

CHAPTER 6

RESPONSE OF ROSE-SCENTED GERANIUM LEAF PHYSIOLOGY AND MORPHOLOGY TO IRRIGATION FREQUENCY

6.1 ABSTRACT

Understanding physiomorphological responses of plants to water stress could be a base for developing suitable crop varieties and/or irrigation strategies for arid and semi-arid regions. Leaf morphological and physiological responses of rose-scented geranium (*Pelargonium capitatum* x *P. radens* cv. Rose) to irrigation frequency were investigated in a glasshouse study at the Hatfield Experimental Farm of the University of Pretoria, Pretoria, South Africa. Daily, and every second, third, fourth and fifth day irrigation were applied as treatments. One week before harvesting, leaves were sampled for electron-microscopic observations. All plants were rewatered at the same time and irrigation was withheld for the last week prior to harvesting. Progressive physiological changes were recorded on a daily basis. Upon rewatering, stomatal conductance (G_s) and transpiration rate (R_t) were significantly lower in the less often irrigated than in the more often irrigated treatments, while leaf water potential (ψ_w) and relative water content (RWC) were the same for all plants. With progress in days of the irrigation withholding period, all the parameters in the more often irrigated treatments dropped at faster rates. Hence, at the end of the stress period, G_s , R_t , ψ_w and RWC were lower in the plants from the more often irrigated than from the less often irrigated treatments. Water stress reduced leaf size, and apparently increased trichome density, while the total number of trichomes per leaf remained more or less the same, indicating that total essential oil yield is mainly affected by leaf number. Stomatal closure was the main water stress avoidant/adaptation mechanism. These results imply that imposing certain water stress levels could enhance water-saving mechanisms and improve water-use efficiency of the crop.

Keywords: Irrigation withholding; leaf water potential; relative water content; rose-scented geranium; stomatal conductance; transpiration rate; trichomes

6.2 INTRODUCTION

Water stress is the most limiting factor in agricultural productivity in arid and semi-arid regions of the world (Chartzoulakis, Patakas, Kofidis, Bosabalidis & Nastou, 2002; Shi-wei *et al.*, 2006). Crop yield losses caused by water stress are estimated to exceed the total yield loss associated with other biotic and abiotic environmental factors (Boyer, 1985). To adapt to or avoid water stress, plant species make a series of physiological, biochemical and morphological adjustments (Chartzoulakis *et al.*, 2002; Lei, Tong & Shengyan, 2006).

Some of the common responses of plants species to water-stressed conditions are increased root depth (Singh & Singh 1995; Niu, Jiag, Wan, Liu, Gao & Li, 2005), reduced cell and leaf sizes, increased cell density (Bosabalidis & Kofidis, 2002; Martínez, Silva, Ledent & Pinto, 2007), and decreases in stomatal conductance, transpiration rate (Chartzoulakis *et al.*, 2002; Heschel & Riginos, 2005) and leaf water potential (Lei *et al.*, 2006). Some plant species also adapt to water stress environments by changing the levels of certain secondary metabolites (Bosabalidis & Kofidis, 2002).

Different plant species or genotypes may use different combinations of the above-mentioned water stress adaptation mechanisms (Wright & Smith, 1983; Gutschick, 1999). Singh and Singh (1995), for instance, reported that because of differences in root water absorbing capacity from the different soil depths, plant growth and yield of pearl millet, sorghum and maize varied with soil water status. In water-stressed conditions, maize extracted more water from the top 45 cm soil depth; sorghum was best in extracting water from soil profiles between 45 and 135 cm. Pearl millet, on the other hand, showed a tendency of taking the same amount of water from all soil layers in the root zone.

Rose-scented geranium (*Pelargonium* species) is an aromatic plant cultivated for its essential oil, which is mainly extracted from leaves by steam or water plus steam distillation-techniques (Rajeswara Rao *et al.*, 1996). Several reports indicated that essential oil yield of rose-scented geranium positively correlates to herbage yield (Rajeswara Rao *et al.*, 1996; Singh, 1999; Motsa *et al.*, 2006). Reducing vegetative growth as a water-stress-avoiding mechanism could be

counterproductive, as was observed in peppermint under severe osmotic stress conditions (Charles, Joly, & Simon, 1990), unless major trade-offs of physiological and morphological change, which would increase yield and/or quality, would take place. A report by Simon *et al.* (1992), for instance, indicated that chemical composition of essential oil of sweet basil was affected by soil water levels. There is also a general understanding that water-stressed conditions favour the production of plant secondary metabolites such as essential oils (Sangwan *et al.*, 2001).

Knowledge of leaf morphological and physiological response of essential oil crops such as rose-scented geranium (commonly cultivated for their herbage extracts) to soil water levels is limited. Studying leaf physiological and morphological responses associated with water stress could be helpful to avoid soil water levels that could result in irreversible damage to the crop. Such knowledge could also be an indispensable contribution to the process of developing irrigation protocols that would increase water productivity by encouraging building up of certain productive crop physiological and morphological adaptation mechanisms to water stress (Liang *et al.*, 2002). Hence, the main objective of these experiments was to examine rose-scented geranium leaf physiological and morphological changes associated with different irrigation levels.

6.3 MATERIALS AND METHODS

The leaf physiological and morphological data were collected from plants grown in glasshouses under five irrigation frequency treatments, namely daily (T1), every second day (T2), every third day (T3), every fourth day (T4) and every fifth day (T5), followed by one week of withholding of irrigation prior to harvesting. Information on planting material, irrigation management and fertiliser application is presented in Chapter 5. On the morning of Day 0 of the one-week irrigation-withholding period all plants were rewatered and data collection started after three to four hours on the same day.

6.3.1 Data recorded

Leaf physiological data

Transpiration rate (R_t), stomatal conductance (G_s) and relative water content (RWC) were monitored on young fully opened leaves on a daily basis during the one-week irrigation-withholding periods. R_t and G_s were measured on the abaxial and adaxial sides of non-detached leaves while in their natural orientation, using an LI-1600 steady-state porometer (LI-COR, Inc. USA) and a leaf porometer (Decagon Device, Washington, USA). Leaf water potential was measured with a portable pressure chamber (Soil Moisture Equipments Corp., Santa Barbara, CA, USA). To avoid water loss, the leaves were mounted to the pressure chamber within 30 seconds after they were detached from the mother plants. The pressure readings were taken when water film/meniscus started to appear on the incised petiole surface protruding from the pressure chamber lid (Lambers *et al.*, 1998).

Relative water content (RWC) was determined gravimetrically. Ten leaf discs of 1 cm² per replication were cut (from progressively stressed plants). After fresh mass was recorded, the leaf discs were floated in distilled water for about 12 hours in the dark to achieve full turgor. Excess water on leaf surface was removed with blotting paper, and the turgid mass for each disc was recorded. For the next 72 hours, the leaf discs were oven-dried at 70°C for dry mass determination. The RWC values were calculated using Equation 6.1 (Barrs & Weatherly, 1962).

$$RWC (\%) = \frac{\text{Fresh leaf mass} - \text{Dry leaf mass}}{\text{Turgid leaf mass} - \text{Dry leaf mass}} \times 100 \quad (6.1)$$

Leaf morphological data

For the electron-microscopic leaf morphological study, fresh samples of about 1 x 1 cm² were cut with surgery blades and fixed in 3% (wt/v) aqueous solution of gluteraldehyde (in 0.05 M phosphate buffer, pH 7.0). After repeatedly being immersed in distilled water, the samples were

post-fixed in osmium tetroxide (1% wt/v) for about two hours and dehydrated in a series of ethanol concentrations [30, 50, 70, 90 and twice 100% (wt/v) for 15 min each]. The samples were then dried in a critical point drying apparatus (Bio-Rad E300, Watford, England), mounted on aluminium stabs with double-sided adhesive tape, and coated with gold under a vacuum unit (Polaron E5200C, Watford, England). The specimens were examined under a JSM-840 scanning electron microscope (JEOL, Tokyo, Japan) at different magnifications, depending on the clarity of the targeted leaf appendage/surface. Stomatal and trichome counting and other measurements were done on digital photos obtained from a computer connected to the scanning electron microscope, by opening the saved photo files in Photoshop 7 Savvy (Sybex, San Francisco, USA), and making specific two-dimensional selections in accordance with the scanning electron-microscopic scales printed on the photos.

For light microscopic observations, samples (of $1 \times 1 \text{ cm}^2$) were fixed in FAA (formalin/acetic-acid/alcohol) for 24 hours. After dehydration in series concentrations of alcohol [once in 30, 50, 70, 90 and twice in 100% (v/v) ethanol for 24 hours each], the samples were immersed in a series of xylene concentrations [once in 30, 50, 70, 90 and twice in 100% (v/v) for 24 hours each]. Following embedding in paraffin wax, the samples were sectioned to about $8 \mu\text{m}$ using a rotary microtome (Reichert-Jung-2040, Reichert-Jung, Germany). The sections were mounted on glass slides, thereafter, stained in safranin and counterstained in Fast Green. Specimens were covered with cover glass over a film of transparent glue. Pictures were taken using an Olympus digital camera (Olympus SZX7, Olympus Optical Co. Ltd, Japan) fitted on a light microscope (Olympus SZX-TR30, Olympus Optical Co. Ltd, Japan).

6.4 RESULTS AND DISCUSSION

6.4.1 Leaf physiological response to water stress

Stomatal conductance

Results obtained during the one-week irrigation-withholding period revealed that irrigation frequency induced changes in stomatal behaviour (Figure 6.1). On Day 0 of the irrigation-withholding period the plants from the less often irrigated treatments (T4 and T5) had lower stomatal conductance rates than the plants in the more often irrigation treatments (T1 and T2).

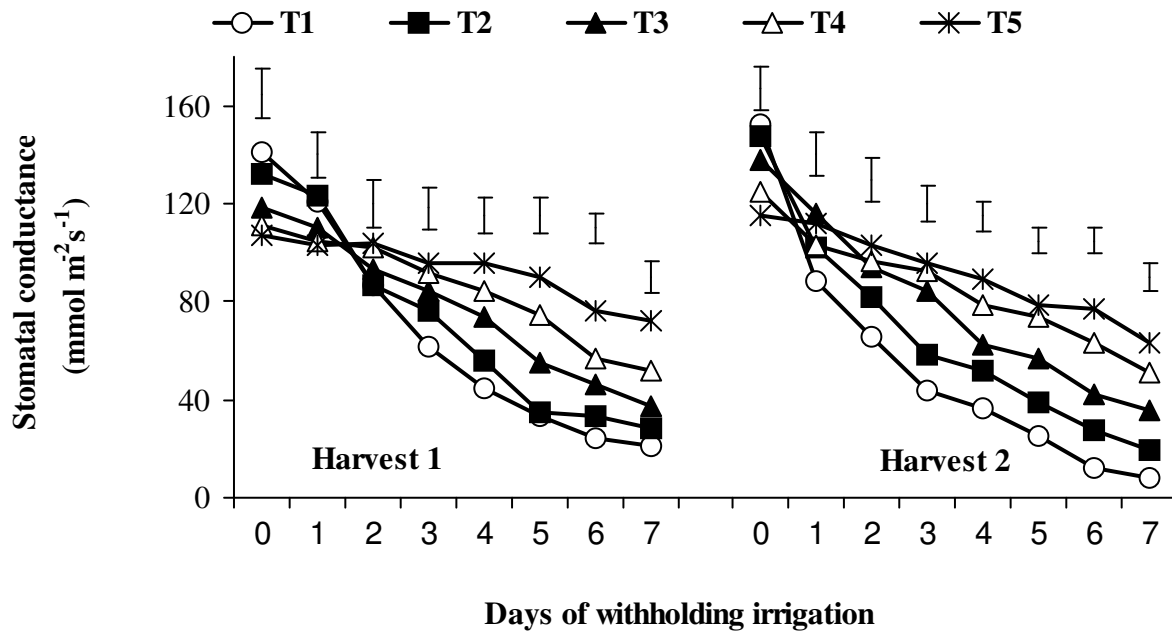


Figure 6.1: Effect of irrigation frequency on stomatal conductance of rose-scented geranium recorded during a one-week irrigation-withholding period. The vertical bars are LSD (at $\alpha = 0.05$); Harvests 1 and 2 were conducted in June and October 2006; T1, T2, T3, T4 and T5 represent daily, every second, third, fourth and fifth day irrigation, respectively

During the first and the second day of the irrigation-withholding period, the stomatal conductance of the plants from more often irrigated treatments declined at a higher rate. Hence, the stomatal conductance ranking order observed upon rewatering was reversed on Day 2 or 3 of the irrigation-withholding period. These results as a whole indicate that water stress induced long term changes in stomatal conductance as an adaptation mechanism. To a certain extent, the results agree with results reported by Liang *et al.* (2002), which showed that in wheat (*Triticum aestivum*) which was relieved from water stress, stomata reopened late. A recent report also characterises reduced stomatal conductance to be a main drought-avoidance mechanism used by *Medicago truncatula* cv. Jemalong plants (Nunes, Araújo, Silva, Fevereiro & Da Silva, 2008).

Transpiration rate

The response of transpiration rate of the plants from the different irrigation frequency treatments to water withholding was similar to that for stomatal conductance (Figure 6.2). At high soil water status (on Day 0 and Day 1 of the irrigation-withholding period), the plants from the high irrigation frequency (T1 and T2) lost water at a higher rate than those from the less frequent irrigation, while the reverse was true after Day 1 (in Harvest 1) or Day 3 (in Harvest 2).

The initial faster declining transpiration rate in T1 and T2 could be attributed to the higher soil drying rate, which resulted from initially higher stomatal conductance (Gutschick, 1999) accompanied by a large canopy size (Figure 6.3). Similarly, Xue, Zhu, Musick, Stewart and Dusek (2006) stated that higher soil water status/irrigation frequency increased evapotranspiration rate in winter wheat.

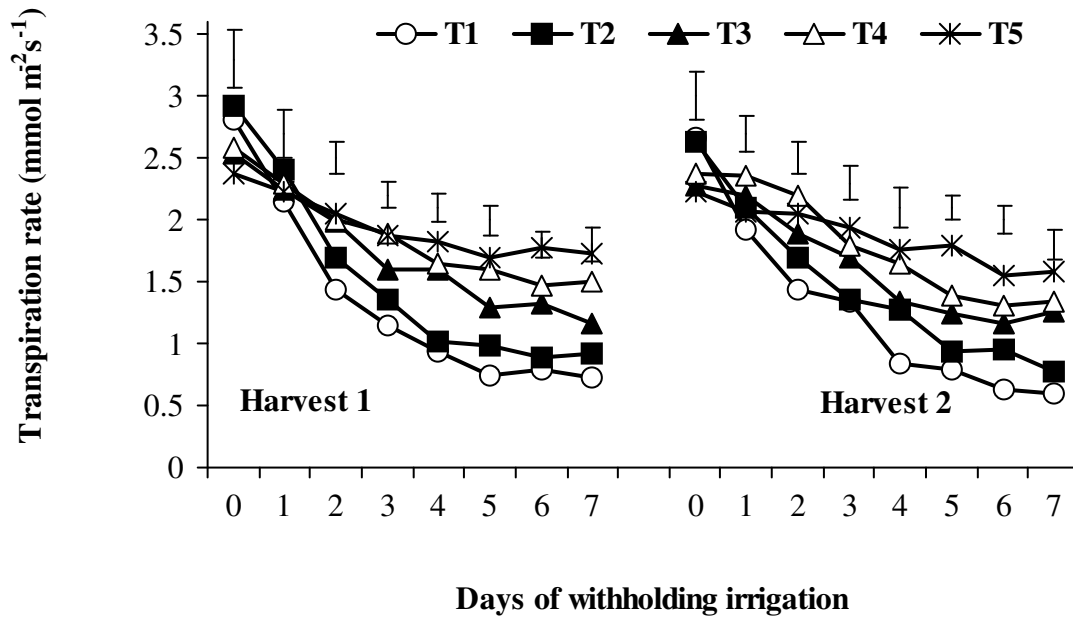


Figure 6.2: Effect of irrigation frequency on transpiration rate of rose-scented geranium leaves recorded during a one-week irrigation withholding period. The vertical bars are LSD (at $\alpha = 0.05$); Harvests 1 and 2 were conducted in June and October 2006, T1, T2, T3, T4 and T5 represent daily, and every second, third, fourth and fifth day irrigation treatments, respectively



Figure 6.3: Rose-scented geranium canopy size as affected by irrigation frequency. Plants from every fifth day (A) and daily (B) irrigation treatments, in Harvest 2 (October 2006)

Relative leaf water content

The results presented in Figure 6.4 show that irrigation frequency did not have a significant effect on leaf water status on Day 0 of the irrigation-withholding period. All plants, regardless of how frequently they were irrigated, had the same relative water content (RWC). With a progress in the days of withholding irrigation, the RWC of the plants from the frequently irrigated treatments showed a faster declining tendency compared to that of the plants from the less often irrigated treatments. The overall results support the declining tendency in RWC with progress in soil depletion levels observed in sunflower (Pankovič, Sakač, Keversan & Plesničar, 1999) and wheat (Liang *et al.*, 2002).

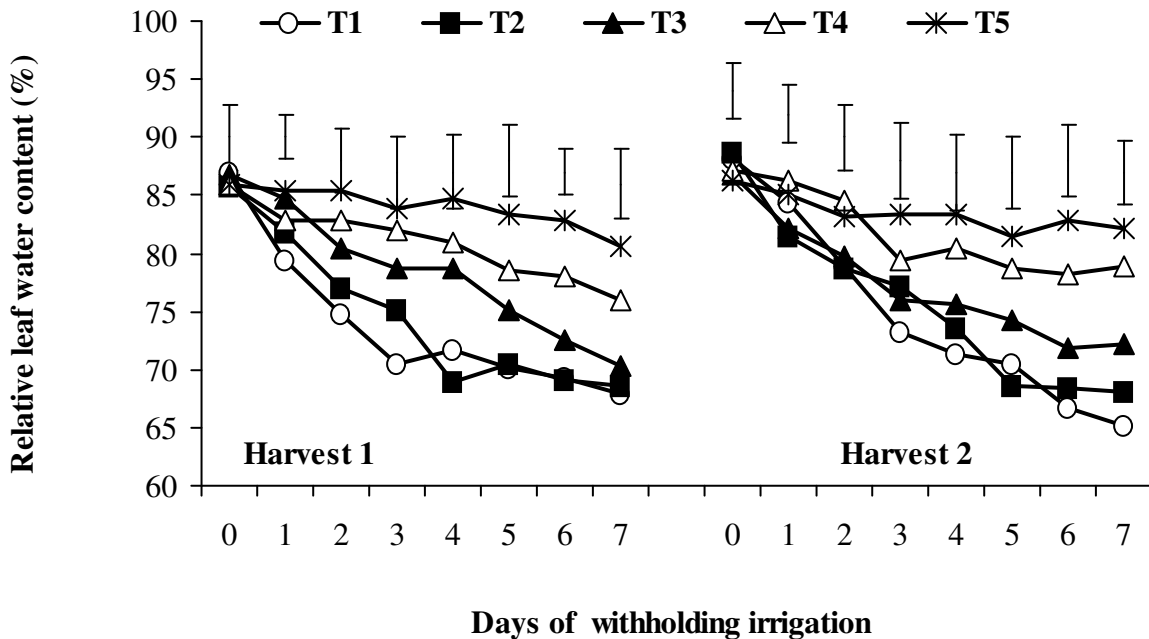


Figure 6.4: Effect of irrigation frequency on relative water content of rose-scented geranium leaves observed during a one-week irrigation-withholding period. The vertical bars are LSD (at $\alpha = 0.05$); Harvests 1 and 2 were conducted in June and October 2006; T1, T2, T3, T4 and T5 represent daily, every second, third, fourth and fifth day irrigation treatments, respectively

Leaf water potential

On Day 0 of the one-week irrigation-withholding period, the magnitudes of the leaf water potential (ψ_w) were the same for all the plants grown under the different irrigation frequencies (Figure 6.5), which could be an indication that irrigation frequency had no long-term effect on leaf water potential. These results are consistent with a previous report (Liang *et al.*, 2002), which stated that water-stress-relieved wheat plants managed to have the same ψ_w as that of control plants within a short time. Similar observations were also reported in avocado (Chartzoulakis *et al.*, 2002) and soybean (Lei *et al.*, 2006).

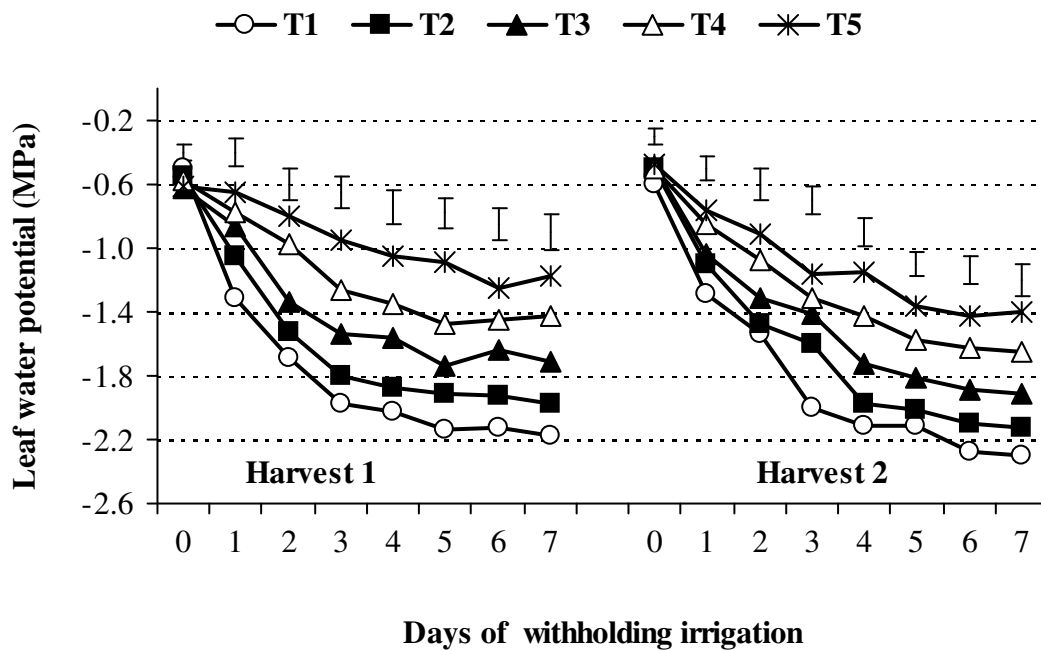


Figure 6.5: After-effect of irrigation frequency on leaf water potential of rose-scented geranium leaves recorded during a one-week irrigation-withholding period. The vertical bars are LSD (at $\alpha = 0.05$); Harvests 1 and 2 were conducted in June and October 2006; T1, T2, T3, T4 and T5 represent daily, every second, third, fourth and fifth day irrigation treatments, respectively

The data recorded on Day 1 show that the ψ_w started to decline in all treatments. The declining rate increased with increase in irrigation frequency. As a result, at the end of the water stress period (on the seventh day of withholding irrigation), the highest and lowest ψ_w were recorded for the T5 (every fifth day of irrigation) and T1 (every-day irrigation) treatments, respectively. In relation to that of the control treatment (T1), the mean improvements in ψ_w induced by the irrigation frequency in both harvests were 8.8, 19, 31, and 42% for T2, T3, T4 and T5, respectively.

The physiological data, as a whole, highlight that in water-stressed conditions the rose-scented geranium plants developed some water-saving mechanisms: water induced a long-term decline in stomatal conductance and transpiration rate, which enabled the plants to maintain higher relative water content and leaf water potential under prolonged water stress. These findings support the general understanding that certain deficit irrigation techniques could induce some physiological adjustments in plants that would contribute to boosting water productivity (Kirida, 2000).

6.4.2 Leaf morphological response to water stress

Trichome morphology

Regardless of irrigation treatments, two groups of glandular (different in shape and size) and one type of non-glandular trichomes were observed in both abaxial and adaxial surfaces of the leaves (Figure 6.6). The small glandular trichomes had nearly a columnar shape with a slightly bent terminal (apical) cell pointing towards the leaf tip.

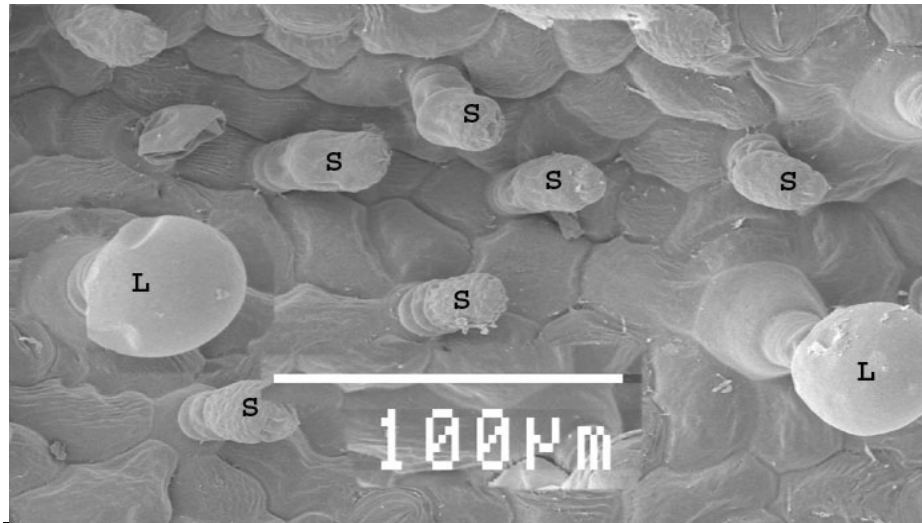


Figure 6.6: Large (L) and small (S) glandular trichomes on a leaf surface viewed under a scanning electron microscope

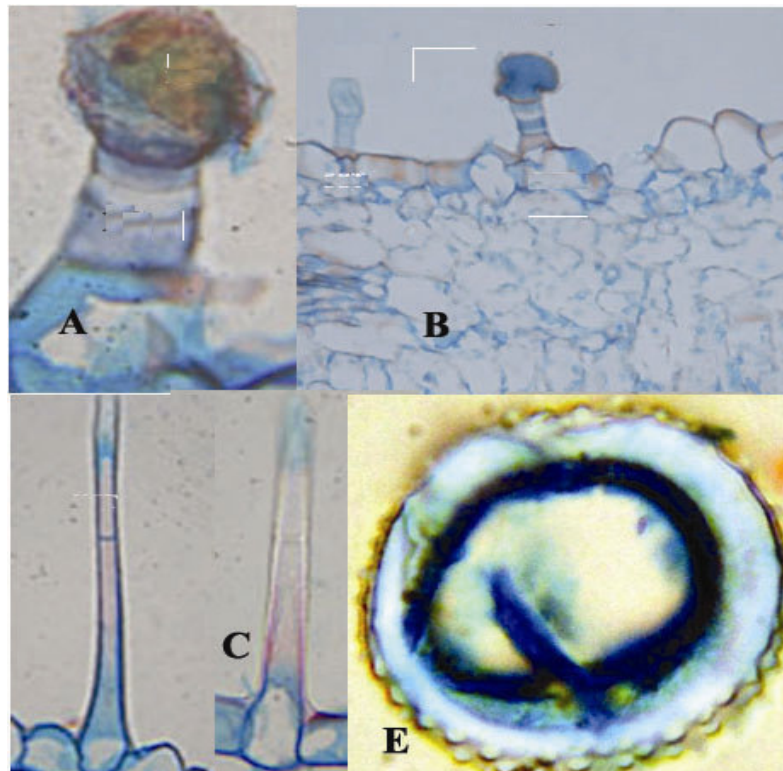


Figure 6.7: Leaf trichomes observed under a light microscope: (A) large glandular trichome, (B) large and small glandular trichomes, (C) non-glandular trichomes and (E) cross-sectional view of a non-glandular trichome

Both groups of glandular trichomes were morphologically of the peltate type, consisting of five cells, one basal, three stalk and one apical (head) cell (Figure 6.7), as previously reported for *Pelargonium scabrum* (Oosthuizen & Coetzee, 1983). It is not clear whether the two groups of glandular trichomes are different. The small trichomes could be miniature trichomes (the same as the larger glandular trichomes) but failed to attain full growth to secrete and/or store essential oils, since the glandular cells looked as if they were shrivelled or lacking stored oil in their sub-cuticular spaces.

The two groups of trichomes may also be different types of glands as was described for *P. scabrum* (Oosthuizen & Coetzee, 1983) and *P. graveolens* and *P. radens* (Van der Walt & Dermane, 1988). *P. radens* is one of the parents of the cultivar used in the present investigations. The small glandular trichomes also look like the columnar glandular trichomes observed in leaves of *Cucurbit pepo* subspecies *pepo* var. *Styrica* (Kolb & Müller, 2004). Consistent with the previous reports (Turner *et al.*, 2000; Sharma, Sangwan & Sangwan, 2003), all the trichomes observed in the current investigation arose from a single epidermal cell (Figure 6.7 B and C).

On average, the diameter of fully expanded apical cells of the large glandular trichome was about 50 μm . Ruptured glandular trichome head cells show that the sub-cuticular space, in which essential oils are stored (Turner *et al.*, 2000; Werker, 2000), is relatively small (Figure 6.8 C and D). Most of the trichomes' globular heads are occupied with solid-like material, presumably the secretory cell (Werker, 2000).

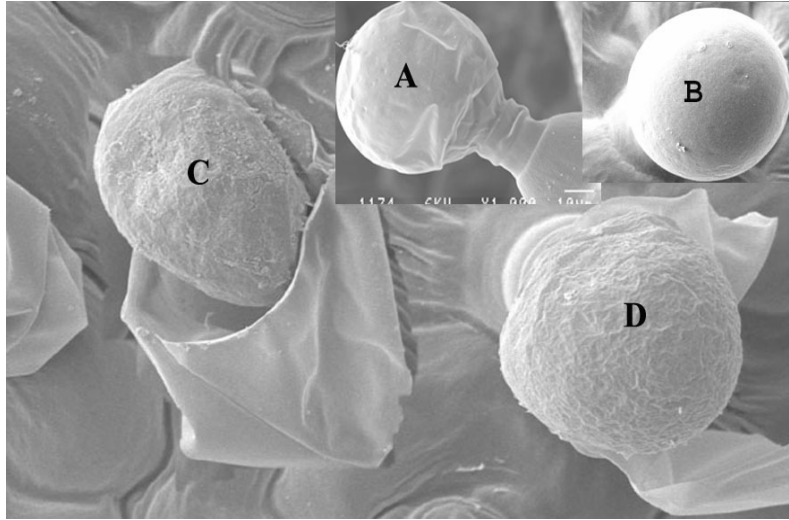


Figure 6.8: Morphology of glandular trichomes observed under a scanning electron microscope: (A) shrivelled, (B) fully expanded and (C and D) ruptured glandular trichomes

Figure 6.9 shows glandular trichomes and stomata on surfaces of an immature leaf (unopened, about seven mm in length) and a mature (open and fully expanded) leaf. Trichome growth remained uniform in size but the density was reduced in the mature leaf. This indicates that trichomes and stomata, which are in the same part of the leaf, were initiated simultaneously. The trichomes appeared fully developed and have their sub-cuticular space turgid (probably filled with oil) even before the leaf opens, whereas the stomata becomes functional later on (Figure 6.9 B and C) as mentioned by Werker (2000). The uniform size of glandular trichomes in the leaves of different age groups implies that there was no further formation of new glands in the later leaf expansion processes.

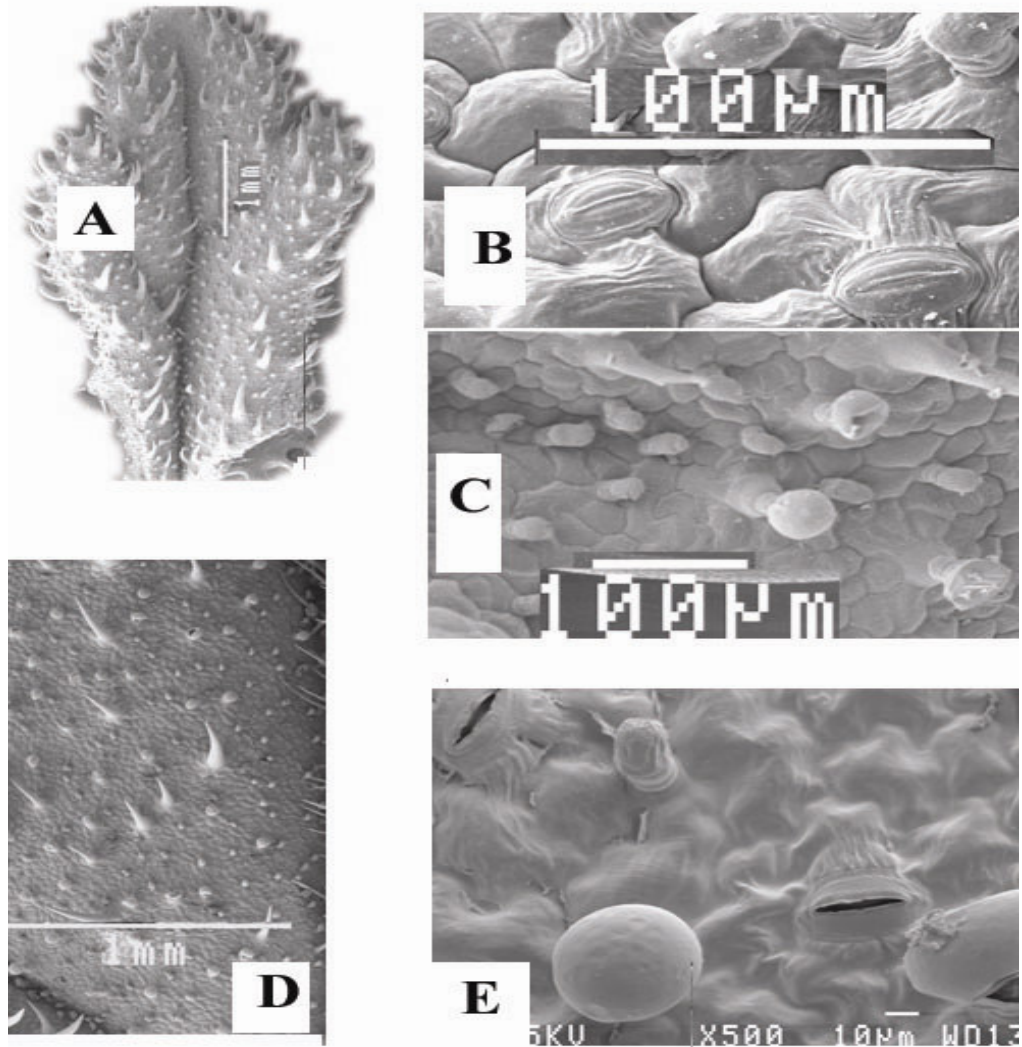


Figure 6.9: Glandular trichomes and stomata in immature leaves (A, B and C) and in mature leaves (D and E)

These observations support a previous report (Werker, Putievsky, Ravid, Dudai & Katzir, 1993), which indicated that trichomes were formed during early leaf formation in *Ocimum basilicum*. In addition, Werker (2000) suggested that formation of glandular trichomes takes place before cell multiplication ceases. Similarly, Valkama, Salminen, Koricheva and Pihlaja (2004) reported that final number of trichomes is reached some time during the early leaf developmental stage in *Betula* species. In contrasting to the above findings and views, formation of glandular trichomes in menthol mint was observed to be non-synchronous and happened throughout the leaf growth phases (Sharma *et al.*, 2003).

Stomatal and non-glandular trichome density as affected by irrigation frequency

In all leaf sections observed under the electron microscope, both leaf hair (non-glandular trichomes) and stomatal densities were higher in the abaxial than in the adaxial leaf surface (Table 6.2.). Irrigation treatments did not have a significant effect on stomatal density on the adaxial surface of the leaf.

Table 6.1: Non-glandular trichome and stomatal density from leaves of rose-scented geranium grown under different irrigation frequencies (for Harvest 2, October 2006)

Treatment	Stomatal number (per mm ²)		Leaf hair number (per mm ²)	
	Adaxial	Abaxial	Adaxial	Abaxial
T1	36.6 a [†]	101.8 b	5.3 c	28.3 b
T2	37.4 a	110.3 b	7.5 c	31.2 b
T3	43.5 a	119.3 ab	10.6 b	37.8 ab
T4	40.5 a	151.9 a	14.0 a	39.3 a
T5	42.0 a	149.6 a	15.3 a	38.8 a
Grand mean	40.0	126.4	10.6	35.1
CV (%)	20.0	22.2	21.0	17.4
LSD ($\alpha = 0.05$)	NS	33.9	2.7	7.4

[†]Values followed by the same letters in a column are not significantly different at 5% level of probability; T1, T2, T3, T4 and T5 represent daily, and every second, third, fourth and fifth day irrigation treatments

On the abaxial leaf surface, significant increases in stomatal and non-glandular trichome densities were observed in the less often irrigated treatments (T4 and T5). The increase in non-glandular trichome density seems to be consistent with the general understanding that in water-stressed conditions, leaf hair density increases to minimise transpiration rate (Lambers *et al.*, 1998) and/or to reflect solar radiation, particularly the ultraviolet wavebands (Holmes & Keiller, 2002). The apparent increase in leaf hair and stomatal densities could be associated with a decrease in epidermal cell size, which could have led to an increase in cell density (Bosabalidis & Kofidis, 2002; Martínez *et al.*, 2007).

The increased stomatal density of rose-scented geranium in less often irrigated treatments is contrary to the behaviour normally observed in succulent plants when adapting to dry environments (Sayed, 1998). The present results indicate that in *Pelargonium* species, the most remarkable water stress adaptation mechanism was partial stomatal closure (Figure 6.10). A study by Bañon, Fernandez, Franco, Torrecillas, Alarcón and Sánchez-Blanco (2004) also revealed that water stress increases stomatal density in *Lotus creticus*.

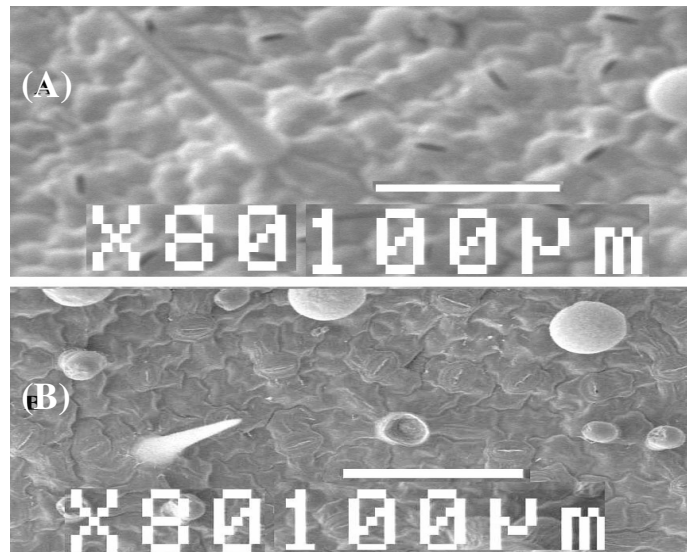


Figure 6.10: Effect of irrigation frequency on stomatal opening observed under a scanning electron microscope: (A) Leaves from the daily irrigated treatment (T1) open on Day 0 and (B) closed stomata on Day 7 of the one-week irrigation withholding period of Harvest 2 (October 2006)

These results also agree with research findings, which indicated that in water-stressed conditions, stomatal density increased but their apertures were reduced in olive cultivars (Bosabalidis & Kofidis, 2002). Niu *et al.* (2005) also described stomatal closure as the major drought-tolerance mechanism used by plant species in semi-arid sandland.

Glandular trichome density

Irrespective of the irrigation treatments, on both leaf surfaces the number of small glandular trichomes was higher than that of the large trichomes (Table 6.2). The abaxial leaf surface was the major site for glandular trichomes. The density of small glandular trichomes was negatively affected by irrigation frequency in both leaf surfaces. On the abaxial surface of the leaves, the density of the large glandular trichomes was significantly higher on the less frequently irrigated plants.

Table 6.2: Glandular trichome density on the abaxial and adaxial surfaces of rose-scented geranium leaves (for Harvest 2, October 2006)

Treatment	Adaxial (per mm ²)		Abaxial (per mm ²)	
	Large	Small	Large	Small
T1	6.5 a [†]	10.4 b	18.2 b	31.9 b
T2	7.8 a	13.7 b	20.0 b	36.5 ab
T3	9.3 a	16.2 ab	25.2 ab	43.0 a
T4	9.0 a	18.0 a	31.7 a	46.7 a
T5	9.7 a	19.3 a	32.4 a	46.3 a
Grand mean	8.0	15.5	25.5	40.9
CV (%)	27.0	18.3	24.0	21.1
LSD ($\alpha = 0.05$)	NS	3.4	7.4	10.3

[†]Values followed by the same letters in a column are not significantly different at 5% level of probability; T1, T2, T3, T4 and T5 represent daily, and every second, third, fourth and fifth day irrigation treatments

The increase in glandular trichome density in the stressed treatments could have resulted from a decrease in epidermal cell size (Bosabalidis & Kofidis, 2002). After exploring the effects of environmental factors on leaf hair density, Roy, Stanton and Eppley (1999) suggested that unlike leaf area, trichome number per leaf is less sensitive to environmental stresses, implying that the apparent increase in trichome density observed in water-stressed conditions mainly arose from a reduction in leaf size. In agreement with these reports, in the current experiments

the size of individual leaves of rose-scented geranium appears reduced with a reduction in irrigation frequency (Table 6.3 & Figure 6.11). These findings highlight that leaf number is the major contributor to total essential yield per plant (hectare).

Table 6.3: Response of petiole and leaf length to irrigation frequency

Treatment	Harvest 1		Harvest 2	
	Petiole length (mm)	Leaf length (mm)	Petiole length (mm)	Leaf length (mm)
T1	137.3 a [†]	89.4 a	110.5 a	68.8 a
T2	127.9 b	91.7 a	100.8 b	64.3 ab
T3	104.2 c	78.7 b	92.0 bc	59.5 bc
T4	86.2 d	70.9 bc	84.0 cd	57.0 c
T5	83.8 d	68.1 c	82.0 d	54.5 c
Grand mean	107.9	79.8	93.9	60.8
CV (%)	5.7	8.9	8.6	6.9
LSD ($\alpha = 0.05$)	7.3	8.6	9.7	5.1

[†]Values followed by the same letters in a column are not significantly different at 5% level of probability; T1, T2, T3, T4 and T5 represent daily, and every second, third, fourth and fifth day irrigation treatments; Harvests 1 and 2 were conducted in June and October 2006, respectively



Figure 6.11: Leaf size of rose-scented geranium as affected by irrigation frequency: Mature leaves sampled from daily (T1), and every second (T2), third (T3) fourth (T4) and fifth (T5) day irrigation treatments in Harvest 2 (October 2006)

6.5 CONCLUSIONS

The current study provides evidence that rose-scented geranium makes physiological and morphological modifications to avoid severe damage from water stress. Less often irrigation induced a long-term decline in stomatal conductance and transpiration rate, which enabled the plants to maintain higher relative water content and leaf water potential under prolonged water stress. Stomatal density apparently increased with a decrease in irrigation frequency implying that stomatal closure is a major water-loss-controlling mechanism in rose-scented geranium. Irrespective of the irrigation frequency, two types of glandular (small and large) and one non-glandular trichome groups were observed in both the adaxial and abaxial surfaces of the leaves. Both glandular trichome sizes showed a synchronised development, indicating that trichomes, at least in the same part of a leaf, are initiated or formed at the same time. The number of small glandular trichomes was higher than that of the large ones in both leaf sides. Trichome density apparently increased with a decrease in irrigation frequency, the opposite was true for leaf size. The trade-offs between leaf size and glandular trichome indicates that leaf number contributes more than leaf size to total essential oil yield.

CHAPTER 7

GENERAL DISCUSSION

Rose-scented geranium (*Pelargonium* species) is an aromatic plant cultivated for its essential oil, which is commonly used in the perfumery, aromatherapy and cosmetic industries. The demand for essential oils is on the increase with population growth and widening of the uses and preferences for the essential oil components. Recent studies indicate that rose-scented geranium oil could contribute to the food-processing (Lis-Balchin *et al.*, 1998; Lis-Balchin & Roth, 2000) and pharmaceutical industries (Dorman & Deans, 2000). The crop is commonly produced under rainfed agriculture and oil yield level per annum is low, 5 to 20 kg/ha (Weiss, 1997). Despite being the centre of origin and diversity for the *Pelargonium* species, the South African geranium oil industry's contribution to international markets is still low (Weiss, 1997), about 20 tons per year (R.A. Learmonth, personal communication).

The available information on the relationship between soil water level and production of secondary metabolites such as essential oils in plants appears contradictory. There is a general understanding that water-stressed conditions increase secondary metabolite production in plants (Yaniv & Palevitch, 1982; Sangwan *et al.*, 2001). Similarly, Weiss (1997) reported that a dry season resulted in a mild increase in rose-scented geranium essential oil yield. Other studies on rose-scented geranium (Rajeswara Rao *et al.*, 1996; Singh, 1999), on the other hand, indicated that irrigation/high soil water improved essential oil yield. Hence, it was hypothesised that rose-scented geranium essential oil yield could be improved though introducing innovative irrigation practices in arid and semi-arid regions such as found in South Africa. The general approach of the study was to grow the crop under different irrigation managements, which would bring certain physiological changes in favour of essential oil yield and/or quality, at the same time increasing productivity of the scarce freshwater resources.

Rose-scented geranium was grown under different maximum allowable depletion (MAD) levels of plant available soil water (Chapter 3). Relatively little water depletion from the root zone between 0.4 and 0.8 m depth indicated that water uptake by the rose-scented geranium root

system was almost limited to the top 0.4 m soil layer. Increasing the soil water depletion level to 60% of the plant available soil water (ASW) and higher resulted in a significant reduction in herbage mass and essential oil yield. An increase in MAD level apparently increased the essential oil concentration (percentage oil on fresh herbage mass basis), but its contribution to total essential oil yield (kg/ha oil) was limited. Up to 28% of irrigation water could be saved by increasing maximum allowable depletion level of ASW from 20 to 40%, without a significant reduction in essential oil yield.

The MAD treatments did not bring significant variations in essential oil composition. Remarkable differences in oil composition among harvests seem to be related to plant age (starting from transplanting date) or the season in which regrowth cycles took place, since the seasonal temperatures were in the order of Harvest 1 < Harvest 2 < Harvest 3. It could be realised that combined geraniol and geranyl format contents were negatively correlated to the combined citronellol and citronellyl formate contents ($R^2 = 0.75$) in the essential oil extracted by the steam-distillation method.

A one-month irrigation-withholding period at different shoot ages (Chapter 4) demonstrated that a significant decline in herbage yield occurs when a water stress period was imposed during the third or fourth month of the regrowth cycles. Essential oil yield was, on the other hand, reduced remarkably only when water stress was imposed during the fourth month of regrowth. The results imply that at certain water stress levels, there could be a trade-off between vegetative growth and essential oil yield (Weiss, 1997), or else essential oil yield has a higher tolerance to water stress. The tendency of essential oil yield to reduce with a decrease in herbage mass confirms that primary and secondary metabolites are positively related (Letchamo *et al.*, 1995; Rajeswara Rao, 2002). With a marginal oil yield loss, it was possible to save about 330 to 460 m³/ha of irrigation water by withholding irrigation during the third month of each regrowth cycle. The overall results highlight that in water-scarce regions, withholding irrigation either during the second or the third month of regrowth in rose-scented geranium could improve water productivity.

Responses of herbage and essential oil yields, and essential oil composition to irrigation frequency and a one-week irrigation-withholding period were investigated in semi-controlled greenhouses (Chapter 5). Essential oil content (percentage oil on fresh herbage mass basis) apparently increased with a decrease in irrigation frequency. Total essential oil yield/ha, however, increased with an increase in herbage yield and irrigation frequency, as was observed in the MAD level of ASW and the one-month irrigation-withholding trials.

Unlike the results recorded for the long-term water stress treatments (irrigation frequency, MAD level and a one-month irrigation withholding period), an increase in essential oil content induced by the one-week irrigation withholding period was high enough to improve essential oil yield per plant. The overall results show that the combination of a high irrigation frequency and a terminal one-week irrigation-withholding period could improve essential oil yield.

The one-week water-withholding period did not affect essential oil composition. Irrigation frequency, on the other hand, affected citronellol, citronellyl formate, and geraniol and geranyl formate levels. The levels of citronellol and its ester (citronellyl formate) consistently increased with a decrease in irrigation frequency, while the opposite was true for the levels of geraniol and its ester (geranyl formate). These results are consistent with the results obtained from the MAD trial in the different harvests. Such a relationship was also true for the one-month irrigation-withholding trial, particularly in Harvest 2 (Figure 4.7B), although in opposite directions, e.g. in the irrigation frequency trials high soil water favoured geraniol and geranyl format levels, while the reverse was true for the one-month irrigation-withholding trials.

The physiological and morphological studies (Chapter 6) revealed that rose-scented geranium adapts to or avoids water stress by making certain changes in leaf physiology and morphology. In these studies, stomatal conductance (G_s), transpiration rate (R_t), leaf water potential (ψ_w) and relative water content (RWC) of plants grown under different irrigation frequencies were investigated during a one-week irrigation-withholding period prior to harvesting. Data recorded on Day 0 (upon rewatering) show that irrigation frequency had an after-effect on G_s and R_t because the magnitudes of these parameters were significantly lower in the plants that were less irrigated compared to the more frequently irrigated plants. Leaf water potential and RWC were

the same for all plants regardless of the irrigation level. With a progress in days of the irrigation-withholding period, all the parameters in the more frequently irrigated treatments declined at higher rates.

The lower G_s , R_{t_s} , ψ_w and RWC in the plants from the more frequently irrigated than the less frequently irrigated treatments at the end of the stress period, highlight that by reducing stomatal conductance, the plants from less irrigated plants managed to minimise transpirational water loss. Thus, they maintained their ψ_w and RWC at higher levels for a longer period in the course of the irrigation-withholding period. From such observation, it could be concluded that stomatal conductance is the main physiological mechanism used by rose-scented geranium to adapt to or avoid water stress conditions. Similar results were also reported for *Triticum aestivum* (Liang *et al.*, 2002) and *Medicago truncatula* (Nunes *et al.*, 2008).

Microscopic observations reveal that irrespective of the irrigation frequency, one type of non-glandular and two types (slightly different in shape and remarkably different in size) of glandular trichomes were observed. Uniform growth and a declining tendency in the density of the glandular trichomes with an advance in leaf expansion imply that new trichome formation stopped with epidermal cell specialisation/division at an early leaf developmental stage. Such observations are consistent with those reported for *Ocimum basilicum* (Werker *et al.*, 1993) and *Brich* species (Valkama, Salminen, Koricheva & Pihlaja, 2003). These findings infer that the total number of glandular trichomes per plant is determined by the total number of leaves, and not by leaf size.

The impacts of the different irrigation treatments considered in the current trials (discussed in Chapters 3, 4, and 5) on water-use efficiency (WUE) were not consistent, and probably affected by seasonal differences (Saeed & El-Nadi, 1997). In the glasshouse trials (Chapter 5), for instance, the highest WUE was recorded for the T2 and T3 treatments in Harvest 1, a growth cycle during the cool season. This result supports the results that indicate that WUE was improved by certain soil water stress conditions in potatoes (Onder, Caliskan, Onder & Caliskan, 2005; Zhang *et al.*, 2006) and in sesame (Uçan, Killi, Gençoğlan & Merdun, 2007). In warm to hot seasons (Harvest 2), on the other hand, WUE increased with an increase in soil

water level, proving the suggestions made by Bessembinder *et al.* (2005) that WUE increases with an increase in soil water level, provided that other environmental factors are in the required range for optimum plant growth.

The results of the water management trials, as a whole, indicate that irrigation increases essential oil yield by boosting vegetative growth. For water saving without a significant reduction in essential oil yield, 40% depletion of the available soil water could be allowed. Withholding irrigation for one month between the 30th and the 90th day of regrowth cycles could also have similar water-saving and possibly energy and labour cost minimising advantages. It is, however, advisable to avoid severe water stress in the fourth month of a regrowth cycle.

At field level, soil water had no remarkable impact on essential oil composition. Despite the fluctuations observed, the citronellol to geraniol ratio (C:G ratio) were relatively high (ranged between 1.9 and 16). Similarly, in data recorded for the same cultivar grown under different nitrogen levels, the lowest and highest C:G ratios were 2.4 and 6.8, respectively (Araya *et al.*, 2006). This slightly contradicts the general understanding that South African geraniol oil is comparable to the geranium oil produced on Réunion Island, namely the Bourbon-type oil (SANDA, 2006), with a C:G ratio of one or close to one (Gupta *et al.*, 2001b; Gauvin *et al.*, 2004). According to Rodolfo, Koroch, Simon, Hitimana, Daka, Ranarivelo and Langenhoven, (2006), South African geranium oil shares similar characteristics with Chinese geranium oil, particularly for the high citronellol and cironellyl formate contents.

From a perfumery point of view, essential oil of this cultivar will not be among the most preferred quality, because of the high C:G ratio. However, C:G ratio may not affect market value for geranium oil in the future because promising results have been discovered in the field of pharmacology (Dorman & Deans, 2000; Deans, 2002) and food-processing industries (Lis-Balchin *et al.*, 1998; Lis-Balchin & Roth, 2000).

The herbage and essential oil yields obtained from the rain shelter were consistently higher than those from the open field of regrowths that experienced similar climatic conditions [e.g.

Harvest 4 from the open field versus Harvest 2 from the rain shelter (Chapter 3) and Harvest 2 from the open field versus Harvest 4 from the rain shelter (Chapter 4)]. These differences, at least partly, resulted from the difference in plant density (16 000 and 30 000 plants/ha in the open field and rain shelter, respectively). These results are consistent with results reported by Rajeswara Rao (2002), which indicated that herbage and essential oil yield consistently increased with increase plant density. The current results together with findings in literature indicate that optimizing the agronomic practices such as plant density, nutrient supply (Araya *et al.*, 2006) and cultivar selection (Gupta *et al.*, 2001b) could enhance oil yield and/or quality, thereby improve water productivity.

This investigation should be followed up by a study that could explain the controversial results obtained from the one-month (Chapter 4) and one-week (Chapter 5) irrigation-withholding trials. Such investigation could help to develop certain irrigation practices by which the high yield at pot level could be achieved in the field.

In addition, results obtained from the trials in the field and in the greenhouse show that responses of geranium oil composition to irrigation level were affected by other factors, most probably by temperature. Irrigation had a clear impact on the greenhouses where temperature was partly regulated, while in the open field (with high diurnal temperature fluctuation), essential oil composition did not respond to soil water levels. Hence, studying the combined effect of temperature and soil water level under controlled conditions could be helpful for further geranium oil quality-improving efforts.