Stomatal Conductance, Growth and Yield of *Pelargonium sidoides* DC. in Response to Watering Frequency and Terminal Water Stress

Motiki Meshack Mofokeng^{1,2}, Hintsa Tesfamichael Araya^{1,2}, Phatu William Mashela² and Christian Phillipus du Plooy¹

1. Agricultural Research Council, Roodeplaat Vegetable and Ornamental Plant Institute (ARC-Roodeplaat VOP), Private Bag X293, Pretoria 0001, South Africa

2. Green Technologies Research Centre, University of Limpopo, Private Bag X1106, Sovenga 0727, South Africa

Abstract: Water is an important factor affecting growth, yield and distribution of different species. Plant response to water deficit can be in the form of physiological disorders, such as reduction in transpiration or assimilating partitioning to root growth. Sustainable use of water has become a priority in agriculture and thus innovative irrigation management practices are critical. The study aimed at investigating how watering frequency and terminal water stress influence growth of *Pelargonium sidoides*, an important medicinal plant in Southern Africa. The trial was a randomized complete block design with three replicates, and treatment factors were watering frequency (everyday, twice and once a week) and terminal water stress (no watering four weeks before harvesting, no watering two weeks before harvesting and no terminal stress). There was an interacting effect of watering frequency and terminal water stress on biomass and fresh root yield. More frequent watering resulted in significantly higher biomass and fresh root yield, compared to other treatments. Watering everyday with terminal or no terminal water stress resulted in higher fresh root yield, compared to other watering treatments with terminal water stress. Plant height and leaf area were significantly affected by watering frequency and terminal water stress, respectively. A significant drop in stomatal conductance of plants watered everyday was observed 240 d after treatment implementation, such that there was no significant difference across all the three watering frequency treatments. In conclusion, farmers can save on irrigation costs by reducing watering frequency, as there was no significant difference on dry root yield.

Key words: African geranium, irrigation, water stress, stomatal aperture, root yield, drought tolerance.

1. Introduction

Water shortage is a serious concern for water scarce countries; South Africa being one of them, where drought (temporary or permanent drought) is already posing a threat and this is due to significant uncertainties about the level of water supply for future generations [1]. Global climate change and increasing occurrence of events, such as El Niño have altered rainfall distribution and intensity in many countries, including South Africa, with the expectation of changing normal growth of wild and cultivated plant

species [2]. The accompanying global warming is expected to escalate water deficits by increasing evapo-transpiration and drought intensity [3]. Temporary or intermittent drought can occur during a growing season when rainfall is inadequate, and terminal drought, which is more permanent, occurs when stored soil moisture is depleted leading to crop senescence [4]. Drought can cause nutrient deficiencies due to reduced mobility and absorbance of individual nutrients, leading to lowered rate of mineral diffusion from the soil matrix to the root [5].

Water is one of the important factors that affect growth and yield, amongst other plant parameters [6], as well as distribution of different species [7], and has

Corresponding author: Motiki Meshack Mofokeng, M.Sc., research field: agronomy.

therefore increased interest in crop development under drought conditions [8]. Many studies have been conducted on the relationship between water stress and land production. However, plant response to such conditions has not been well studied [7].

Plant response to water deficit can be in the form of physiological disorders, such as reduction in photosynthesis and transpiration, although the response varies between species [6]. Many species respond to drought by increasing assimilate partitioning to root growth with related root/shoot ratio increases [2]. This is to extend root growth to reach for soil water deeper in the lower soil profile. Six aspects of plant response to water stress are described by other researchers [3] as drought escape (early completion of plant life cycle), drought avoidance (enhancing the capacity to absorb water), drought tolerance (improving osmotic adjustment and maintenance of tissue turgidity), drought resistance (altering metabolic pathways for survival), drought abandonment (removal of plant parts, such as leaf senescence) and drought prone biochemical-physiological traits (plant evolution under long-term drought). Despite the negative aspects of drought, it can induce adaptations in plants for them to survive, leading to emergence of new functional groups in an ecosystem or important tools for improving agricultural practices and plant breeding [5].

Irrigation is sometimes perceived as highly inefficient, because it is used to grow “water guzzling crops”, while at other times, it is seen as important for production of sufficient food (> 40% of global food production is under irrigation) for the ever growing world population [1]. Sustainable use of water is a priority in agriculture, and therefore innovations relative to irrigation management and practice are critical [9]. It is recommended that irrigation water management, in this period of eminent water scarcity, be carried out most efficiently with the aim of saving water while maximizing productivity [1]. One of the strategies

used in irrigation management is to water at specific intervals where the amount of depleted plant available water is measured and applied to fill the soil profile back to field capacity. The interval can be a specific day or days in a week.

Pelargonium sidoides is a medicinal plant that grows naturally in South Africa and is used for the treatment of cold related ailments, with some claims of anti-HIV-1 properties, according to the study of Helfer et al. [10]. The roots of this species have been exclusively harvested from the wild for local use and for international pharmaceutical companies [11]. Ethanolic fluid extracts of *P. sidoides* are used to treat ear, nose and throat disorders, as well as respiratory tract infections, while traditionally it is used for treatment of gastrointestinal disorders, tuberculosis and wound healing [12].

Research on how plants cope with adverse abnormal climatic variables is crucial in practical management of plant growth [3]. Due to complex interactions of different forms of stress under natural habitat, the underlying survival mechanisms in plants are still not fully understood [5]. Cultivation of medicinal plants as a conservation strategy has been recommended for many years, yet there is not enough information available. The objective of the study was to investigate how watering frequency and terminal water stress influence growth of *P. sidoides* under controlled conditions.

2. Materials and Methods

The study was a glasshouse pot trial, conducted at the Agricultural Research Council—Roodeplaat Vegetable and Ornamental Plants (ARC-Roodeplaat VOP) campus, Pretoria, South Africa (25°59' S, 28°35' E and 1,200 masl). The physical and chemical properties of the soil medium were presented in Table 1. The glasshouse temperature and humidity were monitored using a Tinytag View 2 data logger (Gemini Data Loggers, UK).

Table 1 Chemical and physical properties of the soil used as medium in the pots.

Chemical properties						
P-Bray 1	Ammonium acetate					
P (mg/kg)	K (mg/kg)	C (mg/kg)	Mg (mg/kg)	Na (mg/kg)	Total N (%)	pH H ₂ O
41.3	166	2,228	549	9.8	0.03	8.65
Physical properties						
Sand (%)	Silt (%)	Clay (%)		PWP (%)	FC (%)	BD (%)
76	6	18		0.12	0.24	3.37

PWP: permanent wilting point; FC: field capacity; BD: bulk density.

2.1 Growing System and Plant Culture

Root cuttings of *P. sidoides* were sustainably collected in January 2013 from Golden Gate Highlands National Park, as permitted by the South African National Parks Research Unit. The cuttings were grown in the glasshouse for three months before treatment, to give them time to root and establish. Harvesting was done in February 2014, which was 12 months after planting of the cuttings.

2.2 Fertilizer Application

Potassium (K) (110 kg/ha) and phosphorus (P) (30 kg/ha) application per pot was based on the rose-scented geranium nutrient requirement as described by Mofokeng et al. [6]. Nitrogen (N) was applied at the rate of 100 kg/ha. The plant population of 66,666 plants/ha [6] was used to determine the application per pot. Limestone ammonium nitrate (LAN, 28% N), potassium chloride (50% K) and single-superphosphate (11% P) were used as sources for the three nutrients, respectively. Fertilizer application was done once there was visible vegetative growth, as an indication of root activity.

2.3 Experimental Design and Watering Treatments

The trial was a randomized complete block design with three replicates and two treatment factors. Each treatment combination had four pot plants with soil as a medium. The treatment factors were watering frequency and terminal water stress. The volume of water required to refill the pots to pot capacity, which is water depleted through evapo-transpiration, was

determined by weight. The difference between the pot weight on the day of watering and the weight at pot capacity (which was pre-determined) was the volume of water to be applied (1 kg water = 1 L water). The watering treatments were: irrigating everyday (7 d/week), twice a week (2 d/week) or once a week (1 d/week). Terminal water stress was applied as no watering four weeks before harvesting (4 wks), two weeks before harvesting (2 wks) and a control of no terminal stress (0 wks). The dimensions of pots used to grow the plants were as follows: 30 cm top diameter, 21 cm bottom diameter and 28 cm deep.

2.4 Data Collection and Statistical Analysis

Plant height was measured in centimetres (cm) at 100, 160 and 240 d after treatment, while number of leaves was counted manually at similar intervals. Stomatal conductance was measured with an SC-1 leaf porometer (Decagon Devices, Pullman, USA). Total leaf area (cm²) was measured with a leaf area meter (Li-3100, Li-COR Inc., Lincoln, USA), after harvesting.

Data were subjected to analysis of variance (ANOVA) using GenStat[®], and for least significant difference, treatment means were separated using Fisher's protected *t*-LSD (least significant difference) at 5% level of significance.

3. Results and Discussion

Table 1 shows the physical and chemical properties of the soil medium used in the study. The glasshouse temperature ranged from a maximum of 30 °C to a minimum 18 °C, while relative humidity was between

a maximum of 90% and a minimum of 30%.

The total amount of water used per treatment decreased with a decrease in irrigation frequency (Table 2). Although the average water applied per irrigation event for the more frequently watered treatment (everyday) was lower, the total volume applied over the experimental period increased. Similar results were reported on rose-scented geranium (*Pelargonium* spp.) [13] and annual ryegrass (*Lolium multiflorum*) [14]. This could be attributed to higher evapo-transpiration rate associated with a larger canopy [13].

3.1 Growth Parameters

Plant height and number of leaves, averages across all treatments, increased significantly with growth period as shown in Fig. 1. At 240 d after the implementation of watering frequency, both plant height and number of leaves were significantly higher

than other growth periods. Although the number of leaves was not significantly influenced by watering frequency, the increases showed in Fig. 1 could be attributed to better growth in the well-watered treatment (watered everyday).

Watering frequency had a significant effect on plant height (Fig. 2). The more stressed the plants, through reduced watering frequency, the more reduced was plant height. This is part of plant strategy to survive reduced water supply. Water stress alters plant growth, resulting in reduced height, leaf size and number of leaves [5]. Similar results were reported by Alishah et al. [7], where plant height of *Ocimum basilicum* was significantly reduced under water stress (50% of field capacity) as compared to the non-stressed control. No significant differences were found in number of leaves of *Jatropha curcas*, at the end of their experiment, between the water stress treatment and control [15]. Bambara groundnut (*Vigna subterranean*) responded to

Table 2 The average volume of water applied per irrigation event and the total volume applied over the experimental period, in each watering frequency on *P. sidoides*.

Watering frequency	Average ET on the day of irrigation (L)	Total ET (L)
7 d/week	0.38	74.52
2 d/week	0.88	50.92
1 d/week	1.27	37.96

7 d/week = everyday; 2 d/week = twice a week; 1 d/week = once a week. ET: evapo-transpiration.

Fig. 1 Average plant height and number of leaves of *P. sidoides* as influenced by watering frequency over period across the three watering frequencies.

DAT = day after treatment.

Different letters represent significant difference at 95% confidence level.

Fig. 2 Plant height of *P. sidoides* as influenced by watering frequency.

7 d/week = watering everyday; 2 d/week = watering twice a week; 1 d/week = watering once a week.

Different letters represent significant difference at 95% confidence level.

declining soil water content due to applied water stress, by decreasing plant height, suggesting sensitivity of canopy development to water stress [16].

The total leaf area (cm^2) was significantly influenced by terminal water stress (Fig. 3). No terminal stress resulted in a significantly higher leaf area, with no significant difference between the two terminal water stress treatments. Withholding of irrigation in the third and fourth month of planting resulted in reductions in leaf area index of rose-scented geranium (*P. capitatum* $\times$ *P. radens* “rose”) [17].

3.2 Stomatal Conductance

Watering frequency had a significant effect on conductance from 80 d after treatment implementation (watering frequency) (Fig. 4). At 100 d after treatment, no significant difference was observed between watering everyday and twice a week. Again, at 240 d after treatment implementation, there was a drop in stomatal conductance of plants watered everyday, such that there was no significant difference across all the three watering frequency treatments. The significant drop mainly observed in the plants that were watered daily and twice a week, was because of

the implementation of the terminal stress, which was done 230 d after treatment implementation. The plants that were watered once a week were exposed to long term water stress, which induced changes in stomatal conductance as an adaptation strategy [13]. This mean that the water stressed plants did not have to adapt to terminal water stress as they had already adapted to the drought condition, whereas the well-watered plants had to adapt by closing their stomata, thus resulting in significant drop in stomatal conductance. Partial stomatal closure seems to be the water stress adaptation mechanism in *Pelargonium* species [6, 13].

Terminal water stress also had a significant effect on stomatal conductance (Fig. 5). No terminal water stress resulted in significantly higher conductance, compared to the two terminal water stresses which were not significantly different from each other.

3.3 Yield Parameters

There was an interacting effect of watering frequency and terminal water stress on biomass and fresh root yield (Fig. 6). Watering everyday resulted in significantly higher yield compared to other treatments. Watering twice in a week with no terminal

Stomatal Conductance, Growth and Yield of *Pelargonium sidoides* DC. in Response to Watering Frequency and Terminal Water Stress

Fig. 3 *P. sidoides* leaf area as influenced by terminal water stress.
0 wks = no terminal stress; 2 wks = two weeks' terminal stress; 4 wks = four weeks' terminal stress.
Different letters represent significant difference at 95% confidence level.

Fig. 4 Stomatal conductance of *P. sidoides* as affected by watering frequency and growth period.
DAT = days after treatment implementation.
Different letters represent significant difference at 95% confidence level.

Fig. 5 Stomatal conductance of *P. sidoides* as influenced by terminal water stress.

0 wks = no terminal stress; 2 wks = two weeks' terminal stress; 4 wks = four weeks' terminal stress.

Different letters represent significant difference at 95% confidence level.

Fig. 6 Total biomass (A) and fresh root yield (B) of *P. sidoides* as influenced by watering frequency and terminal water stress.

7 d/week = watering everyday; 2 d/week = watering twice a week; 1 d/week = watering once a week; 0 wks = no terminal stress; 2 wks = two weeks' terminal stress; 4 wks = four weeks' terminal stress.

Different letters represent significant difference at 95% confidence level.

Table 3 Mean dry root yield per plant of *P. sidoides* as influenced by watering frequency and terminal water stress.

Watering frequency	Terminal water stress	Dry root yield (kg)
7 d/week	0 wks	0.08
	2 wks	0.08
	4 wks	0.08
2 d/week	0 wks	0.08
	2 wks	0.07
	4 wks	0.07
1 d/week	0 wks	0.06
	2 wks	0.07
	4 wks	0.07
$p \leq 0.05$		NS

NS = not significant at 95% confidence level.

7 d/week = watering everyday; 2 d/week = watering twice a week; 1 d/week = watering once a week; 0 wks = no terminal stress; 2 wks = two weeks' terminal stress; 4 wks = four weeks' terminal stress.

water stress was not significantly different from watering everyday in both biomass and fresh root yield. There were no significant differences in dry root yield between all the treatments, showing that higher watering frequency did not increase dry root yield (Table 3). The fresh root yield under severe water stress (once a week) was not significantly different from the yield under irrigating twice a week with terminal water stress. This could possibly mean that roots in plants watered twice a week with terminal water stress lost some water during period of no water supply. It has been reported that the more frequently watered *Cucumis myriocarpus* showed a higher root biomass compared to the less frequently watered plants [18]. Similarly, frequent irrigation (once every month, two months and three months) increased fresh bulb weight of *Lycoris haywardii* compared to less frequent irrigation (once every four months and six months), which decreased bulb weight by 21% and 52%, respectively [19].

4. Conclusions

The study showed that *P. sidoides* plants employed a number of strategies when exposed to water stress, including reducing plant growth. When exposed to

prolonged stress, the plants reduced stomatal conductance and were therefore not affected by terminal water stress. Higher watering frequency increased fresh root mass only, but not dry root mass. This is important as the roots, which are the harvested parts, are supplied in a dry state. Farmers can save on irrigation costs by reducing watering frequency, as there was no significant difference on dry root yield. The effect of watering frequency and terminal water stress on chemical composition and medicinal value of the plant should be investigated.

Acknowledgments

Sincere thanks are expressed to the Department of Science and Technology, South Africa (DST-SA) for funding the study. Golden Gate Highlands National Park is acknowledged for the access to plant material. The medicinal plant research team at the ARC-Roodeplaat VOP is acknowledged for their support during the study.

References

- [1] Fereres, E., and Soriano, M. A. 2007. "Deficit Irrigation for Reducing Agricultural Water Use." *J. Exp. Bot.* 58: 147-59.
- [2] González, J. A., Gallardo, M., Hillal, M., Rosa, M., and Prado, F. E. 2009. "Physiological Responses of Quinoa (*Chenopodium quinoa* Willd.) to Drought and Waterlogging Stresses: Dry Matter Partitioning." *Bot. Studies* 50: 35-42.
- [3] Xu, Z., Zhou, G., and Shimizu, H. 2010. "Plant Responses to Drought and Re-watering." *Plant Signaling and Behavior* 5: 649-54.
- [4] Serraj, R., Sinclair, T. R., and Purcell, L. C. 1999. "Symbiotic N₂ Fixation Responses to Drought." *J. Exp. Bot.* 50: 143-55.
- [5] Da Silva, E. C., De Albuquerque, M. B., De Azevedo Neto, A. D., and Da Silva Junior, C. D. 2013. "Drought and Its Consequences to Plants—From Individual to Ecosystem." *INTECH*. doi:10.5772/53833.
- [6] Mofokeng, M. M., Steyn, J. M., Du Plooy, C. P., Prinsloo, G., and Araya, H. T. 2015. "Growth of *Pelargonium sidoides* DC. in Response to Water and Nitrogen Level." *S. Afr. J. Bot.* 100: 183-9.
- [7] Alishah, H. M., Heidari, R., Hassani, A., and Dizaji, A. A. 2006. "Effects of Water Stress on Some Morphological

and Biochemical Characteristics of Purple Basil (*Ocimum basilicum*)." *J. Biol. Sci.* 6: 763-7.

- [8] Bilibio, C., De Assunção Carvalho, J., Hensel, O., and Richter, U. 2011. "Effect of Different Levels of Water Deficit on Rapeseed (*Brassica napus* L.) Crop." *Ciênc. Agrotec. Lavras* 35: 672-84.
- [9] Pereira, L. S., Oweis, T., and Zairi, A. 2002. "Irrigation Management under Water Scarcity." *Agric. Water Manage.* 57: 175-206.
- [10] Helfer, M., Koppensteiner, H., Schneider, M., Rebensburg, S., Forcisi, S., Müller, C., Schmitt-Kopplin, P., Schindler, M., and Brack-Werner, R. 2014. "The Root Extract of Medicinal Plant *Pelargonium sidoides* Is a Potent HIV-1 Attachment Inhibitor." *PLoS ONE* 9 (1): e87487.
- [11] Lewu, F. B., Adebola, P. O., and Afolayan, A. J. 2007. "Commercial Harvesting of *Pelargonium sidoides* in the Eastern Cape, South Africa: Striking a Balance between Resource Conservation and Rural Livelihoods." *J. Arid Environ.* 70: 380-8.
- [12] Gödecke, T., Kaloga, M., and Kolodziej, H. 2005. "A Phenol Glucoside, Uncommon Coumarins and Flavonoids from *Pelargonium sidoides* DC." *Z Naturforsch* 60 (6): 677-82.
- [13] Eiasu, B. K., Steyn, J. M., and Soundy, P. 2012. "Physiomorphological Response of Rose-Scented Geranium (*Pelargonium* spp.) to Irrigation Frequency." *S. Afr. J. Bot.* 78: 96-103.
- [14] Abraha, A. B., Truter, W. F., Annandale, J. G., and Fessehazion, M. K. 2015. "Forage Yield and Quality Response of Annual Ryegrass (*Lolium multiflorum*) to Different Water and Nitrogen Levels." *Afr. J. Range Forage Sci.* 32 (2): 125-31.
- [15] Sapeta, H., Costa, J. M., Lourenço, T., Maroco, J., Van der Linde, P., and Oliviera, M. M. 2013. "Drought Stress Response in *Jatropha curcas*: Growth and Physiology." *Environ. Exp. Bot.* 85: 76-84.
- [16] Mabhaudhi, T., Modi, A. T., and Beletse, Y. G. 2011. "Growth Response of a Bambara Groundnut Landrace to Water Stress." In *Proceedings of the 10th African Crop Science Conference*, 97-102.
- [17] Eiasu, B. K., Steyn, J. M., and Soundy, P. 2008. "Growth and Essential Oil Yield of Rose-Scented Geranium (*Pelargonium capitatum* × *P. radens* 'Rose') as Affected by Withholding Irrigation at Different Times during Regrowth." *New Zealand J. Crop Hort. Sci.* 36: 285-94.
- [18] Nkgapele, R. J., Mphosi, M. S., and Mashela, P. W. 2011. "Effect of Irrigation Amount and Frequency on Phenology, Vine Length and Biomass Partitioning of Wild Cucumber (*Cucumis myriocarpus*) and Wild Watermelon (*Cucumis africanus*)." *Water Res. Manage.* 145: 557-63.
- [19] Bao, C. S., Zhang, P. C., Chen, C., and Zhou, H. 2013. "Growth and Photosynthetic Response of *Lycoris haywardii* Traub to Watering Frequencies." *J. Hort. Forestry* 5 (11): 218-23.