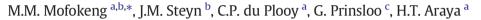
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Growth of *Pelargonium sidoides* DC. in response to water and nitrogen level



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ABSTRACT

Water stress is the most limiting factor in agricultural productivity in arid and semi-arid regions and causes very high losses in crop yield. Regulation of growth and stomatal conductance are the main mechanisms by which plants respond to water stress. Pelargonium sidoides is a medicinal plant that grows in South Africa and is used for the treatment of upper respiratory ailments. Cultivation has been considered as a viable means of reducing the pressure on natural populations of this species, but little to no information is available in this regard. Water and nitrogen supply are two of the most important factors that affect growth and yield of plants. This study therefore aimed at investigating the physiological and morphological response, in relation to growth, of P. sidoides to soil water and nitrogen levels. To achieve this objective, P. sidoides plants were grown under a rainshelter and exposed to three irrigation levels (well watered control, moderate water stress, and severe water stress treatment) and four nitrogen levels (0, 50, 100, and 150 kg \cdot N \cdot ha⁻¹). Nitrogen and water level had no significant interaction effect on measured parameters. Water stress significantly reduced stomatal conductance, while nitrogen had no significant effect on it. The well watered control had a significantly higher leaf area index, plant height, leaf area, and fresh root yield compared to the water stressed treatments. Nitrogen level had a significant effect on number of leaves, where 100 kg \cdot N \cdot ha⁻¹ had a significantly higher number of leaves compared to other nitrogen treatments. The study provides a first report on the response of P. sidoides to water and nitrogen and showed that the plant responds to water stress by closing its stomata and employing other morphological strategies like reducing plant growth.

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1. Introduction

Pelargonium sidoides, which grows naturally in South Africa, is used in the Eastern Cape Province for the treatment of several cold-related ailments in humans and livestock (Lewu et al., 2006; Brendler and van Wyk, 2008). Furthermore, Helfer et al. (2014) also proposed that *P. sidoides* root extract has shown anti-HIV-1 properties. Up to now, *P. sidoides* plant material for medicinal use has almost exclusively been harvested from the wild. However, there has been an increase in demand for the plant for traditional use as well as by local and international pharmaceutical companies (Lewu et al., 2007). As a result, cultivation has been considered as a viable means of reducing the pressure on natural *P. sidoides* populations. Information on the cultivation of medicinal plants such as *P. sidoides* is, however, very limited, and therefore further research was needed.

Water and nutrient supply are two of the most important factors that can affect growth, biomass yield, and chemical composition of

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plants, and therefore, this study focused on these two production factors. Exchange of water and carbon dioxide (CO₂) between leaves and the ambient air are important plant processes by which heat is dissipated through transpiration, while a primary substrate for photosynthesis is taken up (Streck, 2003). The ability of plants to adjust gaseous exchange through stomata permits them to control water relations and carbon assimilation, and the opening of the stomatal pore reflects a compromise between the photosynthetic requirement for CO₂ and the availability of water (Tricker et al., 2005). Regulation of leaf expansion and stomatal conductance are the main mechanisms by which plants respond to soil water deficit (Liu and Stützel, 2002; Eiasu et al., 2012). High transpiration causes stomatal closure, possibly by increasing the water potential gradient between the guard cells and other epidermal cells or by lowering the leaf water potential, either of which directly decrease the turgor pressure of guard cells relative to other epidermal cells or affect hormonal distribution (Bunce, 1996).

Water deficit in plants leads to physiological disorders, such as a reduction in photosynthesis and transpiration (Petropoulos et al., 2008a,b); however, the effects vary between species (Karkanis et al., 2011). Both photosynthesis and transpiration, which are closely related to dry matter production, are regulated by a stomatal feedback control



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Table 1	
Physical properties of the experimental site soil	Ι.

Soil depth (cm)	^a Sand				^a Silt		^a Clay	aPWP	aFC	aBD
	Coarse	Medium	Fine	Very fine	Coarse	Fine				
	mm (PS)									
	2-0.5	0.5-0.25	0.25-0.106	0.106-0.05	0.05-0.02	0.02-0.002	< 0.002			
Top soil (0–20) Sub soil (20–40)	4.7 3.6	17.2 15.9	26.2 23.2	11.9 12.1	10.9 7.2	10.4 13.8	16.5 22.1	10.3 12.9	19.9 25.5	1.59 1.56

^a Percentage, PWP: permanent wilting point, FC: field capacity, BD: bulk density, PS: particle size.

mechanism which, in turn, is influenced by water deficits (Kumar et al., 1994; Bota et al., 2004). The limitation of plant growth enforced by low water availability is mainly due to decreases in plant carbon balance, which is dependent on the balance between photosynthesis and respiration (Flexas et al., 2006). An early response to water stress supports immediate survival, whereas acclimation, calling on new metabolic and structural capabilities mediated by altered gene expression, help to improve plant functioning under stress (Chaves et al., 2002). Shoot growth is more sensitive to water deficit than root growth and the mechanisms underlying the sustained root growth under water stress include osmotic adjustment and an increase in the loosening capacity of the cell wall (Chaves et al., 2002).

Nitrogen increases leaf area index (LAI) and also improves the physiological properties of the plant (Kara and Mujdeci, 2010). Nitrogen is a component of many biological compounds that plays a major role in photosynthetic activity and crop yield capacity, and its deficiency constitutes one of the major yield limiting factors for production (Hokmalipour and Darbandi, 2011). Nitrogen deficiency leads to loss of green color in the leaves, decreased leaf area and intensity of photosynthesis, leading to reduced photosynthate production and thus lower yields (Alva et al., 2006; Bojović and Marković, 2009). Overapplication of nitrogen causes many environmental pollution problems (Lee et al., 2011) and can lead to decreased yields due to luxury consumption (Alva et al., 2006). Leaf area influences the interception and utilization of solar radiation of crop canopies (Hokmalipour and Darbandi, 2011), but it also plays an important role in water use (Liu and Stützel, 2002). LAI is a significant feature for the determination of plant photosynthetic activity and is a crucial structural characteristic of plants due to the role of green leaves in controlling many biological and physical processes in plant canopies (Kara and Mujdeci, 2010).

Deficit irrigation is becoming an important strategy to reduce agricultural water use in arid and semi-arid regions (Ayana, 2011). It is the practice of deliberately under-irrigating crops to reduce water consumption while minimizing adverse effects of extreme water stress on yield (Ayana, 2011). Deficit irrigation does not always decrease yield, as deficits properly applied in some development stages may even increase crop yield (Bilibio et al., 2011).

Plants take up inorganic nitrogen contained in the water absorbed from soil solution through their root systems, and thus, the fate of nitrogen is certainly coupled to that of water reaching the soil in the root zone (Alva et al., 2006). Water and nitrogen deficiency induces alterations of many morphological and physiological processes (Shangguan et al., 2000). Information on the response of *P. sidoides* to different water stress and nitrogen deficiency levels is not known and thus the objective of this study was to investigate the effect of water stress and nitrogen level on physiology and morphology of *P. sidoides*.

2. Materials and methods

The trial was conducted in a rainshelter at the Agricultural Research Council – Roodeplaat Vegetable and Ornamental Plant Institute (ARC-Roodeplaat VOPI), Pretoria, South Africa (25°59'S; 28°35'E and 1 200 m.a.s.l.). Soil samples were collected from the experimental site for analysis. The physical and chemical properties of the soil are presented in Tables 1 and 2, respectively, while a summary of the weather data recorded by a weather station (Campbell Scientific, USA) at the experimental site during the experiment period is shown in Table 3.

2.1. Plant material and experimental design

The mother material was acquired from a nursery at the Golden Gate Highlands National Park, in the Free State province of South Africa in 2010 and grown under shade-net (40% shade effect, grey color) at ARC-Roodeplaat VOPI. Root cuttings were made from the mother plants in January 2012.

Rooted cuttings of *P. sidoides* (4 months old) were transplanted to the rainshelter in May 2012 and harvested in June 2013. The trial was a factorial experiment designed as a randomized complete block design. The two factors were water and nitrogen levels. Each treatment plot was 4.5 m² in size, with 30 plants planted at a spacing of 0.5 m between the rows and 0.3 m in the row. The treatments were replicated three times and each replicate had 12 treatment plots.

2.2. Irrigation and fertilizer application

A neutron probe (Waterman, Probe Version 1.6, 2005, Geotech) was used to monitor soil water loss. The instrument was calibrated against different soil water contents determined gravimetrically to a depth of 1.0 m, at intervals of 0.2 m and calibration functions were developed (Shenkut et al., 2013).

The predetermined water treatments applied were 30, 50, and 70% allowable depletion level (ADL) of plant available water (PAW), where a specific percentage was allowed to deplete from the effective rooting depth before refilling the soil profile back to field capacity. Since the standard practice is to irrigate when 30% of PAW is depleted, this treatment will be referred to as the well watered control, while the 50 and 70% ADL will be the water stress treatments. The effective rooting depth was determined as 400 mm, from previous observations. A non-regulated drip irrigation system (Netafim, South Africa) with a discharge rate of 2000 ml per hour and maximum pressure of 270 kPa was used for irrigation. The treatments were applied from 7 months after planting to give the plants enough time to establish.

Table 2

Chemical properties of the experimental site soil.

Soil depth (cm)	Fe	Mn	Cu	Zn	Ca	Mg	Na	Р	К	Total N	pH H ₂ O
	mg∙kg									%	
Top soil (0–20)	13.74	44.10	9.24	14.00	980	298	24.7	80.9	134	0.028	7.26
Sub soil (20-40)	9.74	28.50	5.64	7.43	1201	370	39.4	60.4	94	0.026	7.44

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