

Salinity Effects on External and Internal Morphology of Rose Geranium (*Pelargonium graveolens*L.) Leaf

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Abstract: Salinity is an environmental condition that induces stress on plants especially under high soil salts levels. It limits crop metabolic activities, hampers plant growth and synthesis of secondary metabolites. It also affects osmotic potential in the plant root zone. A complete randomized block design was used to evaluate the effect of salinity applied at 1.6, 2.4, 3.2 and 4.0 mS cm⁻¹ on external and internal morphology of rose geranium (*Pelargonium graveolens* L.) leaf and treatments were replicated three times. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to evaluate the morphology of the leaf. Salinity levels induced the development of capitate trichome. Abaxial leaf position had the highest number of trichomes than the adaxial leaf position. A strong polynomial ($r^2=0.97$) relationship was found between capitate trichome and salinity. High densities of capitate trichomes were found at a high salinity level. Although the development of asciiform trichome was induced, it was in an insignificant level, trichomes densities are therefore not affected by salinity. It was therefore concluded that rose geranium might have some degree of tolerance to salinity.

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1. Introduction

Salinity is an environmental condition that induces stress on plants especially under high soil salts levels. It limits crop metabolic activities, hampers plant growth and synthesis of secondary metabolites. It also affects the osmotic potential in the root zone of plants (Baatour et al. 2012). It induces ion toxicity due to excessive uptake of sodium ions (El-Baz et al. 2003). The storage and pathway of essential oil to the oil glands synthesis of sweet marjoram (*Origanum majorana* L.) and pennyroyal (*Menthapulegium* L.) were affected by salinity. These effects take place in the plant cell, leaf surface and the trichomes. Salts present in irrigation water originate from dissolution or weathering of the rocks and soil (lime, gypsum and other slowly dissolved soil minerals). The suitability of water for irrigation is determined not only by the total amount of salt present but also by the kind of salt (Ayers and Westcot 1994).

Rose geranium produces two types of glandular trichomes; brevicollate, asciiform trichomes and the non-glandular trichomes. Brevicollate trichomes are classified as large trichomes and asciiform trichomes are classified as small trichomes (Sedibe 2012; Motsa et al. 2006). Most of these trichomes are found on the adaxial and abaxial of a leaf surface (Sedibe 2012). Essential oil are only stored on brevicollate and asciiform trichomes (Sedibe 2012; Motsa et al. 2006). Moreover, oil yield is correlated to leaf size and to foliage yield (Sedibe 2012).

Synthesis of the essential oils occurs within the plants cell, especially the palisade cells. Plant cells contain organelles such as bladder cells which appear as huge vacuole and other cell organelles which are pushed onto the plant cell wall. Most essential oil synthesis activities occur inside the cell and stored in the vacuole (Blumwald 2000). Plants species have a specific genetic mechanism that control the trichome morphology and density (Roy et al. 1999; Payne 1978).

Environmental stress are reported to have an effect on trichomes density as was found on wormwood (*Artemisia annua* L.) and wormwood (*Artemisia campestris* L.) plants by Kjaer et al. (2012) and Ascensao and Pais (1987), respectively. This effect was never tested on rose geraniums, therefore, the objective of this study was to determine the effect of salinity applied at 1.6, 2.4, 3.2 and 4.0 mS cm⁻¹ salts levels on internal and external morphology of rose geranium leaf.

2. Material and Methods

The experimental plots were laid out in a randomized complete block design. Four levels of salinity induced by NaCl were evaluated at 1.68, 2.40, 3.20 and 4.0 mS cm⁻¹ salts levels (Table 1). Salinity was initiated gradually on a weekly sequence on the third month after transplanting at 25, 50, 75 and 100% until constant levels were met for all the levels. The pH was maintained at 5.5 for all the experimental units. The study was conducted under a

corrugated fiber glass plant house described by Sedibe and Allemann (2012).

Table 1 Macro-nutrients concentrations and micro-nutrients used in the salinity study subjected to 1.6, 2.4, 3.2 and 4.0 mS cm⁻¹ salinity level

Cations					
Salinity	Na ⁺	NH ₄ ⁺	K ⁺	Ca ⁺	Mg ²⁺
1.6	1.31	1	5.5	6.5	2.5
2.4	8.49	1	5.5	6.5	2.5
3.2	16.5	1	5.5	6.5	2.5
4.0	24.5	1	5.5	6.5	2.5
Anions					
Salinity	NO ₃ ⁻	H ₂ PO ₄ ⁻	SO ₄ ²⁻	Cl ⁻	HCO ₃ ⁻
1.6	11.04	0.1	3.8	1.31	0.4
2.4	11.04	0.1	3.8	8.49	0.4
3.2	11.04	0.1	3.8	16.5	0.4
4.0	11.04	0.1	3.8	24.5	0.4
Micro					
Salinity	Fe ²⁺	Mn ³⁺	B	Cu ²⁺	Mo ²⁺
1.6	1.12	0.54	0.03	0.02	0.05
2.4	1.12	0.54	0.03	0.02	0.05
3.2	1.12	0.54	0.03	0.02	0.05
4.0	1.12	0.54	0.03	0.02	0.05

Rooted rose geranium cuttings were transplanted during autumn and pieces of leaf samples were harvested in summer. Irrigation system was scheduled using methods of Sedibe (2012) in a custom built small scale growing units (450 x 800 x 215 mm). The irrigation systems had six dripper tubings with a flow rate of 4 L h⁻¹; these drippers were allocated to 6 potted plants.

Leaf external morphology analysis

Leaf microscopic data measurements were conducted using a scanning electron microscopy (SEM) as described by Eiasu (2009) and Motsa et al. (2006). Pieces of leaf samples (1 cm²) were collected from the leaf apex and fixed in a 3% glutardialdehyde (sodium phosphate buffer 0.1 M at pH 7.0) and post fixed for 2 hours in osmium tetroxide (1%), prior rinsing with distilled water. These samples were dehydrated once with a series of ethanol concentrations of 50, 70, 95 and 100% (twice) for 15 minutes each, followed by drying in a Tousimis critical point drier apparatus (Bio-Rad E300 Rockville). Dried samples were mounted on aluminium stabs using double-sided adhesive tape and thereafter coated with gold using a vacuum unit (BIO-RAD Microscience Division coating system United Kingdom). The critical point dryer was pressured with CO₂ liquid at 37°C to replace ethanol. These samples were examined using a Shimadzu SSX-550 (Kyoto Japan) scanning electron microscope set at the magnifications of x300 and

x400 μm. Sizes and morphology of the glandular and non-glandular trichomes were distinguished by the description characteristics described by Sedibe (2012), Motsa et al. (2006) and Payne (1978).

Leaf internal morphology analysis

A sample of 1 cm² was collected from the apex of the leaf and fixed in a 0.1 M sodium phosphate buffer solution at pH 7 containing 3% of glutardialdehyde and post fixed for 2 hours in osmium tetroxide (1%), followed by a wash with distilled water. The dehydration processes were followed with acetone in a series of 30, 50, 70 and 95% for 10-30 minutes in each stage. Acetone (100%) was conducted twice for a final dehydration process and each process lasted for 15-30 minutes. The process was followed by embedding the dehydrated samples with epoxy (100% for 8 hours at 70°C in vacuum desiccators overnight) to make thin sections for the microscopy study. Sections were cut with Leica glass knives (EM KMR3) using a Leica ultra-microtome (EM UC7 [Vienna, Austria] between 60-90 nm (1000 nanometer [nm] = 1 microtome)). Sections were stained with 6% uranyl acetate and lead citrate and rinsed with water. A Philips (FEI Netherlands) CM100 transmission electron microscopy (TEM) was used for examination of the sections (Zhang et al. 2012). The epidermal cell morphology was determined using digital images obtained from a computer mounted on the system (Eiasu 2009).

Analysis of variance was determined using the general linear model (GLM) of SAS statistical software version 9.2 (SAS 2008). Significant results were compared using Tukey's least significant difference (LSD_T), described by Steel and Tourie (1980). Statistically difference between treatment means was determined at the 5% level of significance.

3. Results and Discussion

Leaf external morphology

Three types of trichomes (asciform, brevicolate and non-glandular trichomes) were described on rose geranium leaf by Sedibe (2012) and Motsa et al. (2006). Payne (1978) described different types of trichomes which were specifically described in this study. Four types of trichomes were observed on the leaves of rose geranium i.e. asciform, brevicolate, non-glandular trichomes and the type of trichomes not commonly found on rose geranium, capitate trichome. Capitate trichomes are characterized by an elongated segment flask-shape body that consist of a round head (Figure 1a).

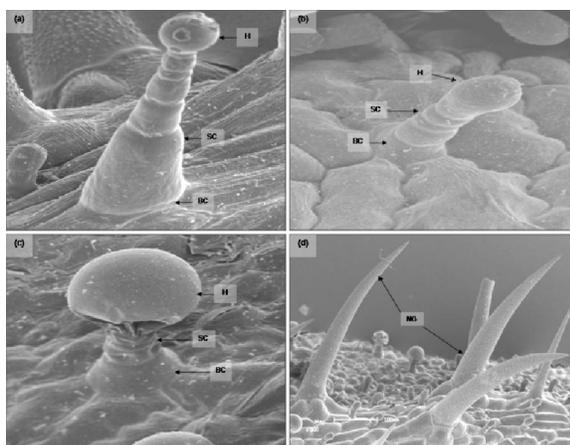


Figure 1. Different types of trichomes found on the leaf of rose geranium subjected to salinity at 1.6, 2.4, 3.2 and 4.0 mS cm^{-1} salt levels, observed under scanning electron microscope (300x and 400x magnifications) a) Capitulate trichome b) Asciform trichome c) Brevicollate trichome d) Non-glandular trichome (NG), Basal cell (BC), Unicellular stalk cell (SC), Head (H)

The densities of most trichomes were not affected by salinity. However, there was a strong polynomial ($r^2=0.97$) relationship found between capitulate trichome and salinity (Figure 2). Capitulate trichomes density were significantly increased at a high salinity level of 4.0 mS cm^{-1} , but this increase did not affect the total trichome density (data not presented) (Table 2). The development process of capitulate trichome occurs through elongation of asciform trichome (Tisser 2012). Trichome development is initiated by a hypertrophy process and an anti-clinal division of the protodermal cells followed by the development of glandular head cells. Growth on the epidermis occurs through a basal cell (Sugiyama et al. 2006; Berta et al. 1993). Although the developmental stages of this parameter was not measured in this study. The formation of asciform trichome is attributed to the effect of salinity during the development stage of this trichome. The trichome head grow round during the first stage of development, thereafter, followed by oil accumulation (Sugiyama et al. 2006). To date, no literature found has shown the effect of salinity during cell division of trichomes during developmental stages. However, increased trichomes density due to salinity was reported on pennyroyal and sweet marjoram by Baatour et al. (2012) and Karray et al. (2009), respectively.

As shown in Figure 3 the density of trichomes on the leaf surface varied according to the trichome type. Most glandular and non-glandular trichomes occurred on the abaxial leaf surface of rose geranium (Figure 3). The asciform trichome and capitulate

trichome were not significantly influenced by the leaf position. Most brevicollate and non-glandular trichomes densities were significantly increased on the abaxial leaf surface (Figure 3). Asimilar trend on density of trichomes was also reported on wormwood subjected to external stress (Kjaer et al. 2012; Ascensao and Pais 1987).

Table 2 The effect of salinity (1.6, 2.4, 3.2 and 4.0 mS cm^{-1}) on trichome density of rose geranium

Salinity	Trichome density			
	Brevicollate	Asciform	Capitate	Non-glandular
1.6	74.83 ^a	86.50 ^a	0.83 ^c	55.67 ^a
2.4	55.83 ^a	105.00 ^a	1.50 ^{bc}	51.67 ^a
3.2	60.83 ^a	92.00 ^a	4.67 ^{ab}	39.83 ^a
4.0	59.83 ^a	108.00 ^a	7.50 ^a	50.83 ^a
<i>P</i> value	<i>ns</i>	<i>ns</i>	0.01*	<i>ns</i>
<i>LSD</i> _{T(0.05)}	33.77	22.78	3.27	18.69
<i>CV</i> %	2.14	2.14	2.14	2.14

Means followed by the same letter in the same column are statistically non-significant at $P<0.05$ according to the least significant difference, ($LSD_{T(0.05)}$), $CV\%$ = coefficient of variation, *ns* = not significant at $P<0.05$, **F*-ratio probability of $P<0.01$

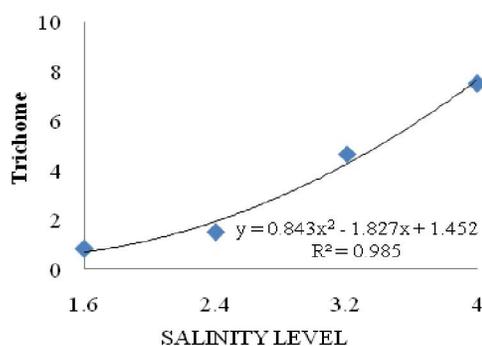


Figure 2. Polynomial relationship between capitulate trichome density and salinity levels (1.6, 2.4, 3.2 and 4.0 mS cm^{-1})

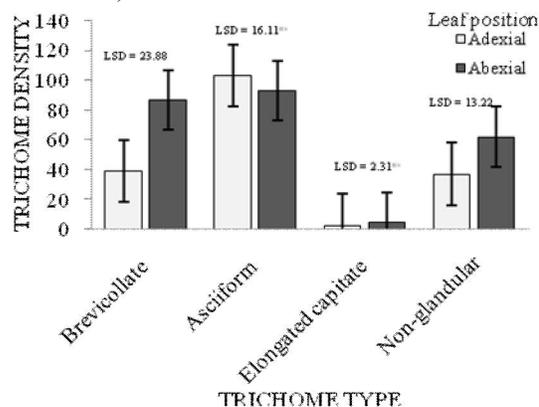


Figure 3. Trichome density measured on the adaxial and abaxial leaf surface of rose geranium subjected to salinity at 1.6, 2.4, 3.2 and 4.0 mS cm^{-1} salt levels

Leaf internal morphology

Plant leaves develop thicker upper palisade, spongy parenchyma, a thicker lower epidermis and densely spaced trichomes on plants subjected to stress (Ennajeh et al. 2010). The transmission electron micrograph in Figure 4 (a-d) shows the effect of salinity on epidermal cells morphology of rose geranium leaf. In Figure 4 (micrograph a) 1.6 mS cm^{-1} illustrated with darker coating is associated with stored essential oil (EO) on the surface of the tonoplast. Figure 4 (micrograph b), illustrates a sac associated with EO in the vacuole, which is visible where salinity was applied at 2.4 mS cm^{-1} salt level. Furthermore, on Figure 4 (micrograph b) the edge of the palisade cell, an organic cellular activity (OCA) demonstrates biosynthesis of EO on the surface of the plasma-membrane (Kjaer et al. 2012; Marty 1999). In level 3.2 and 4.0 mS cm^{-1} salt level, palisade cells shows a decay of the EO compounds, on the surface of tonoplast and this effect is attributed to increased salt levels (Figure 4 micrograph a to d) (Horie and Schroeder 2004).

Most activities are associated with biosynthesis of EO and they take place within a specialized section located in the palisade cells (PAL), cell organelles, cytosol, mitochondria (MIT), and chloroplasts (CHL) (Kjaer et al. 2012; Marty 1999). Accumulation of EO in the trichome head occur during the third stage of trichome formation, whereby, EO accumulate in the trichome head directly from the vacuole of the palisade mesophyll cell (Kjaer et al. 2012; Sugiyama et al. 2006). The accumulated EO are identified by dark patches around the tonoplast of the palisade cells (Figure 4 micrograph a-d).

Although the effect of salinity on internal morphology of trichome was not investigated in this study, only the cross section of rose geranium leaf internal morphology was observed to illustrate the accumulation of EO on the epidermal cells. The synthesis and storage of EO has been outlined, salinity has shown to have an effects on the storage of EO rose geranium leaf cells (Figure 4 micrograph a to d).

Cell vacuole regulates of cytoplasmic ions and pH within the plant cell (Marty 1999). Essential oil observed on the surface of the tonoplast where salts was applied at 1.6 and 2.4 mS cm^{-1} in Figure 4 (micrograph a and b) give explanation of vacuole functions as an anti-porter and has been reported to play a significant role in salt regulation (Xu et al. 2009). Vacuole regulates Na uptake, whereby excess Na ions are transported and stored in the vacuole (Blumwald 2000). Moreover, Figure 4 (micrograph c and d) shows the decayed EO compound as a result of increased salt levels found at 3.2 and 4.0 mS cm^{-1}

and this is associated to plant nutrient deficiency stress (El-Baz et al. 2003).

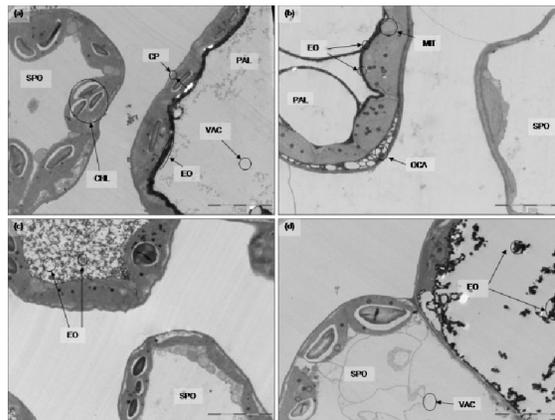


Figure 4. Internal leaf morphology of rose geranium subjected to salinity at 1.6, 2.4, 3.2 and 4.0 mS cm^{-1} salt levels observed under transmission electron microscopy (5 μm) showing SPO (Spongy mesophyll cell), PAL (palisade mesophyll cell), CHL (chloroplast with starch grains), MIT (mitochondria), VAC (vacuole), EO (essential oil), OCA (organic cellular activity), CP (cell wall and plasma membrane)

Conclusions

Salinity induced the development and density of capitate trichome. However, the density of most trichomes types were not affected, it was therefore concluded that rose geranium might have some degree of tolerance to salt stress.

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