Research Article

Morphology and density of trichomes and stomata of *Trichosanthes cucumerina* (Cucurbitaceae) as affected by leaf age and salinity

Odunayo Clement ADEBOOYE*, Mauricio HUNSCHE, Georg NOGA, Christa LANKES

Institute of Crop Science and Resource Conservation, University of Bonn, Bonn – GERMANY

Abstract: We investigated the relationship between leaf age and trichome/stomata densities in the snake tomato (*Trichosanthes cucumerina* L.). In a second experiment, the effects of different salinity levels on trichome and stomatal densities and their micromorphology were investigated. The leaf age investigation utilised young, semiold, and old leaves, while the salinity study utilised 0, 60, 120, and 180 mM NaCl for the investigation. Two morphotypes (Variant A-Green and Variant B-Light Green) of *T. cucumerina* were used for the salinity experiment while only Variant B-Light Green was used for the leaf age investigation. This study identified 2 discernible types of trichomes in *T. cucumerina*, namely conical trichomes and globular-headed trichomes, which are segmented into 3 or more distinct sections. A major observation in this study was that while salinity did not significantly ($P \geq 0.05$) affect the densities (abaxial and adaxial) of the conical trichomes in Variant A, the same parameter was significantly reduced in Variant B due to salinity treatment. Results showed that there were significantly ($P \geq 0.05$) more stomata on the abaxial surface than the adaxial surface, irrespective of the morphotype and salinity treatment. Under the control treatment (no salinity), Variant B-Light Green had significantly more stomata on the abaxial side than Variant A-Green, while on the adaxial side, the reverse was found to be true. Results also showed that the conical and globular-headed trichomes' densities decrease as leaf age increases. The leaf petioles of the 2 variants are dominated by globular-headed trichomes. On the abaxial surface, analyses showed that there were 28% and 58% more stomata on the young leaf compared to semiold and old leaves, respectively. Further studies into the physiologically mediated mechanisms that are responsible for these observations in *T. cucumerina* are crucial.

Key words: *Trichosanthes cucumerina*, trichomes, stomata, salinity, leaf age

Introduction

Trichomes are known to be present on the surfaces of leaves, stems, fruits, and sepals and also on the margins of leaves and sepals. Previous reports have shown that there are glandular and nonglandular trichomes that function in plants to reduce heat load, increase tolerance to freezing, aid seed dispersal, enhance water absorption, protect plant structures from the harmful effects of UV-B, serve as taxonomical criteria, serve as insect repellent, and offer a means of protection against herbivores and pathogens (Johnson, 1975; Mauricio & Rausher, 1997; Werker, 2000; Kennedy, 2003; Wagner et al., 2004; Simmons & Gurr, 2005; Serna & Martin, 2006).

Cucurbitaceae is a family of plants known to consist of several members with hairy structures.

* E-mail: adebooye@uni-bonn.de

The presence of different types of glandular trichomes in some Cucurbitaceae species was reported by Kolb and Müller (2004). According to those researchers, the botanical literature contains more than 300 descriptions of trichome types. The trichome appendages arise from a series of anticlinal and periclinal divisions of epidermal cells to form specialised structures that function as glandular or nonglandular trichomes. Simply defined, such integral elements of the plants' surface, which are all outer growths of the epidermis, are termed "trichomes" (Kolb & Müller, 2004). Inamdar and Gangadhara (2008) studied the vegetative and floral trichomes in 9 genera and 12 species of Cucurbitaceae and classified them into glandular and nonglandular types. In all, 13 main types and 26 subtypes of glandular and nonglandular trichomes were reported. These authors showed that all of these types of trichomes originated from a single papillate hair.

An important member of the Cucurbitaceae family is the Nigerian underexploited and indigenous African snake tomato (*Trichosanthes cucumerina* L.), otherwise known as the snake gourd. It is one of the species that possess prominent abaxial and adaxial pubescence properties. Scientific evidence has shown that the fruit of *T. cucumerina* is a viable substitute for the solanaceous tomato (*Lycopersicon esculentum* (L.) Mill.) (Adebooye et al., 2005; Oloyede & Adebooye, 2005; Adebooye & Oloyede, 2007; Adebooye, 2008). Specific studies on the oxidative stress response mechanism of *T. cucumerina* at the cellular level have been carried out (Adebooye et al., 2008a, 2008b). However, none of these studies considered the effects that induced stress could have on the leaf trichomes of *T. cucumerina*. On the basis of this gap in information, we hypothesise that salinity stress and leaf age would affect trichome density and structure in *T. cucumerina*.

Materials and methods

Plant material and growth medium

The study was carried out at the Institute of Crop Science and Resource Conservation- Horticultural Science at the University of Bonn in Germany. Two morphotypes of *T. cucumerina* (Variant A-Green and

Variant B-Light Green), which had previously been described by Adebooye et al. (2005), were used for the study. The study was set up in a digitally controlled growth chamber maintained at an air temperature of 20 ± 1 °C and relative humidity of 60 ± 5%, and which was supplied with sodium fluorescent lamps providing photosynthetic active radiation of 160 μmol s^{-2} m^{-1} for a 14-h light period daily throughout the duration of the experiment. A 50 × 80 cm propagator (three-in-one-propagator, Jemp Engineering Ltd., UK) equipped with adjustable heating control elements was used for raising the plants at a root zone temperature of 30 °C. The seeds were treated with Aatiram® fungicide (Stähler Agrochemie, Germany) at a rate of 0.5 g 100 g seed^{-1} before planting. Seeds were sown in plastic cups filled with peat-based compost, which contained, per litre, 4.0 mmol NO_3, 1.3 mmol P, 2.2 mmol K, 1.0 mmol Ca, 1.0 mmol Mg, 0.15 mmol Fe, and 0.5 mmol Mn. The propagator was stuffed with inert perlite to serve as insulation and conserve the heat in order to maintain the required root zone temperature. The plastic cups were inserted into the insulated propagator. There was one seedling per cup.

Experiment 1: Salinity study

Seedlings emerged 7 days after planting and they were left to grow for an additional 7 days (3-leaf stage). At this stage, treatment of the plants with salt solution (sodium chloride: NaCl, MW 58.44 g) commenced. There were 3 salt concentrations (60, 120, and 180 mM) and 1 control (0 mM). The control treatment received only water. Each treatment was applied in 4 replications to each *T. cucumerina* morphotype. There were 10 cups per treatment. Every other day, each cup received 30 mL of the appropriate treatment solution. At 12 days after the commencement of the salinity treatments, samples of the first generation (oldest) leaves, located just above the ground level, were collected for stomata and trichome counts on both the abaxial and the adaxial sides using scanning electron microscopy (SEM).

Experiment 2: Leaf age study

Seedlings of the 2 morphotypes of *T. cucumerina* were raised as described above but without salinity treatment. At 5 weeks (35 days after emergence), 3 generations of leaves were sampled as follows: 1) Old leaves were samples from the first generation

leaves close to the collar. They were well lignified, fully expanded, and approximately 30 days old. 2) Semiold leaves were sampled from the second generation leaves located in the middle of the vine length. They were semilignified, fairly expanded, and approximately 20 days old. 3) Young leaves were sampled from the apex of the vine. They were soft, not fully opened, and approximately 10 days old. All of these leaves were subjected to SEM as described below.

Scanning electron microscopy (SEM)

Leaf samples were carefully harvested without causing any damage to the surfaces. For each treatment, 10 leaf samples were taken. Leaf discs (0.276 mm^2) were punched out from the sampled leaves and fixed on double-sided adhesive on electrically conductive carbon planchets (Plano GmbH, Wetzlar, Germany) previously mounted on aluminium SEM stubs. Adaxial and abaxial sides were investigated using a scanning electron microscope (ESEM XL 30 FEI, Philips, Eindhoven, the Netherlands) at the environmental modus (pressure = 4.5 Torr) with an acceleration beam voltage of 15 kV and a working distance of 10 mm (= amplification of ×200). A Peltier cooling element maintained the specimen temperature close to 5 °C. Electronically stored SEM images were used to determine the density of the stomata and trichomes using microscope-specific software (XL 30 Microscope Control).

Statistical analysis

All data collected were subjected to analyses of variance and means were separated using Duncan's multiple range test at a 5% level of probability as well as simple percentage error for the charts.

Results and discussion

Stomatal density due to salinity

General observations of the leaves of *T. cucumerina* showed that leaf surfaces (abaxial and adaxial) are soft, with no visible wax crystals. It was also noted that the trichomes are soft and easily susceptible to damage by the SEM beam and the vacuum produced in the microscope chamber. The stomatal densities (Table) vary with the morphotype, leaf surface, and salinity treatments. Generally, there

were significantly more stomata on the abaxial than the adaxial surface, irrespective of the morphotype and salinity treatment. It was also noted that under the control treatment (no salinity), Variant B-Light Green had significantly more stomata on the abaxial side than Variant A-Green, while on the adaxial side, the reverse was observed. This is a perfect indication of genotypic difference even for the same plant (species), but with different morphotypes. Results showed that stomatal density on both the adaxial and abaxial sides of Variant A-Green was not significantly affected by the salinity treatment. However, for Variant B-Light Green, on the abaxial side the control plants had approximately 38% more stomata than the plants that were treated with salt (60-180 mM), while the stomatal count on the adaxial side was not significantly affected by salinity treatment.

Stomatal and trichome density due to leaf age

We report the stomatal and trichome densities only for Variant B-Light Green of *T. cucumerina*. Results (Figure 1) showed that on the abaxial leaf surface, as the leaf age increases, the density of stomata decreases. Analyses showed that there were 28% and 58% more stomata on the abaxial surface of young leaves compared to semiold and old leaves, respectively. We suspect that the number of stomata on the abaxial surface is programmed genetically and established during the first stage of cell differentiation. As the leaf grows and expands, wider spacing is created between the stomata, thus resulting in fewer numbers of stomata per given leaf area. It must be noted that for all leaf ages, a sample size of 0.276 mm^2 was used for SEM analyses. In contrast, it is interesting to note that the stomata count on the adaxial surface was not significantly affected by leaf age (Figure 1). For this observation, it is suspected that more stomata are produced on the adaxial surface as the leaf grows and expands since leaf expansion is expected to result in wider spacing between the stomata, especially when the same sample size (0.276 mm^2) was used for all of the SEM analyses. The physiological mechanism that is responsible for this type of development needs further investigation.

As shown in Figures 2 and 3, the conical and globular-headed trichomes' densities decrease as leaf age increases. In an earlier study on *Tetradenia riparia* (Hochst.) Codd (Lamiaceae), Gairola et

Table. Influence of variety and salt treatments on the stomata and trichome densities of the adaxial and abaxial *Trichosanthes cucumerina* leaf surfaces.

| Variety | Salt treatments | Leaf side | Stomatal density[1] | Trichome density 0.276 mm^{-2} | |
				Conical trichomes	Globular-headed trichomes
Green	Control	Adaxial	7.0 ± 0.8c	3.0 ± 0.4cd	1.1 ± 0.4a
		Abaxial	22.1 ± 3.0b	5.3 ± 0.7b	1.1 ± 0.3a
	60 mM	Adaxial	9.4 ± 1.2c	3.5 ± 0.4cd	1.4 ± 0.2a
		Abaxial	26.4 ± 3.3b	6.1 ± 0.8b	1.3 ± 0.6a
	120 mM	Adaxial	6.2 ± 0.7c	2.6 ± 0.5d	1.3 ± 0.3a
		Abaxial	22.9 ± 1.9b	5.2 ± 0.5bc	0.4 ± 0.2a
	180 mM	Adaxial	6.5 ± 0.9c	4.5 ± 0.2c	1.6 ± 0.5a
		Abaxial	30.8 ± 2.1b	5.8 ± 0.7bc	1.4 ± 0.3a
Light green	Control	Adaxial	4.1 ± 0.7c	6.8 ± 0.4b	1.5 ± 0.3a
		Abaxial	43.7 ± 3.6a	13.2 ± 0.7a	1.0 ± 0.2a
	60 mM	Adaxial	5.5 ± 0.9c	2.2 ± 0.3d	1.2 ± 0.2a
		Abaxial	29.3 ± 1.6b	4.0 ± 0.4bc	1.1 ± 0.2a
	120 mM	Adaxial	4.8 ± 0.8c	4.3 ± 0.3c	2.1 ± 0.4a
		Abaxial	27.1 ± 3.0b	9.1 ± 0.8b	0.9 ± 0.3a
	180 mM	Adaxial	5.7 ± 0.5c	1.6 ± 0.3d	1.7 ± 0.2a
		Abaxial	28.3 ± 2.3b	6.7 ± 0.8bc	0.8 ± 0.3a

[1]Evaluated area (0.6 × 0.46 mm) = 0.276 mm^2. Presented values = mean ± SE, n = 10.
Means in each column are separated by Duncan's multiple range test at a 5% level of probability.
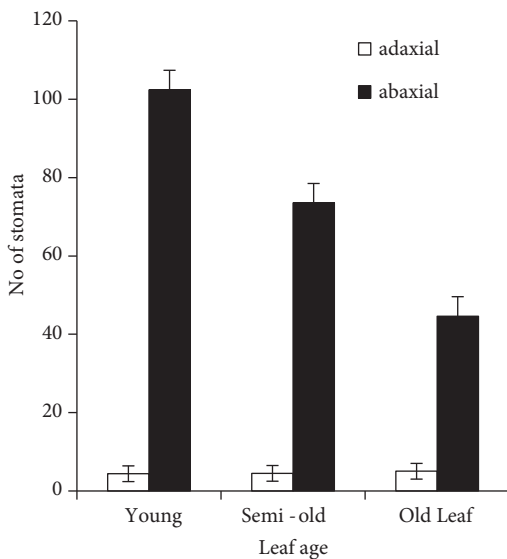

Figure 1. Relationship between leaf age and stomatal count on the adaxial and abaxial leaf surfaces of *T. cucumerina* for the variant B Light-Green morphotype.
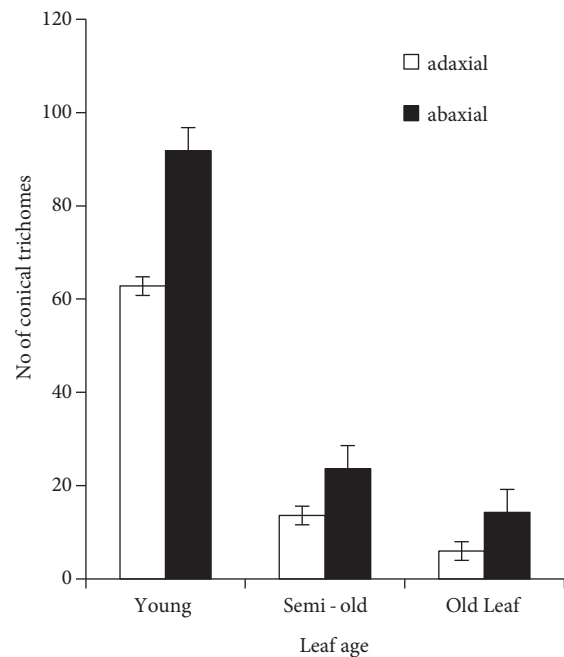

Figure 2. Relationship between leaf age and conical trichome count on the adaxial and abaxial leaf surfaces of *T. cucumerina* for the variant B Light-Green morphotype.

al. (2009) showed that young leaves were densely covered with trichomes; however, the density of these trichomes decreased progressively with leaf maturity. In separate studies, Werker et al. (1993) and Ascensão et al. (1995) showed that trichome density was high in young leaves but decreased rapidly with leaf expansion. While some studies have shown that trichomes remain functional in mature leaves (Werker, 2000), other contrasting works have reported that at leaf maturity, the functional role of trichomes becomes less important and they therefore senesce or shrivel (Gairola et al., 2009). We note especially that the decrease in the number of conical trichomes (adaxial-to-abaxial ratio) in relation to leaf age is almost proportional (Figure 2). This observation is thought to be due to proportionate leaf expansion and growth, resulting in proportionate spacing between the conical trichomes. For all leaf ages, the number of conical trichomes (Figure 2) on the abaxial surface was significantly higher than on the adaxial surface. In contrast, the number of globular-headed trichomes (Figure 3) on the adaxial surface was significantly higher than on the abaxial surface in young leaves. For the semiold and old

leaves, there was no difference between globular-headed trichomes on the adaxial and abaxial leaf surfaces. All of the above differences could be related to genetic and physiologically mediated mechanisms operating within this plant, which need further investigation.

Trichome types and density due to salinity

This study identified 2 discernible types of trichomes in *T. cucumerina* (Figure 4), namely conical trichomes and globular-headed trichomes. We note that all of the trichomes exist as either tall or short structures (Figure 5) and that they are segmented into 3 or more distinct sections (Figure 6). The leaf petioles of the 2 variants are dominated by globular-headed trichomes (Figure 7). Earlier reports (Meyberg et al.,

Figure 4. SEM showing a mixture of conical and globular-headed trichomes on a *Trichosanthes cucumerina* leaf.
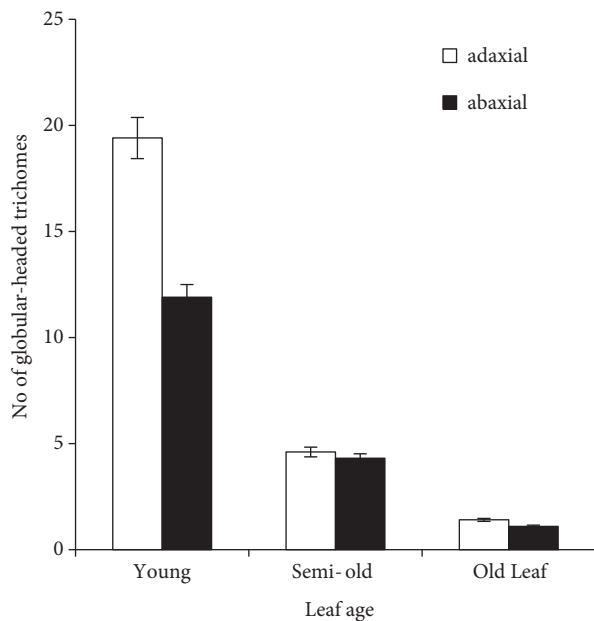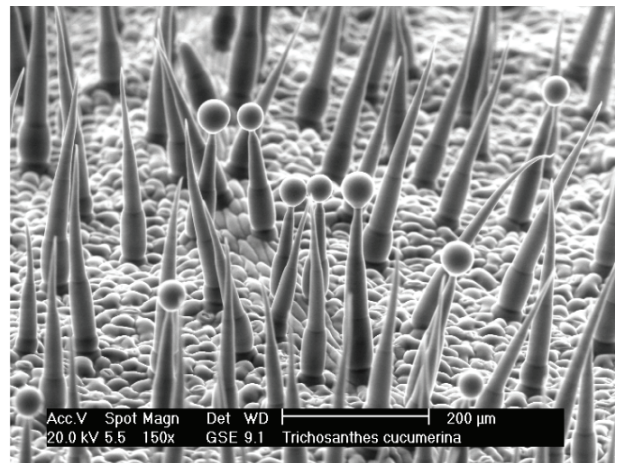

Figure 3. Relationship between leaf age and globular-headed trichome count on the adaxial and abaxial leaf surfaces of *T. cucumerina* for the variant B Light-Green morphotype.
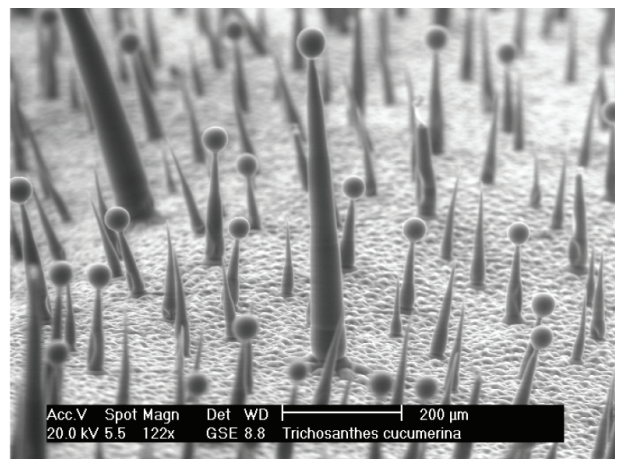

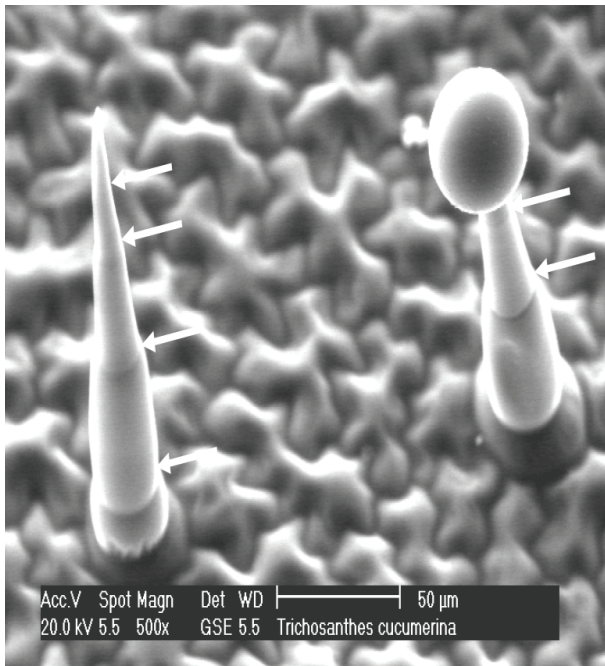Figure 5. SEM showing short and tall trichomes on a *Trichosanthes cucumerina* leaf.

Figure 6. SEM with arrows to show the segmentation of trichomes on a *Trichosanthes cucumerina* leaf.
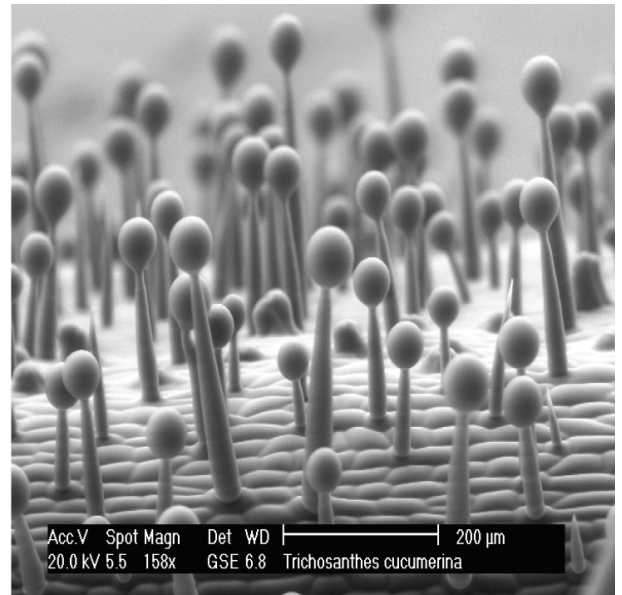

Figure 7. SEM showing the dominance of globular-headed trichomes on petioles of a *Trichosanthes cucumerina* leaf.

1991; Kolalite, 1998; Ascensão et al., 1999; Rapisarda et al., 2001; Kaya et al., 2003; Kolb & Müller, 2004; Serna & Martin, 2006; Kaya et al., 2007) showed that different types of trichomes can be produced by the same plant. Results from the present study (Table) showed that for both morphotypes, there were significantly more conical trichomes on the abaxial than the adaxial sides, irrespective of the salinity treatment. Under the control treatment, it was noted that Variant B-Light Green had a significantly higher number of conical trichomes (abaxial and adaxial) than Variant A-Green.

A major observation in this study is that while salinity did not significantly affect the conical trichomes (abaxial and adaxial) in Variant A, the same parameter was significantly reduced in Variant B due to salinity treatment. The mechanism responsible for this observation needs further investigation. However, we postulate that the trichomes that disappeared in Variant B may have played a role in the detoxification of the salts. Reporting on the effects of salinity on tobacco (*Nicotiana tabacum* L.), Choi et al. (2004) stated that when tobacco was exposed to 0.05 M NaCl, the trichomes developed irregularly.

In a previous study, Luttge (1971) reported that some salt-tolerant species secrete salts to the outer epidermal layer through specialised trichomes or salt glands, thereby providing resistance to salinity. Gonzales et al. (2008), in their study on Chilean tarweed (*Madia sativa* Molina (Asteraceae)), showed that stress induced by drought and deliberate plant damage resulted in overall increases in trichome density, and that the type of trichomes induced after stress induction varied from that of the control plants. Indeed, some reports (among others, Cano-Santana & Oyama, 1992; Sandquist & Ehleringer, 1997; Pérez-Estrada et al., 2000) indicated that water shortage is closely related to an increase in leaf pubescence in some plant species. Working with *Arabidopsis thaliana* (L.) Heynh., Nagata et al. (1999) showed that stress induced by gamma radiation resulted in a significant increase in the number of trichomes on the leaf.

Our findings in this study, especially with respect to the effects of salinity on the conical trichomes of Variant B, are in contrast to an earlier report by Pritchard and Amthor (2005), which suggested that it is unlikely that salinity has much of an effect

on trichome density or structure in field crops. Those researchers, however, stated that a number of nondomesticated halophytes possess specialised trichomes that function in salt storage and/or secretion and that the presence of these specialised trichomes enables them to successfully grow and reproduce in high-salt environments.

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