highly concentrated phytochemicals with biological activity of interest to many industries (Nishizawa et al., 1992; Duke, 1994; Turner et al., 2000). The essential oil produced by glandular trichomes is one of the characteristic features for Lamiaceae family (Ascensão et al., 1995). The ultrastructure and micromorphology of *S. greggii* have not been studied previously and also such investigations on *Salvia* species are rather very limited. Therefore, the present study aims to investigate the morphological features of *S. greggii* leaf, to determine the structure and distribution of the foliar appendages and to evaluate the possible usefulness of the structures.

MATERIALS AND METHODS

Plant material

The leaves of *S. greggii* were collected from a natural habitat around the University of Fort Hare, Alice campus, Eastern Cape Province , South Africa (Latitude 32° 46' 60 S and Longitude 26° 52' 60 E) in September 2010. The plant was identified by the curator of the Selmar Schönland Herbarium at Rhodes University, Grahamstown, South Africa. A voucher specimen (Dyubeni 2010/2001) was prepared and deposited at the Giffen Herbarium, University of Fort Hare, Alice campus.

Scanning electron microscopy (SEM)

Fresh leaves were cut into segments of about 4 to 6 mm in length and fixed for 24 h in 6% glutaraldehyde in 0.05 M sodium cacodylate, rinsed in 0.05 M cacodylate buffer (pH 7.5) and dehydrated in a graded series of ethanol (20 to100% X 3) at 20 min per rinse. This was followed by critical point drying with liquid CO_2 in Hitachi HCP-2 critical point dryer. Each dried sample was mounted onto aluminum specimen stubs with double-sided carbon coated adhesive discs and sputter-coated with gold-palladium (Eiko IB · 3 Ion Coater). Both the adaxial and abaxial surfaces of the leaves were examined at varying magnifications using a JEOL (JSM-6390LV) scanning electron microscope (SEM), operated at 10 – 15 kV acceleration voltage. All the representative features examined were captured digitally using microsoft image programme for windows. The energy dispersive X-ray spectroscopy-SEM, involved both the fixing and dehydration procedures as in SEM, while an EDX Analyzer (FEI QUANTA 200 Oxford) was used for the analysis.

RESULTS

The micromorphology of the leaf surfaces of *S.greggii* is presented in Figures 1 to 3. The leaves were characterized by two types of trichomes, glandular (peltate and capitates glandular) and non glandular. Short-stalked multicellular glandular trichomes were sparsely and evenly distributed on both leaf surfaces (Figure 2). Glandular trichomes were more abundant than the non glandular trichomes on both the abaxial and adaxial leaf surfaces (Figures 2 and 3). Each glandular trichome is composed of basal cell which is in line with the epidermal cells, the stalk cell and the oval shape glandular head which is characterized with cuticular sac (Figures 1, 2 and 3). Like other members of the Lamiaceae, *S. greggii* was characterized by both peltate and capitate glandular trichomes (Figure 1).

The SEM revealed that the plant contains uniseriate and multicellular non-glandular trichomes (Figures 1, 2 and 3). The distribution of these non-glandular trichomes appeared to differ in different areas of the leaf surface (Figures 2 and 3). A high number of non glandular trichomes compared to glandular trichomes have been observed along the midrib of the abaxial leaf surface (Figure 2). These non glandular trichomes were multicellular, uniseriate, short trichomes, with swollen basal epidermal cell, enlarged bases, thick cell wall and acute apices (Figure 2).

The leaves were also characterized by anisocytic stomata which were more abundant on the abaxial than adaxial epidermis (Figures 2 and 3). Some crystal deposits were observed on the leaf surfaces of *S. greggii* and some were deposited near the stomata (Figure 4). The chemical nature of the crystals on the leaf surfaces of *S. greggii* showed that they were predominantly composed of Ca, Mg, Si, Al, Fe, O, C, K and Na.

DISCUSSION

The present study sought to provide useful information on the foliar micromorphology of *S. greggii.* Like other members of the Lamiaceae, *S. greggii* carries both peltate and capitates glandular tricomes, as well as non glandular ones. Peltate glandular trichomes of Lamiaceae often comprise a broad head of several secretory cells (up to 16), a wide short stalk and a basal epidermal cell (Hallahan, 2000; Kahraman et al., 2009; Celep et al., 2011). Other species of Lamiaceae such as *Salvia chamelaeagnea* have been reported to contain, with up to sixteen head cells (Kamatou et al., 2006). Capitate glandular trichomes constitute a significant taxonomic character of the Lamiaceae and form part of the floral specialized properties for pollination (Navarro and El Oualidi, 2000; Kahraman et al., 2009).

Like the peltate trichomes, the capitates ones are very common in many species of *Salvia* (Corsi and Bottega, 1999; Siebert, 2004; Kahraman et al., 2009). Capitate trichomes are assumed to secrete varying amounts of polysaccharides along with essential oils (Werker, 1993). The material within the cuticular sac of capitate trichomes

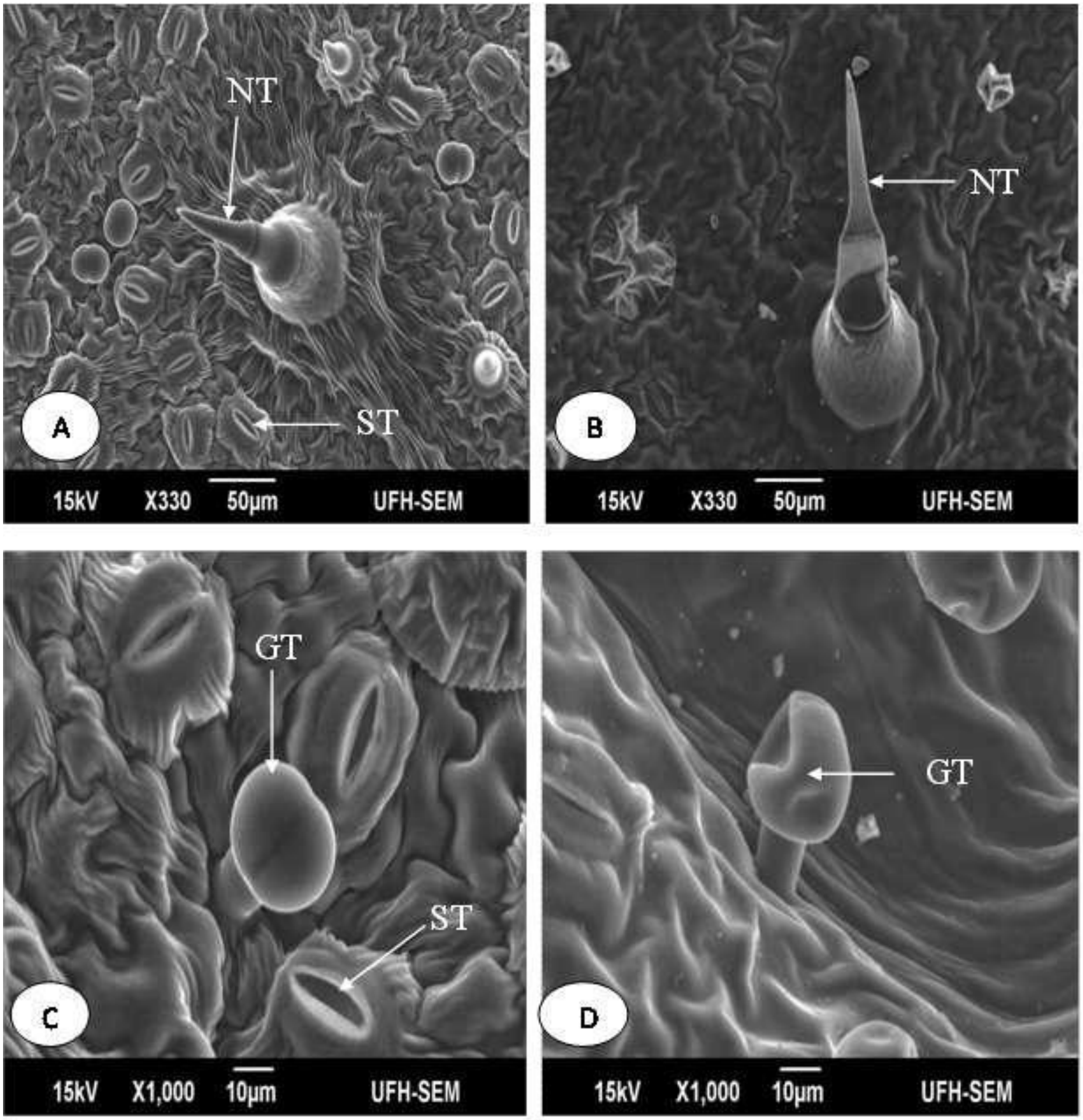

Figure 1. Micromorphology of glandular and non- glandular trichomes of *S. greggii* on both leaf surfaces. (A) NT on the abaxial surface. (B) NT on the adaxial surface. (C) GT on the abaxial surface. (D) GT on the adaxial surface. (NT = Non glandular trichome, GT = Glandular trichome and ST = Stomata).

is stored in the form of lipophilic substances such as terpenes, lipids, waxes and flavonoid aglycones (Mayekiso et al., 2008). When the cuticular sac ruptures due to external pressures these substances are released to the outside of the cuticular sac and kept on the surface layer of the plant. These substances (secondary metabolites) may provide the plant with chemical and physiochemical protection against various types of herbivores and pathogens by entrapping, deterring and poisoning (Wagner, 1991). The development of trichomes from the epidermis usually results from the differential enlargement and subsequent divisions of the epidermal cells and their derivatives (Carlquist, 1958).

Non glandular trichomes are associated with protection of the foliar appendages of the plant by covering the layer of epidermis possibly against foraging insects and airborne propagules of fungi (Harborne, 1990; Afolayan and Meyer, 1995; Aliero et al., 2005; Mayekiso et al.,

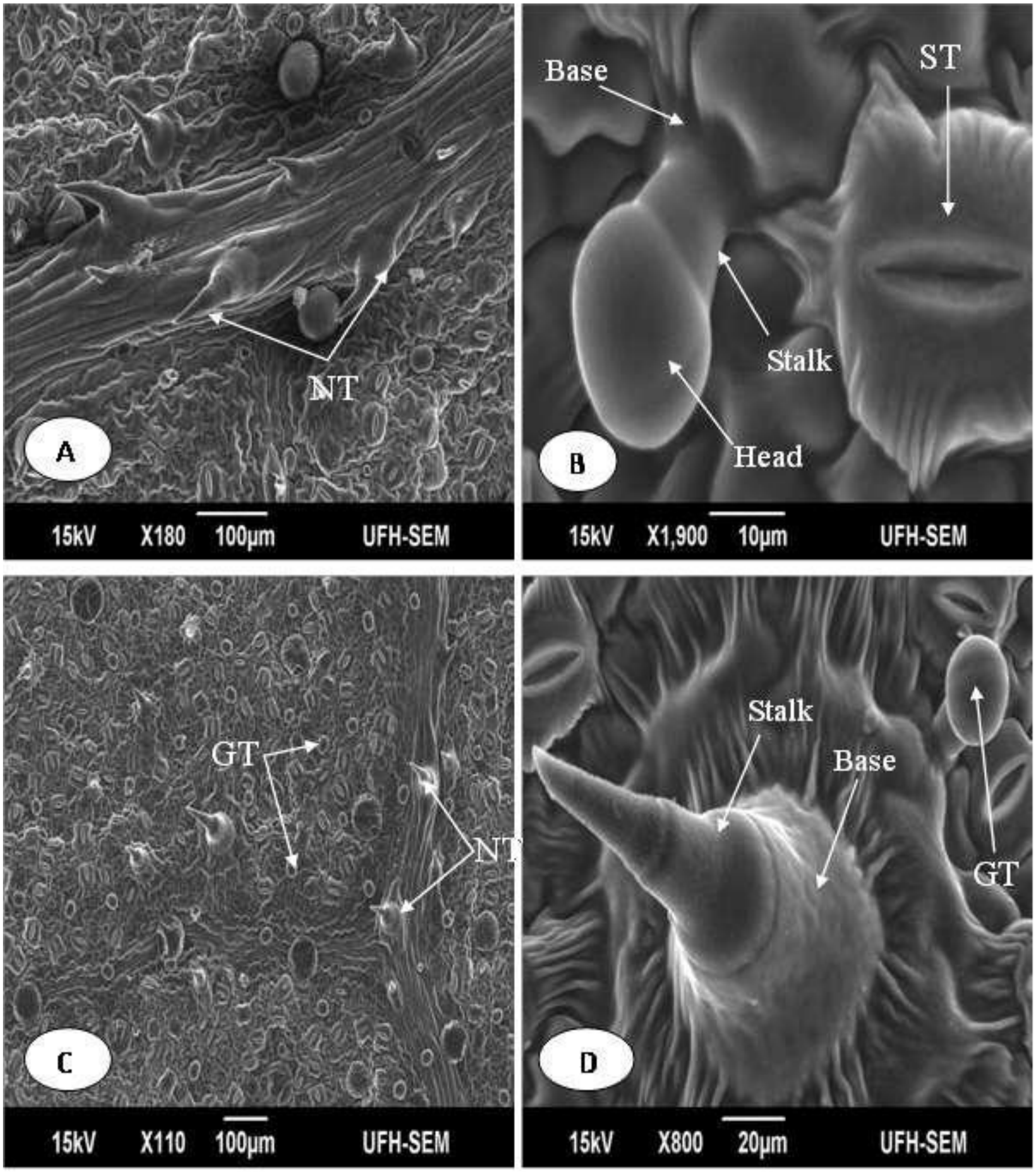

Figure 2. Trichome distribution and a trichome shown on a high magnification. (A) Abaxial surface displaying distribution of NT on the midrib. (B) Peltate GT morphology showing head, stalk and base. (C) Abaxial epidermal appendages. (D) A high magnification of NT on the leaf edges on the adaxial surface. (NT = Non glandular trichome, GT = Glandular trichome and ST = Stomata).

2008; Mayekiso, 2009). This is a natural phenomenon in most angiosperms (Fahn, 1967).

Energy dispersive X-ray spectroscopy-SEM analysis of *S. greggii* has shown that it contains foliar crystals. The primary function of these crystals is believed to aid in plant protection. Ions reported to be secreted by glandular trichomes on the leaf surface include Na, Cl, Ca, Cd, Zn, Mo, Pb, Pi, Si, S and others which significantly contributes to the toxic effects of the substance formed by that particular species (Salt et., 1995; Choir et al., 2001; Aliero et al., 2005). Some of these elements such as, potassium and calcium (macro

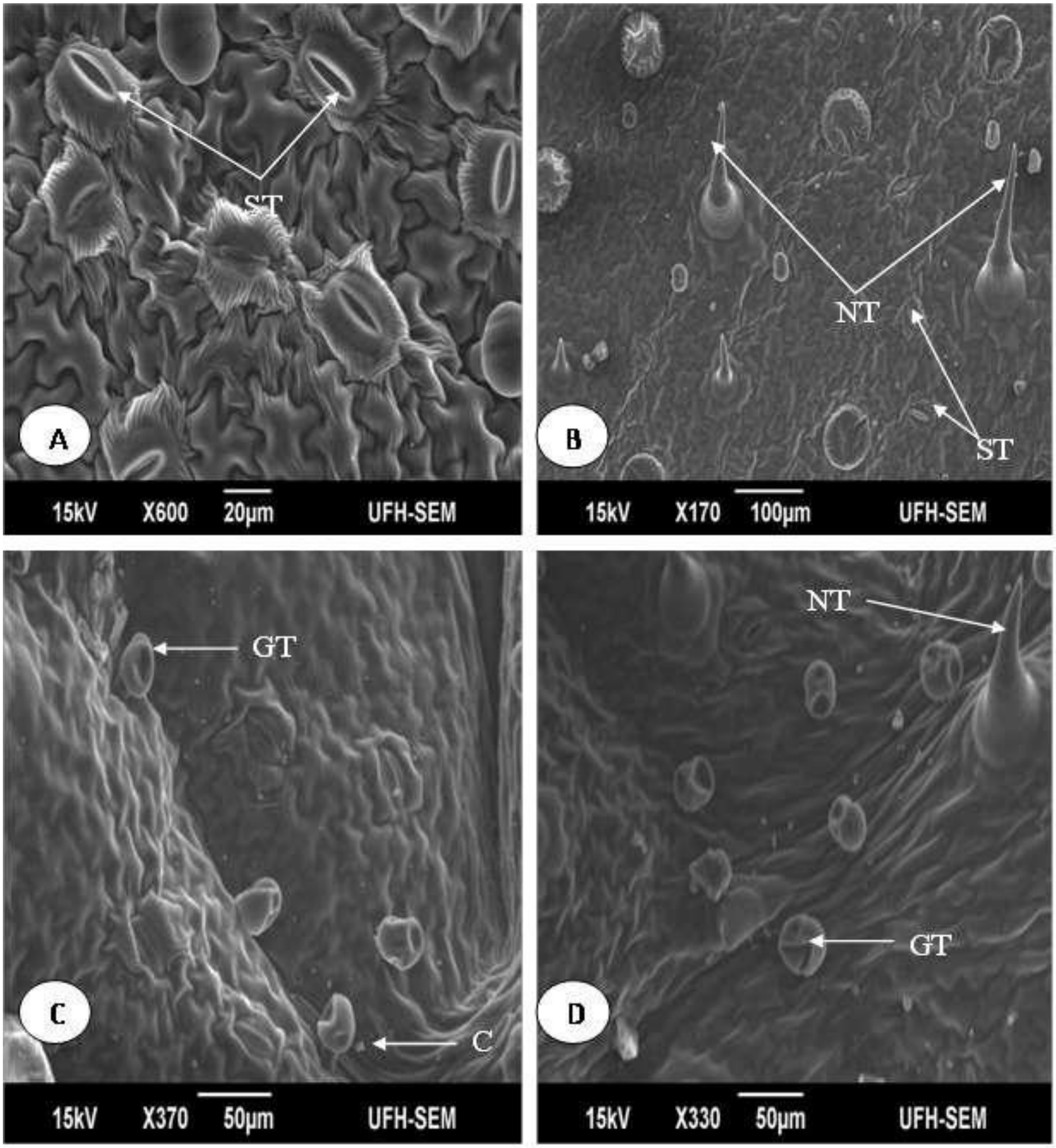

Figure 3. Trichome and stomatal distribution. (A) Distribution of stomata on the abaxial surface. (B) NT and stomatal distribution on adaxial surface (NB. short and long NT). (C) Peltate GT and some crystal deposits on the epidermal surface. (D) NT and GT on the midrib (adaxial). (NT = Non glandular trichome, GT = Glandular trichome, ST = Stomata and C = Crystal).

elements); sodium, aluminum and silicon (microelements) are very essential for plant growth. The mechanical stability of the leaf appendages is probably increased with the presence of these elements in the trichomes of *S. greggii*.

ACKNOWLEDGEMENTS

Authors are grateful to the Govan Mbeki Research and Development Center (GMRDC) and the National Research Foundation (NRF) for the financial support.

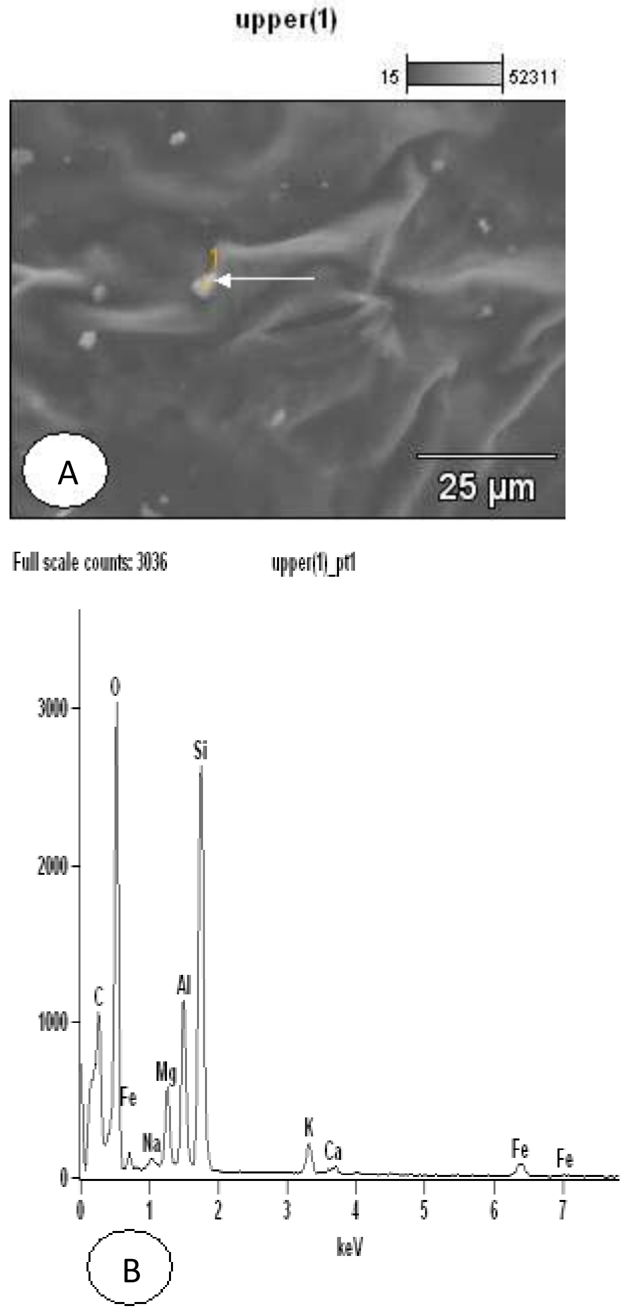

Figure 4. (A) Epidermal surface of *S .greggii* (The arrow points to the crystal analyzed). (B) Spectra of energy dispersive X-ray (EDX) spectroscopy of the crystal.

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