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Full Length Research Paper

Anatomy and morphology of *Nicotiana glauca* with regard to its crystals characterization

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***Nicotiana glauca* belongs to the Solanacese family of which *Nicotiana tabacum* and *Nicotiana rustica* are best known for their use as tobacco. *N. glauca* attains the greatest abundance and diversity in Central Argentina where it is considered native, and has been known as a weed in South Africa since the 19th Century. Extensive work on toxic effect of secondary metabolites extracted from this plant was conducted but their sources of origin and structure were not known. The leaves of *N. glauca* were characterized by the presence of solitary crystals and crystal-sand which originated from the cytosol and vacuoles. The presence of the compact tissues, isobilateral leaf with thick-cuticular epidermis and abundant crystals was assumed to be of ecological importance as this species appeared to be adapted to the xeromorphic habitats.**

Key words: Anatomy, morphology, crystals in *Nicotiana glauca*.

INTRODUCTION

It is known that, traditional medicine plays a very significant role in rural communities as a source of various remedies (Magwa et al., 2005). Recently, an enhanced interdisciplinary and research program with botanical, chemical, pharmacological and clinical studies has been undertaken by scientist to explore the potential of drug plants as a source of new medicines (Pei-gen and Shan-Lin, 1997; Van Wyk, 1997). It has been estimated that, over 250 000 species of plants are used and more of these are yet to be explored for their usage in the welfare of societies.

These species are common in the diverse families of flowering and non-flowering plants. Among the flowering plant families, the family of Solanaceae has many species of high medicinal value. This family is characterized by flowering plants of which most of them are of agricultural importance. They are the source of food to human kind; however, some of these plants within this family are toxic to human and animals. Those species that are regarded as toxic are often rich in alkaloids whose toxicity to living organisms ranges from mildly irritating to fatal in small quantities (Magwa et al., 2005; Knobloch et al., 1988; Uribe et al., 1985).

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Nicotiana glauca is a common weed beside inland rivers and is often found as a garden plant. The first investigation on toxic effects of secondary metabolites extracted from this plant was initiated as early as the 1930's (Steyn, 1934). In the late seventies, the four major pyridine alkaloids, that is, nicotine, anabasine, anatabine and nornicortine were produced from this species (Hawley, 1977; Waller and Edmund, 1978). Anabasine is derived by extraction from both *Anabasis apylla* and *N. glauca* or by synthetic means and is used as an insecticide (Hawley, 1977; Duke, 1985).

Nicotine is derived via distillation of tobacco with milk of lime and extracted with ether. *N. glauca* reported to be anodyne and hirucidal is a folk remedy for boils, headaches, piles, sores and wounds (Duke, 1985). Although, there is extensive work that was conducted on toxic effects of the secondary metabolites of *N. glauca*, their sources of origin are not clearly identified. In addition, there is very little information concerning the anatomical and morphological structure of this species, a preliminary study on anatomy of this plant is therefore reported in this paper.

MATERIALS AND METHODS

Plant collection

Nicotiana glauca was collected from the Hogsback Forest about 20

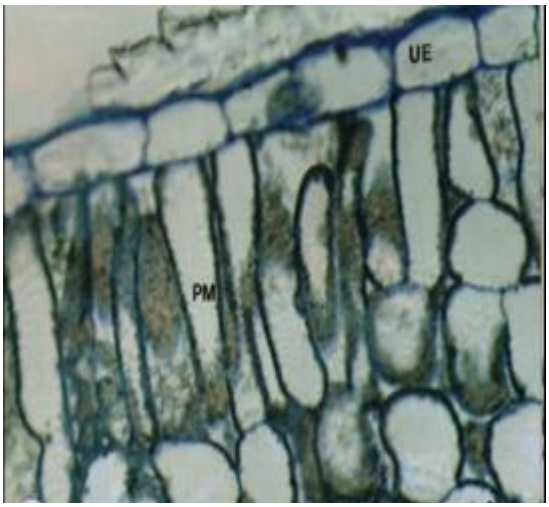

Figure 1. Anatomical features of *N. glauca* leaf showing upper epidermis and palisade mesophyll cells with crystals.

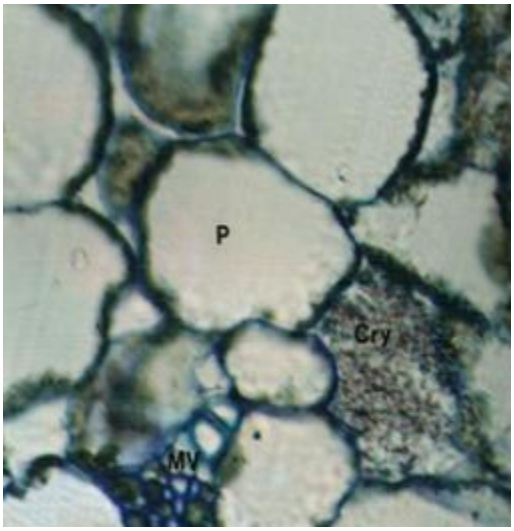

Figure 2. Parenchymatous cells contain crystals and a minor vein.

km North of Alice, in the Eastern Cape Province, South Africa, at a height of approximately 1200 m above the sea level.

Light microscopy (LM)

The leaf portions were cut into smaller sections of approximately 0.5 mm and were fixed in a buffered of 6% glutaraldehyde and kept in a refrigerator overnight. The sections were then rinsed in 50 M Sodium Cacodylate buffer and were then post fixed in 2% Osmium tetroxide in 50 mM Na-cacodylate buffer, pH 7.3, overnight at 4°C, infiltrate in graded series resin. Thin sections (about 0.5 - 2.0 μm^2) were cut with glass knives on a LKB Ultramicrotome, stained with Uranyl acetate followed by lead citrate and observed in a Hitachi Transmission electron microscopy at 75 - 100 kV. Some of the sections were stained with 0.05% toluidine blue and examined with Zeiss Photo-microscopeIII.

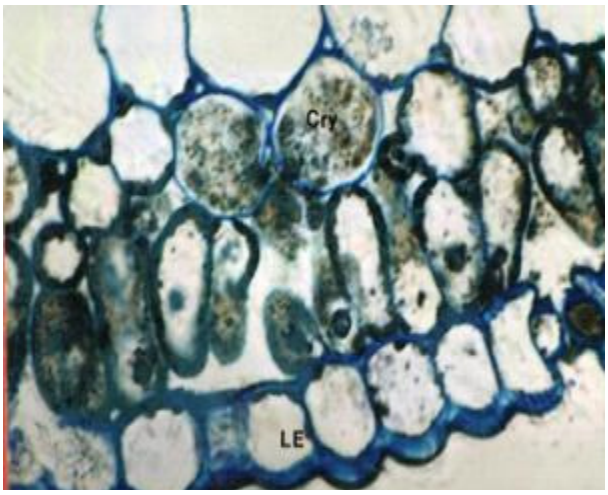

Figure 3. Displayed lower epidermis and a spongy mesophyll tissue whose cells are associated with solitary crystals and crystal sand. UE = Upper epidermis. LE = lower epidermis, PM = palisade mesophyll, Cry = crystal, MV = minor vein and P = parenchymatous cells.

Scanning electron microscopy (SEM)

Sections of leaves and stem (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 Eiko IB-3 ion Coater, a sputter coater and viewed at 15 KV with a Hitachi S-450 Scanning Electron Microscope. For energy dispersive spectroscopy (EDS), the fixing and dehydration followed the same procedure as in SEM while a FEI QUANTA 200 EDS was used for the analysis.

RESULTS

Anatomical investigation showed that the leaves of *N. glauca* were characterized by a single layer of thick cutinized epidermal cells on both sides of the leaf (Figures 1 and 3) and a continuous layer of palisade mesophyll tissue (Figure 1). These parenchymatous cells occupied almost the entire space of the adaxial side and therefore appeared to be the main photosynthetic tissue as the chloroplast occurred in great abundance in this tissue (Figure 1)

The spongy mesophyll tissue was characterized by intercellular spaces which were assumed to occur as a result of cell lysis (Figure 2). The crystal sand and solitary crystal types were often found in these intercellular spaces (Figures 2, 3, 4, 5 and 6). However, some of the crystal sand and solitary crystals occurred as idioblasts in various tissues. The common example is the mesophyll cells which were characterized by the presence of cluster of crystals (Figure 3) together with parenchymatous pith of the stem (Figures 7 and 8). In some instances, these crystals were so large to the extent that some cells

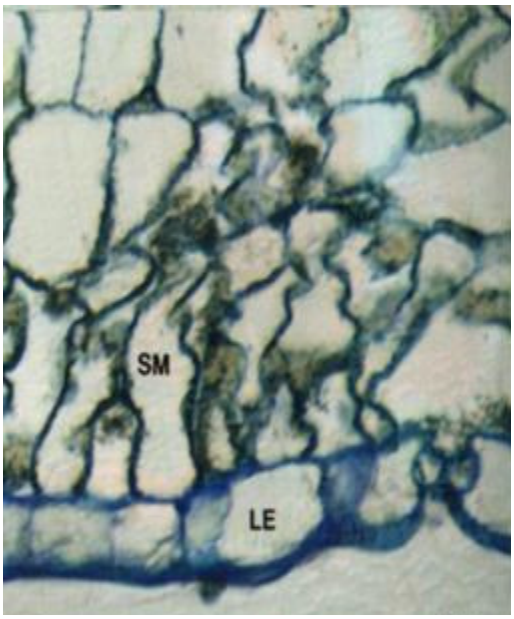

Figure 4. Displayed lower epidermis and a spongy mesophyll tissue whose cells are associated with solitary crystals and crystal sand. UE = Upper epidermis. LE = lower epidermis, PM = palisade mesophyll, SM = spongy mesophyll, MV = minor vein and P = parenchymatous cells.

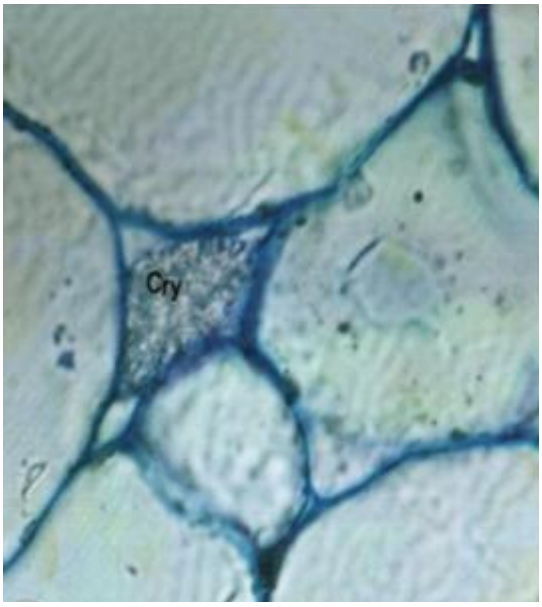

Figure 5. Exhibited the localization of crystals in the intercellular space whilst.

appeared to be deformed in shape (Figures 2 and 7). It appeared that some of the crystals which were contained in the intercellular spaces were also associated with the vascular tissue, both the xylem (Figure 9) and the phloem (Figure 10).

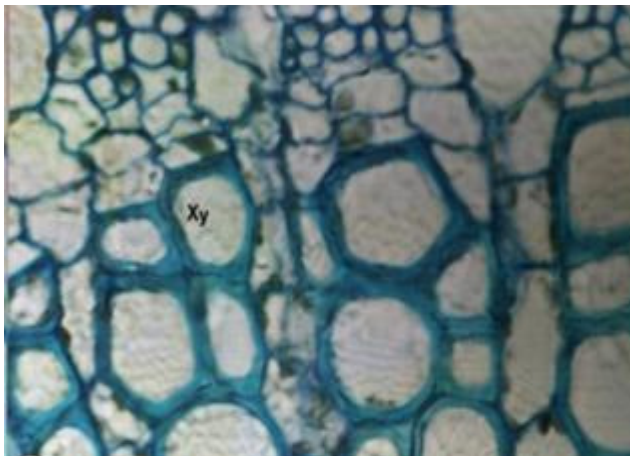

Figure 6. The presence of crystals in the ray parenchyma of the xylem tissue.

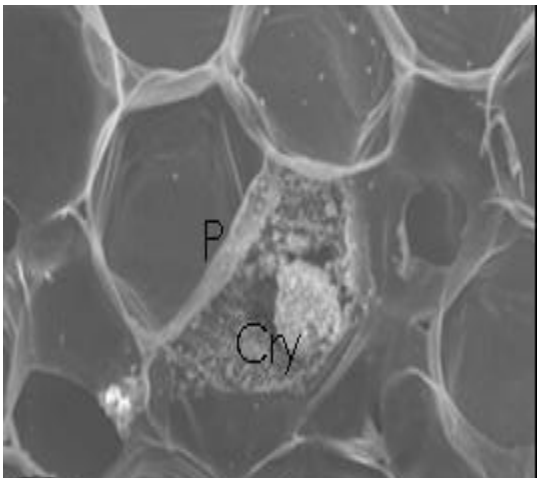

Figure 7. SEM micrographs of the stem pith. Note the distribution of sand crystals (Cry) within the parenchymatous cells (P).

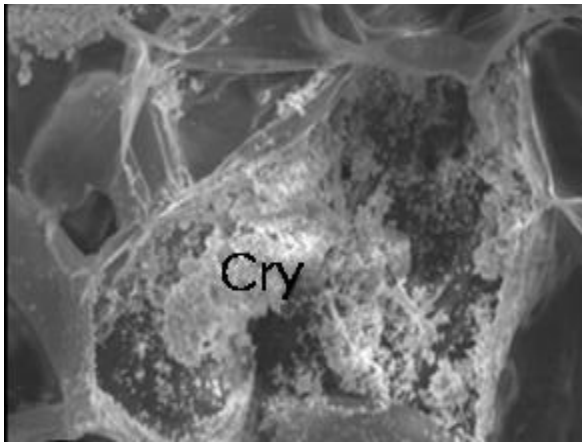

Figure 8. SEM micrographs of the stem pith with distribution sand crystals (Cry).

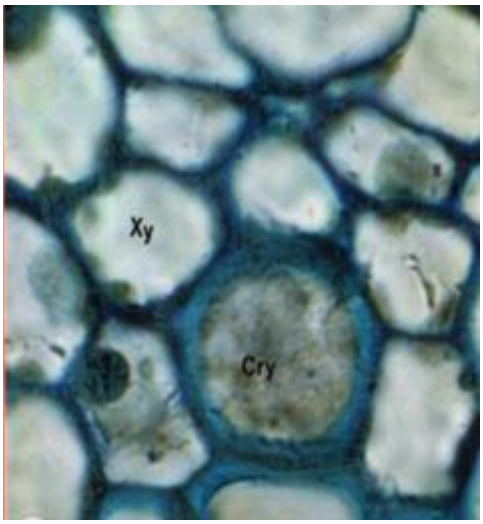

Figure 9. Displayed of the abundance of crystals in the xylem parenchyma.

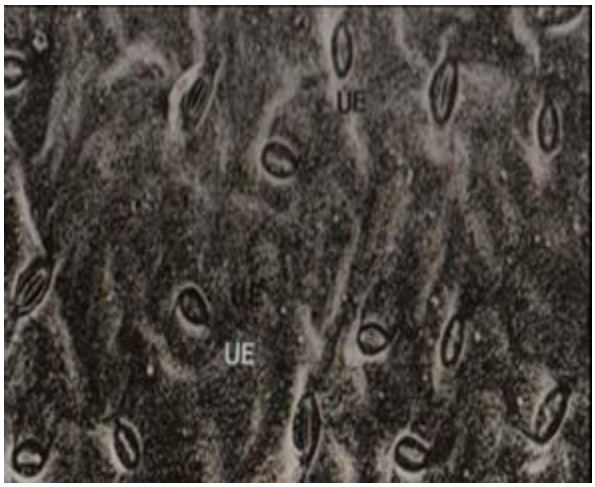

Figure 12. The SEM micrographs of the upper epidermis of a leaf. Note: the distribution of stomata on abaxial and adaxial surfaces, respectively.

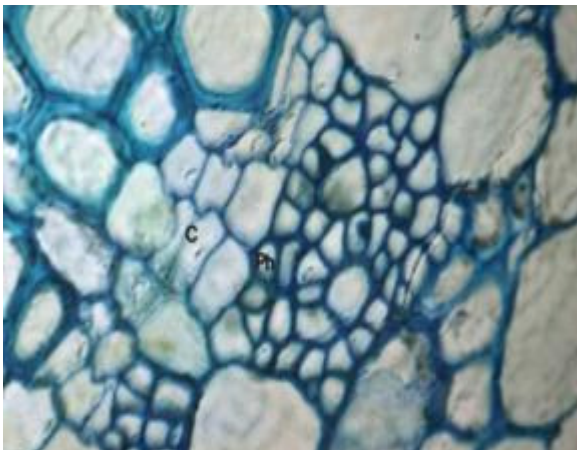

Figure 10. Crystals are in the cambium and the phloem parenchyma. Cry = crystal, Xy = xylem, C = cambium and Ph = phloem.

Figure 13. An elevated stomata on the adaxial surface whilst.

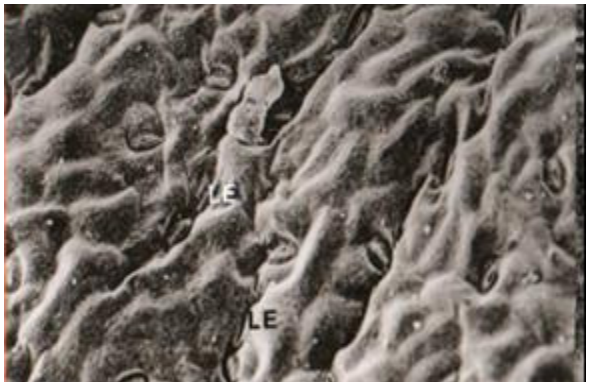

Figure 11. The SEM micrographs of the lower epidermis of a leaf. Note: the distribution of stomata on abaxial and adaxial surfaces respectively.

Scanning Electron Microscope (SEM) results showed that the leaves of *N. glauca* were characterized by high distribution of stomata on both sides of the leaf (Figures 11 and 12). This amphistomatic system exhibited the elevated and sunken stomata (Figures 13 and 14). These stomata were covered by the solitary crystals which were strategically positioned within the stomata pores (Figures 14 and 15). These crystals appeared to originate from the cytosol and vacuoles and were occasionally found on the exterior surfaces of plant organs as epidermal appendages (Figure 16). The morphological studies have shown that these crystals appeared to be more dominant on the epidermal layer of leaf which was deliberately injured (Figures 17 and 18).

Energy Dispersive Spectroscopy (EDS) showed that

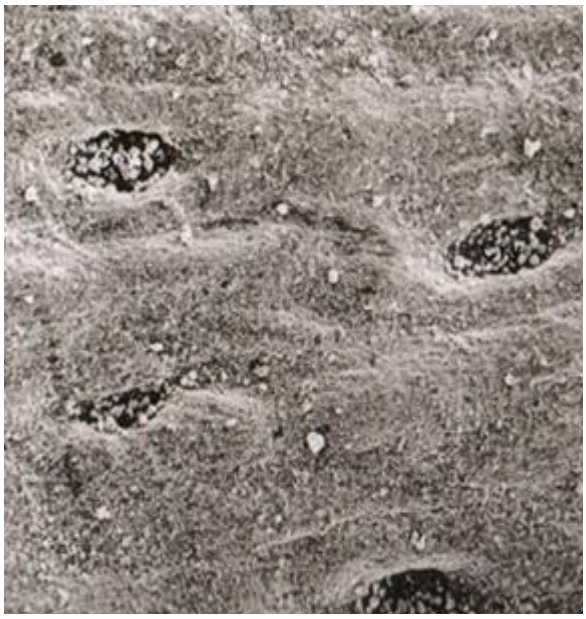

Figure 14. Displayed crystals which are concentrated within the sunken stomata. ST = stomata, UE = upper epidermis, LE= lower epidermis and Cry = crystal.

Figure 15. SEM micrographs of a leaf showing a high distribution of crystals on the leaf epidermal stoma.

the foliar crystals are predominantly composed of calcium (32.46%), oxygen (58.97%), sulphur (7.78%), potassium (0.99%) and chlorine (0.84%). These macro elements are very essential to plant growth. The EDS also showed that calcium, oxygen and potassium were the major constituents of the crystals found in the parenchymatous pith of *N. glauca* stem (Figures 19 and 20).

Figure 16. SEM micrographs of a leaf showing a high distribution of crystals on the guard cells.

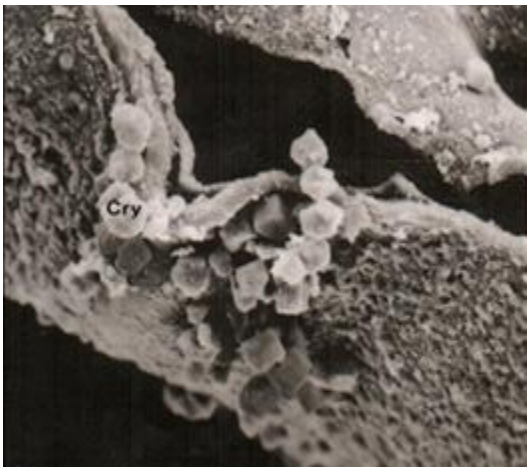

Figure 17. The micrograph of a deliberately injured leaf, which showed the production of the solitary crystals from the sap of a wounded tissue.

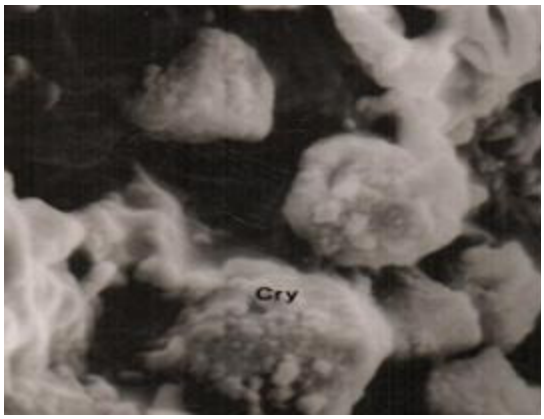

Figure 18. The crystals of a wounded tissue at a higher magnification. ST = stomata and Cry = crystal.

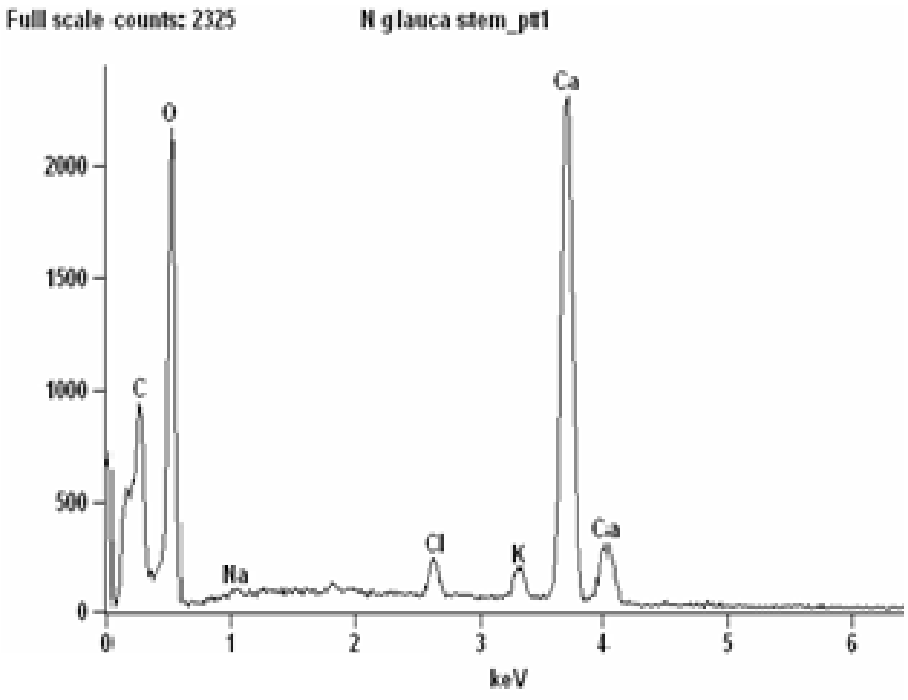

Figure 19. Typical EDS scan of *N. glauca* stem and leaf. Note the presence of calcium, oxygen and chlorine as major constituents of the crystals analyzed in both the stem and the leaf.

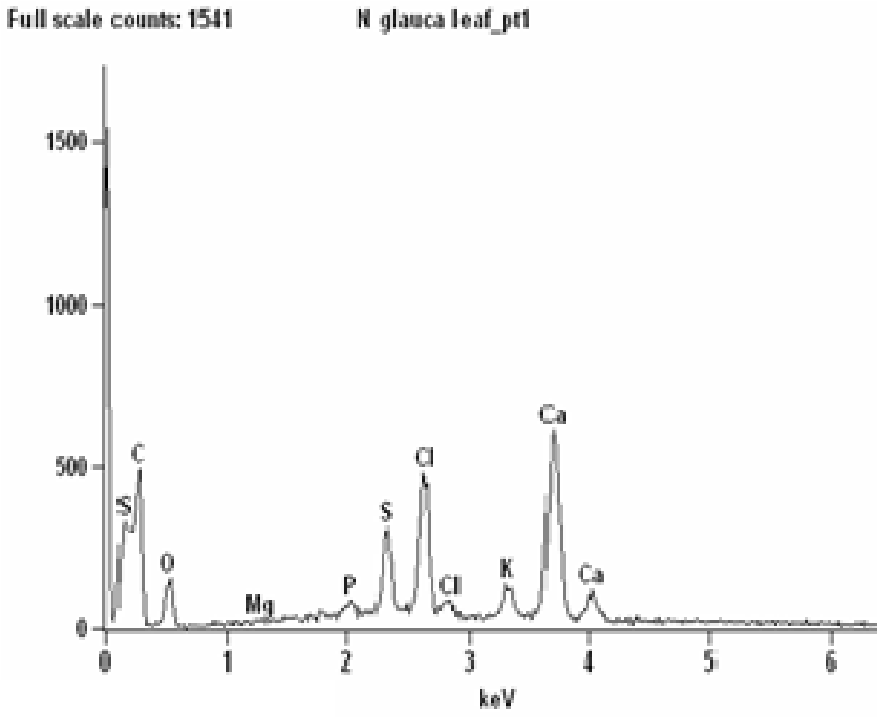

Figure 20. Typical EDS scan of *N. glauca* stem and leaf. Note the presence of calcium, oxygen carbon, sulphur and chlorine as major constituents of the crystals analyzed in both the stem and the leaf.

DISCUSSION

N. glauca was characterized by waxy cuticle, a compact palisade mesophyll tissue and a loosely arranged spongy mesophyll tissue. This spongy mesophyll tissue was associated with vascular tissue. Such association was characterized with the intercellular spaces which contained the crystal sand (mass of tiny individual crystals) and solitary crystals. Some of the obvious features of this species were thickness of the cell wall, the greater density, both the vascular system and the stomata, and the increased development of the palisade tissue at the expense of the spongy tissue. These structural characteristics have been shown in other species belonging to this family and are assumed to be associated with arid habitats (Fahn, 1986). It is also believed that the arrangement of these tissues within the leaf is responsible for maximum utilization of light (Fahn, 1982). The other important factor which is assumed to enhance the photosynthetic efficiency was the presence of well-developed systems of intercellular spaces which might be involved in facilitating rapid gas exchange. However, the significance of association of vascular tissue and the intercellular spaces, both of which contained crystals in *N. glauca* that is not clear.

One of the diagnostic microscopical characters of *N. glauca* included the presence of crystals that were occasionally found on the exterior surfaces of plant organs as epidermal appendages. These crystals appeared to originate from the intracellular compartments and were transported to outside the plant through the epidermis. The combination of crystal sand and solitary crystals appeared to be predominantly associated with the intercellular spaces, vascular tissue, spongy and palisade mesophyll tissues, whilst the large solitary crystals were more pronounced on the epidermal surfaces.

The crystal formation within the mesophyll cell often became so large that the cell appeared to be deformed and ultimately had the same shape as the crystal when viewed with the light microscope. An interesting observation was that, similar large crystals were rapidly produced both internally and externally in a deliberately injured leaf. It is assumed that a further growth of these crystals within the two adjacent cells resulted to cell lysis and subsequently in the formation of large intercellular spaces containing crystals (Fanceshi and Horner, 1980).

Energy Dispersive Spectroscope (EDS) showed that the foliar crystals were predominantly composed of calcium, oxygen, potassium and chlorine. These elements are very essential to plant growth and their presence are most probably increases the mechanical stability of the leaf appendages. It is feasible that the anionic contents of the crystals, possibly produced by *N. glauca* leaves contribute to the toxic effects of secondary metabolites extracted from this plant (Maiti et al., 2002). It is therefore, assumed that these secondary metabolites might serve in defense mechanism. In this regard, the anatomical and morphological characteristics could be used as the additional tool in the classification of this species with the genus Nicotiana.

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