Physiomorphological response of rose-scented geranium (*Pelargonium* spp.) to irrigation frequency

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Abstract

The effect of irrigation frequency on leaf physiomorphological processes of rose-scented geranium (*Pelargonium capitatum x P. radens* cv. Rose*)* was investigated in a glasshouse study at the Hatfield Experimental Farm of the University of Pretoria, Pretoria, South Africa, from November 2005 to October 2006. Daily, and every 2nd, 3rd, 4th, and 5th day irrigation were applied as treatments. Leaf samples for electron-microscopic observations were taken one week prior to harvesting, whereafter all plants were re-watered. For each of the irrigation frequency treatments, 50% of the plants were then exposed to a one-week irrigation withholding period (brief stress treatment) prior to harvesting. During this period, physiological properties were recorded on a daily basis to identify or monitor change. Higher irrigation frequency and a brief water stress period increased essential oil yield. Lower irrigation frequency tended to increase the citronellol to geraniol (C:G) ratio to unacceptably high levels (C:G > 3). Upon re-watering, stomatal conductance (G_s) and transpiration rate (R_t) were significantly lower for the lower irrigation frequency treatments, compared to the higher irrigation frequency treatments, while no noticeable differences were observed in water potential (ψ_w) and relative water content (RWC). At the end of the one-week stress period, G_s, R_t, ψ_w and RWC were lower for the plants that were more frequently irrigated compared to the less frequently irrigated treatments. Water stress reduced leaf size, and apparently increased trichome density, whereas 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 (and not by leaf size or trichome number). Stomatal closure was the main water stress avoiding/adaptation mechanism. These results demonstrate that rose-scented geranium plants can make physiomorphological adaptations to save water. However, such a water saving strategy was counter-productive, since it resulted in lower essential oil yield and lower water-use efficiency.

Keywords: Irrigation withholding period; leaf water potential; relative water content; stomatal conductance; transpiration rate; trichomes

1. Introduction

Water stress is the most limiting factor in agricultural productivity in arid and semi-arid regions of the world (Shi-wei et al., 2006). Crop yield losses caused by water stress exceed the total yield loss associated with other biotic and abiotic environmental factors (Boyer, 1985). To adapt to or avoid water stress, plants make a series of physiomorphological and biochemical adjustments (Lei et al., 2006). Some of the common plant responses to water-stressed conditions are increased root depth (Niu et al., 2005), decreases in cell and leaf sizes (Martínez et al., 2007), and lower stomatal conductance, transpiration rate and leaf water potential (Heschel and Riginos, 2005; Lei et al., 2006).

Rose-scented geranium (*Pelargonium* species) is an aromatic plant cultivated for its essential oil, which is mainly extracted from leaves by steam and/or water distillation techniques (Rajeswara Rao et al., 1996). Reports indicated that essential oil yield of rose-scented geranium positively correlates to herbage yield (Eiasu et al., 2009; Motsa et al., 2006; Rajeswara Rao et al., 1996; Singh, 1999). Any decline in vegetative growth as a water-stress-avoiding mechanism could be counterproductive to oil production, as in the case of peppermint under severe osmotic stress conditions (Charles et al., 1990), unless major trade-offs in physiological and morphological adjustments could take place. Simon et al. (1992) 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 on leaf morphological and physiological response to water supply could make an indispensable contribution to the process of developing irrigation protocols. Encouraging certain crop physiological and morphological adaptation mechanisms to water stress would increase water productivity (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.

2. Materials and methods

2.1 Growing system

Pot trials were conducted in a glasshouse at the Hatfield Experimental Farm of the University of Pretoria, Pretoria, South Africa (Latitude 25° 45'S and Longitude 28° 16'E, and altitude of 1372 m), from November 2005 to October 2006. Shading effects due to the roof and sidewall glass panels ranged between 30 and 35%. A computerised cooling system was set to regulate temperature when it rose higher than 18°C. The highest maximum temperature recorded during the experimental period was about 33°C.

2.2 Plant culture

Rose-scented geranium (*Pelargonium capitatum x P. radens*) stem cuttings were taken from healthy growing plants (in a tunnel) and raised in seedling trays filled with peat in a mist bed for 40 days. The plantlets were transplanted into 10-L plastic pots, filled with a sandy clay soil (52:10:38 coarse sand, silt and clay, respectively) on 29 October 2005. Water holding capacity of the growing medium was about 29 and 17% (v/v) at field capacity and permanent wilting point, respectively. Before applying treatments, the plants were allowed to grow for six months until uniform growth was attained, during which the plants were cut back twice. Irrigation frequency treatments were imposed for two months, starting one month after the last cut back of plant shoots.

2.3 Treatments and experimental design

Five irrigation frequency treatments (T1 to T5) were imposed, consisting of irrigating daily or every second, third, fourth or fifth day. The irrigation frequency treatments were arranged in a complete randomised block design and replicated six times. There were six rows of 40 pots each, representing the six blocks or replications. Each of the five irrigation treatments was randomly assigned to a group of eight pots in each row. The space between adjacent rows was 1 m, and the plants within a row were 0.30 m apart.

The plants appeared to be sensitive to water stress during the first month after cutting. Hence, in the first month of regrowth, no water stress was applied. Cultivation practices such as fertilizer application and pest control measures (e.g. for white fly and red spider mite) were also done within that period. Irrigation treatments were then applied during the subsequent two months (Months 2 and 3) of the regrowth period.

For each of the irrigation frequency treatments, 50% of the plants were exposed to a one-week irrigation withholding period (brief stress treatment) prior to harvesting (only on cloudless periods). On day 0 of the one-week irrigation withholding period, all pots were irrigated and physiological data collection started after two hours.

2.4 Cultivation practices

During each regrowth period, each plant received 3 g nitrogen (N), 4.5 g phosphorus (P), and 3 g potassium (K) [in the form of 2:3:2 (22) NPK fertiliser granules] as a split in Week 1 and Week 7 of each regrowth cycle. In addition, 1 g N (as ammonium nitrate) and 1 g K (as potassium chloride) were applied to each pot in Week 9 of each regrowth cycle. To avoid salt accumulation, plants were over-irrigated on the first and second day of each regrowth cycle to induce leaching.

2.5 Irrigation management

The pots were placed on top of parallel metallic/wooden bars, which were supported by bricks to allow sufficient space for water-collection cans. At each irrigation event, a measured volume of water was applied. The volume of water that was required to refill the pots to pot capacity for each irrigation event (water volume depleted through evapotranspiration) was determined by supplying an excess volume and subtracting the volume of drained water from the applied volume. To minimise nutrient losses, the drainage water was recycled on the next irrigation event.

2.6 Data recorded

Data for two regrowth cycles, from 26 March to 29 June 2006 (Harvest 1) and from 30 June to 10 October 2006 (Harvest 2) were recorded. Transpiration rate (R_t), stomatal conductance (G_s) and leaf relative water content (RWC) were monitored on young fully-expanded leaves on a daily basis during the one-week irrigation-withholding periods (before harvesting). R_t and G_s were measured on the abaxial and adaxial sides of intact leaves in their natural orientation, using an LI-1600 steady-state porometer (LI-COR, Inc. USA) and a leaf porometer (Decagon Devices, Pullman, USA), respectively. Leaf water potential (ψ_w) was measured with a portable pressure chamber (Soil Moisture Equipments Corp., Santa Barbara, USA). To avoid water loss, the leaves were mounted in the pressure chamber within 30 seconds after they were detached from the mother plants. The pressure readings were taken when a water film meniscus started to appear on the incised petiole surface protruding from the pressure chamber lid (Lambers et al., 1998).

Relative water content of leaves was determined gravimetrically. For RWC determination, ten leaf discs of 1 cm^2 per each replication were cut (from progressively stressed plants). After fresh mass was recorded, the leaf discs were floated on distilled water for about 12 hours in the dark to achieve full turgor. Excess water on the leaf surfaces was removed with tissue paper, and the turgid mass for each disc was recorded. For the next 72 hours, the leaf discs were oven-dried at 70°C to determine their dry mass. The RWC values were calculated using Equation 1 (Barrs and Weatherly, 1962).

$$RWC\ (\%) = \frac{Fresh\ leaf\ mass - Dry\ leaf\ mass}{Turgid\ leaf\ mass - Dry\ leaf\ mass} \times 100 \qquad \text{(Equation 1)}$$

For electron-microscopic leaf morphological studies, leaves were sampled one week prior to harvesting. Fresh samples of about 1 cm^2 each were cut with surgical blades and fixed in 3% (mass/volume) 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 tetraoxide (1% mass/volume) for about two hours and dehydrated in a series of ethanol concentrations [30, 50, 70, 90 and 100% (twice) (mass/volume) for 15 min each]. The samples were then dried in a critical point drying apparatus (Bio-Rad E300, Watford, England), mounted on aluminium stubs with double-sided adhesive tape, and coated with gold under a vacuum unit (Polaron E5200C, Watford, England). The specimens were then examined under a JSM-840 scanning electron microscope (JEOL, Tokyo, Japan) at different magnifications. Stomatal and trichome counting, and other measurements were done on digital photos obtained from the scanning electron microscope (using Photoshop 7 Savvy, Sybex, San Francisco, USA) by making specific two-dimensional selections in accordance with the scanning electron microscopic scales printed on the photos.

For light microscopic observations, samples of 1 cm^2 each were fixed in FAA (formalin acetic-acid alcohol for 24 hours), dehydrated in series concentrations of alcohol [once in 30, 50, 70, 90 and twice in 100% (v/v) ethanol for 24 hours each], and 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 μm using a rotary microtone (Reichert-Jung-2040, Reichert-Jung, Germany), mounted on glass slides, stained with safranin and counterstained with Fast Green. The specimens were then covered with a glass cover over a film of transparent glue. Images were obtained 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).

During harvesting, plant shoots were cut to a height of about 15 to 20 cm above the surface of the growing medium. Fresh herbage mass was measured immediately after cutting, and samples (2.5 - 5 kg each) were sent for oil content determination (by steam distillation) and oil composition analysis. From the oil content and fresh herbage mass, the oil yield per treatment was calculated. Oil samples were pooled per treatment (the same amount of oil taken from each replication of the same treatment was mixed together) and analysed by gas chromatography (GC), as described by Eiasu et al. (2008).

Water use efficiency (WUE) was determined using the relationship between oil yield (mg/plant/regrowth) and total water volume used (litre/plant/regrowth) (Equation 2).

$$WUE\ (mg/litre) = \frac{Oil\ yield}{Total\ water\ used} \qquad \text{(Equation 2)}$$

The recorded data were subjected to analysis of variance (ANOVA) using Mstatc (MSTAT-C, 1991). Where applicable, treatment means were separated by a LSD (least significant difference) test at $\alpha = 0.05$ level.

3. Results and discussion

3.1 Essential oil yield and composition

Essential oil yield

The combination of high irrigation frequency (daily and/or every second day irrigation) and a brief water stress period (one-week irrigation withholding before harvest) gave the highest essential oil yield (Fig. 1). In general, the irrigation frequency and brief water stress effects were more prominent in Harvest 2 (warm season regrowth cycle) than in Harvest 1 (cool season regrowth cycle). These results are consistent with the findings of a previous study with similar irrigation treatments (Eiasu et al., 2008).

Essential oil composition

Results of essential oil composition in response to irrigation frequency are presented in Fig 2. Since oil composition results were not replicated (pooled samples per treatment), statistical analysis could not be performed on the data. However, there was a tendency that less frequent irrigation (T3, T4 and T5) favoured the production of citronellol and citronellyl formate. An increase in the levels of these compounds in the oil was associated with a decrease in geraniol and geranyl formate levels. These results are in agreement with the findings of Eiasu et al. (2008). Citronellol to geraniol (C:G) ratio ranged between 2.4 in higher irrigation frequency treatments (T1 and T2) and 4.8 in lower irrigation frequency treatments (T3, T4 and T5). The C:G ratio for the T1 and T2 treatments was consistently within the acceptable range (C:G ratio < 3) (Motsa et al., 2006). The high C:G ratio in the lower irrigation frequency treatments (T3, T4 and T5) will probably make the oil unsuitable for the perfume industry (Motsa et al., 2006). There was no clear indication that the one-week irrigation-withholding period affected oil composition.

3.2 Leaf physiological responses to water stress

Stomatal conductance

Results obtained during the one-week irrigation-withholding period revealed that irrigation frequency influenced stomatal conductance behaviour differently (Fig. 3). On Day 0 of the irrigation-withholding period, plants from the less often irrigated treatments (T4 and T5) had lower stomatal conductance than plants from the more often irrigation treatments (T1 and T2).

During the first and the second day of the irrigation-withholding period, stomatal conductance of the plants from the more often irrigated treatments declined at a faster rate. Hence, the stomatal conductance ranking order observed upon rewatering (Day 0) was reversed on Day 2 and 3 of the irrigation-withholding period. These results indicated that long term water stress induced changes in stomatal conductance as an adaptation mechanism. Values of Gs recorded on Day 0 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 compared to well-watered plants. Nunes et al. (2008) also demonstrated stomatal conductance to be the main drought-avoidance mechanism used by *Medicago truncatula* cv. Jemalong plants.

Transpiration rate

At high soil water status (on Day 0 and Day 1 of the irrigation-withholding period), transpiration rate was significantly higher for the plants from the high irrigation frequency treatments (T1 and T2), compared to those from the less frequently irrigated treatments (T4 and T5). The reverse was true after Day 1 (in Harvest 1) or Day 3 (in Harvest 2) (Fig. 4). The initial fast decline in transpiration rate for T1 and T2 could be attributed to the higher soil drying rate, which resulted from initially higher stomatal conductance (Gutschick, 1999). As a result, transpiration rate dropped to about 30% of its initial value for T1, compared to ± 80% for T5.

Relative water content

On Day 0 of the irrigation-withholding period, irrigation frequency treatments did not affect leaf water status or relative water content (RWC) (Fig. 5). With a progress in the days of withholding irrigation, the RWC of the plants from the frequently irrigated treatments tended to decline at a faster rate compared to that of the plants from the less often irrigated treatments. The overall results support the tendency of declining RWC with progression in soil depletion level observed in sunflower (Panković et al., 1999) and wheat (Liang et al., 2002).

Leaf water potential

Immediately after irrigation (Day 0), there were no differences in water potential (ψ_W) among the plants grown under the different irrigation frequency treatments (Fig. 6). These results are consistent with a previous report (Liang et al., 2002), which stated that water-stress-relieved wheat plants managed to attain 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).

On Day 1 ψ_W started to decline in all treatments. The declining rate was highest in the plants of the highest irrigation frequency (T1). As a result, on the seventh day of withholding irrigation, the highest and lowest ψ_W were recorded for the T5 and T1 treatments, respectively. The respective mean ψ_W for T2, T3, T4 and T5 were 8.8, 19, 31, and 42% higher than that of T1 (the most frequently irrigated treatment).

The physiological data, as a whole, highlight that in water-stressed conditions, the plants developed some water-saving mechanisms, i.e. water stress 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 could contribute to improved water productivity (Kirda, 2000).

3.3 Leaf morphological response to water stress

Shape and type of trichomes

Regardless of irrigation treatments, two types of glandular (different in shape and size) and one type of non-glandular trichome were observed on both adaxial and abaxial surfaces of the leaves (Fig. 7a-c). The small glandular trichomes had nearly a columnar shape with a slightly bent terminal (apical) cell pointing towards the leaf tip. Both types of glandular trichomes were morphologically of the peltate type, consisting of five cells, i.e. one basal, three stalk and one apical (head) cells (Fig. 7a), as previously reported for *Pelargonium scabrum* (Oosthuizen and Coetzee, 1983). It is not clear whether the two groups of glandular trichomes are different in morphology. 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 lacked 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 and Coetzee, 1983) and *P. graveolens* and *P. radens* (Van der Walt and 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 and Müller, 2004). Consistent with previous reports (Turner et al., 2000; Sharma et al., 2003), each trichome in this investigation arose from a single epidermal cell (Fig. 7b).

On average, the diameter of fully expanded apical cells of the large glandular trichomes was about 50 µm. Ruptured glandular trichome head cells showed that the sub-cuticular space, in which essential oils are stored (Turner et al., 2000; Werker, 2000), is relatively small (Fig. 7d), i.e. most of the trichomes' globular heads are occupied with solid-like material, presumably the secretory cell (Werker, 2000).

Trichome density

Electron-microscopic observations showed that leaf hair (non-glandular trichomes) and stomatal densities were higher in the lower (abaxial) than on the upper (adaxial) leaf surface (Table 1). Irrigation treatments did not have a significant effect on stomatal density of the adaxial surface of the leaf. On the abaxial leaf surface, however, stomatal and non-glandular trichome densities were higher for 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 and Keiller, 2002).

The apparent increase in leaf hair and stomatal densities could be associated with a decrease in epidermal cell size (total number of stomata and leaf hair per leaf probably remained the same), which could have led to an increase in hair density (Bosabalidis and 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. A study by Bañon et al. (2004) also revealed that water stress increased stomatal density in *Lotus creticus*. Similarly, Bosabalidis and Kofidis (2002) reported that stomatal density increased but their apertures were reduced in olive cultivars exposed to water-stressed conditions. Niu et al. (2005) also described stomatal closure as the major drought-tolerance mechanism used by plant species in semi-arid sandlands.

Irrespective of the irrigation treatments, the number of small glandular trichomes was higher than that of the large trichomes on both leaf surfaces (Table 2). The abaxial leaf surface was the major site for glandular trichomes. On both surfaces, density of small glandular trichomes decreased with an increase in irrigation frequency.

The increase in glandular trichome density in the stressed treatments could have resulted from a decrease in epidermal cell size (Bosabalidis and Kofidis, 2002). Roy et al. (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, the size of individual rose-scented geranium leaves was found to be affected by irrigation frequency (Table 3). These findings suggest that leaf number is a major contributor to total essential yield per plant (and hectare).

3.3 Water use and water-use efficiency (WUE)

Water usage decreased with a decrease in irrigation frequency (Table 4). The greater water use for the more often irrigated treatments could be attributed to higher evapotranspiration rate associated with a larger canopy and increased water availability. In agreement with this observation, Şimşek et al. (2005) reported that crop evapotranspiration rate of cucumber (*Cucumbis sativus*) decreased with a decrease in irrigation level. Wallace (2000) also indicated that more frequent irrigation encourages water loss/use.

In general, WUE tended to increase with an increase in irrigation frequency, which was more pronounced during the warm season regrowth cycle (Harvest 2). In contrast, Kirda (2000) and Liang et al. (2002) reported that mild water stress improved WUE. However, our results agree with Bessembinder et al. (2005), who reported that WUE increases with increasing soil water levels, provided that other factors such as the essential nutrients are not limiting.

4. Conclusions

A combination of high irrigation frequency and a brief water stress period one week before harvest enhanced essential oil yield. High irrigation frequency resulted in a favourable citronellol to geraniol ratio (C:G < 3). The current study also provides evidence that rose-scented geranium makes physiological and morphological adaptations to avoid severe damage from water stress. Low irrigation frequency 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 type of non-glandular trichomes were observed on 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. Trichome density apparently increased with a decrease in irrigation frequency, and the opposite was true for leaf size. The trade-offs between leaf size and glandular trichome density implies that leaf number contributes more than leaf size to total essential oil yield. A longer irrigation interval consistently reduced water usage. These results demonstrate that rose-scented geranium plants can employ physiomorphological adaptation mechanisms to save water. However, such water saving strategy is counter-productive, since it will result in lower essential oil yield and water-use efficiency as a result of lower herbage yield.

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References

Bañon, S., Fernandez, J.A, Franco, J.A., Torrecillas, A., Alarcón, J.J., Sánchez-Blanco, M.J., 2004. Effects of water stress and night temperature preconditioning on water relations and morphological and anatomical changes of *Lotus creticus* plants. Scientia Horticulturae 101, 333-342.

Barrs, H.D., Weatherly, P.E., 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. Australian Journal of Biological Science 15, 413-428.

Bessembinder, J.J. E., Leffelaar, P.A., Dhindwal, A.S., Ponsioen, T.C., 2005. Which crop and which drop, and the scope for improvement of water productivity. Agricultural Water Management 73, 113-130.

Bosabalidis, A.M., Kofidis, G., 2002. Comparative effects of drought stress on leaf anatomy of two olive cultivars. Plant Science 163, 375-379.

Boyer, J.S. 1985. Water transport. Annual Review of Plant Physiology 36, 473-516.

Charles, D.J., Joly, R.J., Simon, J.E., 1990. Effects of osmotic stress on the essential oil content and composition of peppermint. Phytochemistry 29, 2837-2840.

Chartzoulakis, K., Patakas, A., Kofidis, G., Bosabalidis, A., Nastou, A., 2002. Water stress affects leaf anatomy, gas exchange, water relations and growth of two avocado cultivars. Scientia Horticulturae 95, 39-50.

Eiasu, B.K., Soundy, P., Steyn, J. M., 2008. High irrigation frequency and brief water stress prior to harvest enhances essential oil yield of rose-scented geranium (*Pelargonium capitatum x P. radens*). Hort Science 43, 500-504.

Eiasu, B.K., Steyn, J. M., Soundy, P., 2009. Rose-scented geranium (*Pelargonium capitatum* x *P. radens*) growth and essential oil yield response to different soil water depletion regimes. Agricultural Water Management 96, 991-1000.

Gutschick, V.P., 1999. Research reviews of biotic and abiotic consequences of differences in leaf structure. New Phytology 143, 3-18.

Heschel, M.S., Riginos, C., 2005. Mechanisms of selection for drought stress tolerance and avoidance in *Impatiens capensis* (Balsaminaceae). American Journal of Botany 92, 37-44.

Holmes, M.G. , Keiller, D.R., 2002. Effects of pubescence and waxes on the reflectance of leaves in the ultraviolet and photosynthetic wavebands: a comparison of a range of species. Plant, Cell and Environment 25, 85-93.

Kirda, C., 2000. Deficit irrigation scheduling based on plant growth stages showing water stress tolerance. FAO Water Report No. 22, pp. 3-10.

Kolb, D., Müller, M., 2004. Light, conventional and environmental scanning electron microscopy of *Cucurbit pepo* subsp. *pepo* var. S*tyriaca* and histochemistry of glandular secretory products. Annals of Botany 94, 513-526.

Lambers, H., Chapin III, F.S., Pons, T.L., 1998. Plant physiological ecology. Springer, New York, USA, p. 343.

Liang, Z., Zhang, F., Shao, M., Zhang, J., 2002. The relationship of stomatal conductance, water consumption, growth rate to leaf water potential during soil drying and rewatering cycle of wheat (*Triticum aestivum*). Botanical Bulletin Academia Sinica 43, 187-192.

Lei, W., Tong, Z., Shengyan, D., 2006. Effect of drought and rewatering on photosynthetic physioecological characteristics of soybean. Acta Ecologica Sinica 26, 2073-2078.

Martínez, J.P., Silva, H., Ledent, J.F., Pinto, M., 2007. Effect of drought stress on the osmotic adjustment, cell wall elasticity and cell volume of six cultivars of common bean (*Phaseolus vulgaris* L.). European Journal of Agronomy 26, 30-38.

Motsa, N.M., Soundy, P., Steyn, J.M., Learmonth, R.A., Mojela, N., Teubes, C., 2006. Plant shoot age and temperature effects on essential oil yield and oil composition of rose-scented geranium (*Pelargonium* sp.) grown in South Africa. Journal of Essential Oil Research 18, 106-110.

MSTAT-C, 1991. A microcomputer program for design management and analysis of agronomic research experiments. Michigan State University, East Lansing, MI, USA.

Niu, S.L., Jiang, G.M, Wan, S.Q., Liu, M.Z., Gao, L.M., Li, Y.G., 2005. Ecophysiological acclimation to different soil moisture in plants from a semi-arid sandland. Journal of Arid Environment 63, 353-365.

Nunes, C., Araújo, S.S., Silva, J.M., Fevereiro, M.P.S., da Silva, A.B., 2008. Physiological responses of the legume model *Medicago truncatula* cv. Jemalong to water deficit. Environmental and Experimental Botany 63, 289-296.

Oosthuizen, L., Coetzee, J., 1983. Morphogenesis of trichomes of *Pelargonium scabrum*. South African Journal of Botany. 2, 305-310.

Panković, D., Sakač, Z., Kevrešan, S., Plesničar, M., 1999. Acclimation to long-term water deficit in the leaves of two sunflower hybrids: photosynthesis, electron transport and carbon metabolism. Journal of Experimental Botany 50, 127-138.

Rajeswara Rao, B.R., Kaul, P.N., Mallavarapu, G.R., Ramesh, S., 1996. Effect of seasonal changes on biomass yield and terpenoid composition of rose-scented geranium (*Pelargonium* species). Biochemica Systematics Ecology 24, 627-635.

Roy, B.A., Stanton, M.L., Eppley, S.M., 1999. Effects of environmental stress on leaf hair density and consequences for selection. Journal of Evolutionary Biology 12, 1089-1103.

Sangwan, N.S., Farooqi, A.H.A., Shabih, F., Sangwan, R.S., 2001. Regulation of essential oil production in plants. Plant Growth Regulation 34, 3-21.

Sayed, O.H., 1998. Phenomorphology and ecophysiology of desert succulents in eastern Arabia. Journal of Arid Environment 40, 177-189.

Sharma, S., Sangwan, N.S., Sangwan, R.S., 2003. Developmental process of essential oil glandular trichome collapsing in menthol mint. Current Science 84, 544-550.

Shi-wei, G., Yi, Z., Na, S., Qi-rong, S., 2006. Some physiological processes related to water use efficiency of higher plants. Agricultural Science in China 5, 403-411.

Şimşek, M, Tonkaz, T, Kaçira, M, Çömlekçioğlu, N., Doğan, Z., 2005.The effect of different irrigation regimes on cucumber (*Cucumbis sativus* L.) yield and yield characteristics under open field conditions. Agricultural Water Management 73, 173-191.

Simon, J.E., Riess-Bubenheim, D., Joly, R.J., Charles, D.J., 1992. Water stress induced alterations in essential oil contents and composition of sweet basil. Journal of Essential Oil Research 4, 71-75.

Singh, M., 1999. Effect of soil moisture regime, nitrogen and modified urea materials on yield and quality of geranium (*Pelargonium graveolens*) grown on alfisols. Journal of Agricultural Science 133, 203-207.

Turner, G.W., Gershenzon, J., Croteau, R.B., 2000. Development of peltate glandular trichomes of peppermint. Plant Physiology 124, 665-679.

Van der Walt, J.J, Dermane, F., 1988. *Pelargonium graveolens* and *P. radens*: a comparison of their morphology and essential oils. South African Journal of Botany 54, 617-622.

Wallace, J.S., 2000. Increasing agricultural water use efficiency to meet future production. Agriculture, Ecosystems and Environment 82, 105-119.

Werker, E., 2000. Trichome diversity and development. Advances in Botanical Research 31, 1-35.

Table 1

Non-glandular trichome and stomatal density on the adaxial and abaxial leaf surfaces 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 in a column followed by the same letter do not differ significantly at $P < 0.05$; T1, T2, T3, T4 and T5 represent daily, and every second, third, fourth and fifth day irrigation treatments; CV = coefficient of variation; LSD = least significant difference at $\alpha = 0.05$

Table 2

Glandular trichome density on the abaxial and adaxial leaf surfaces of rose-scented geranium grown under different irrigation frequencies (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 in a column followed by the same letter do not differ significantly at $P < 0.05$; T1, T2, T3, T4 and T5 represent daily, and every second, third, fourth and fifth day irrigation treatments; CV = coefficient of variation; LSD = least significant difference at $\alpha = 0.05$

Table 3

Response of rose-scented geranium petiole and leaf length to irrigation frequency treatments.

Treatment	Harvest 1		Harvest 2	
	Petiole length (mm)	Leaf length (mm)	Petiole length (mm)	Leaf length (mm) (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 in a column followed by the same letter do not differ significantly at $P < 0.05$; 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; CV = coefficient of variation; LSD = least significant difference at $\alpha = 0.05$

Table 4

Water use and water-use efficiency (on essential oil yield basis; mg of oil/litre of water used) of rose-scented geranium grown under different irrigation frequencies and a one-week irrigation-withholding period.

Treatments	**Harvest 1**			**Harvest 2**		
	Oil yield (mg/plant)	Water usage (litre/plant)	WUE (mg/litre)	Oil yield (mg/plant	Water usage (litre/plant)	WUE (mg/litre)
T1	534.9 a[†]	42.70 a	12.76 ab	739.1 a	48.15 a	15.68 a
T2	551.6 a	39.07 b	14.30 a	558.6 a	41.43 b	13.76 b
T3	466.2 b	34.26 c	13.82 a	411.8 b	36.18 c	11.51 c
T4	348.6 c	28.08 d	12.59	335.3c	30.97 d	10.91cd
T5	331.6 c	27.65 d	12.03 b	261.9c	28.73 d	9.29 d
Grand mean	446.6	34.37	13.10	461.3	37.09	12.10
CV (%)	7.2	11.95	15.78	5.9	10.68	13.30
LSD ($\alpha = 0.05$)	35.6	3.50	1.76	39.0	3.48	1.47

[†] Values followed by the same letters within a column are not significantly different at $P < 0.05$; Harvests 1 and 2 were conducted in June and October 2006 in the glasshouse; T1, T2, T3, T4 and T5 represent daily, every second, third, fourth and fifth irrigation treatments; CV = coefficient of variation; LSD = least significant difference at $\alpha = 0.05$

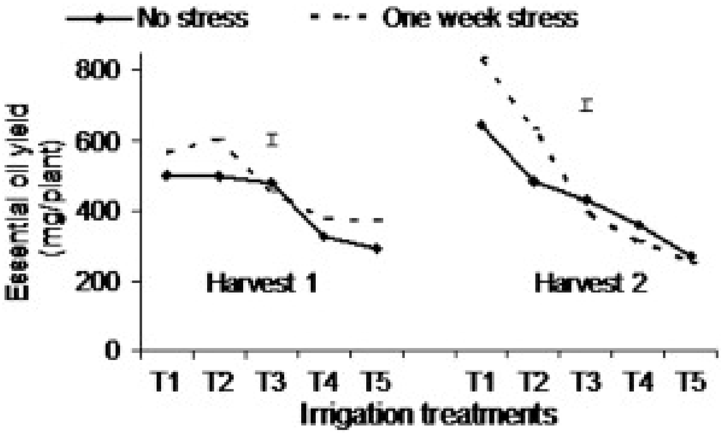

Fig. 1. Essential oil yield of rose-scented geranium grown at different irrigation frequencies and with (stress) or without (no stress) a one-week water withholding period prior to harvesting. The vertical bars are LSD (at $\alpha = 0.05$); Harvests 1 and 2 were conducted in June and October 2006, respectively; T1, T2, T3, T4 and T5 represent daily, and every second, third, fourth and fifth day irrigation treatments, respectively

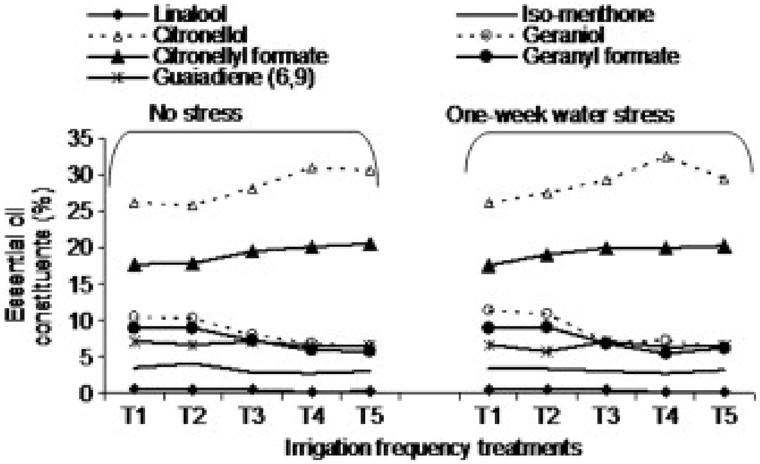

Fig. 2. Essential oil composition of rose-scented geranium as affected by irrigation frequency and one week irrigation withholding period prior to harvesting. T1, T2, T3, T4 and T5 represent daily and every second, third, fourth and fifth day irrigation, respectively

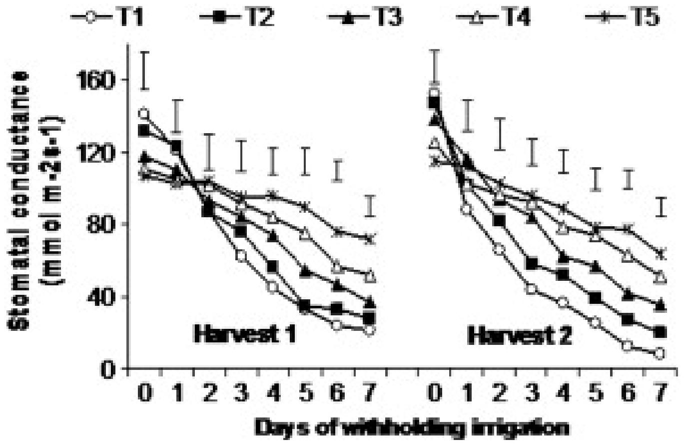

Fig. 3. Effect of irrigation frequency on stomatal conductance of rose-scented geranium recorded during a one-week irrigation-withholding period. The vertical bars are LSD (at ∝ = 0.05); Harvests 1 and 2 were conducted in June and October 2006; T1, T2, T3, T4 and T5 represent daily, every second, third, fourth and fifth day irrigation, respectively

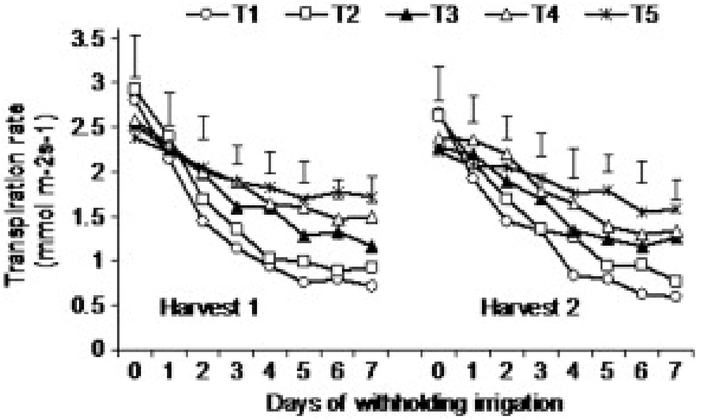

Fig. 4. Effect of irrigation frequency on transpiration rate of rose-scented geranium leaves recorded during a one-week irrigation-withholding period. The vertical bars are LSD (at ∝ = 0.05); Harvests 1 and 2 were conducted in June and October 2006; T1, T2, T3, T4 and T5 represent daily, and every second, third, fourth and fifth day irrigation treatments, respectively

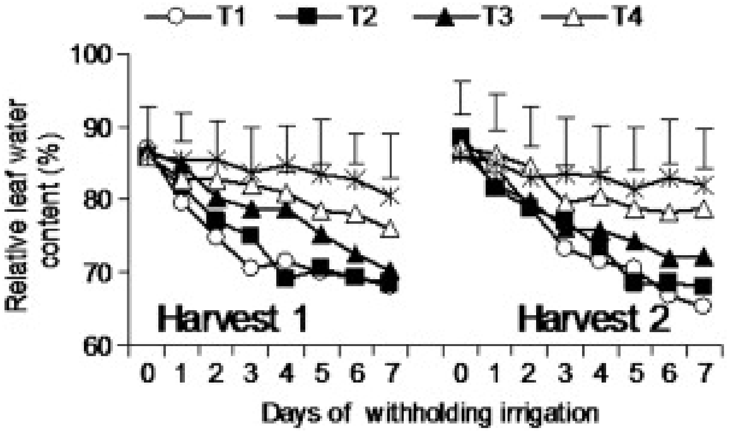

Fig. 5. Effect of irrigation frequency on relative water content of rose-scented geranium leaves observed during a one-week irrigation-withholding period; The vertical bars are LSD (at ∝ = 0.05); Harvests 1 and 2 were conducted in June and October 2006; T1, T2, T3, T4 and T5 represent daily, every second, third, fourth and fifth day irrigation treatments, respectively

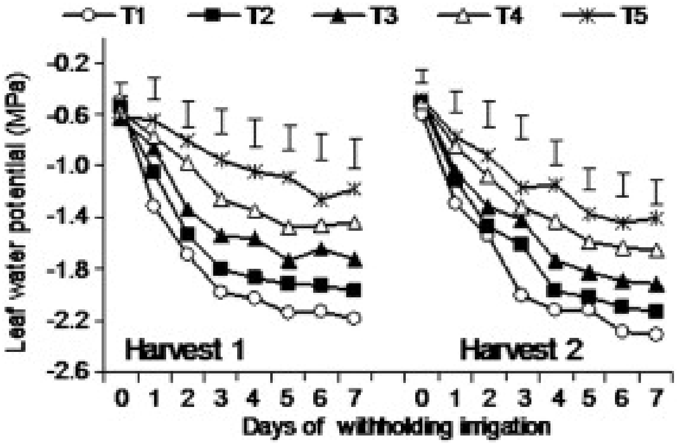

Fig. 6. After-effect of irrigation frequency on leaf water potential of rose-scented geranium leaves recorded during a one-week irrigation-withholding period. The vertical bars are LSD (at ∝ = 0.05); Harvests 1 and 2 were conducted in June and October 2006; T1, T2, T3, T4 and T5 represent daily, every second, third, fourth and fifth day irrigation treatments, respectively

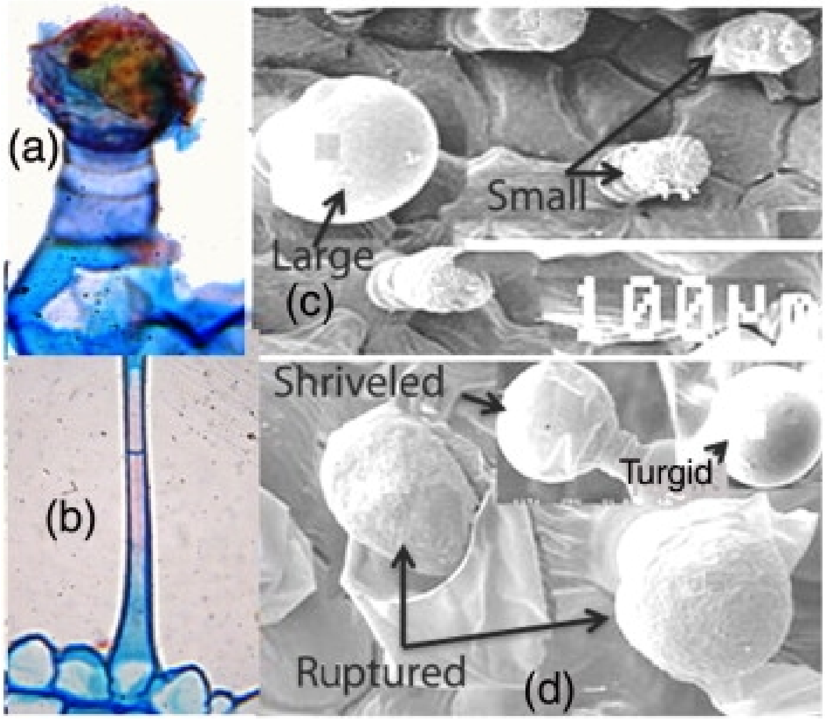

Fig. 7 – Trichome morphology: (a) glandular trichome, (b) non-glandular trichome, (c) two types of glandular trichomes (large and small), and (d) internal and external structure of oil gland cells