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 is the main mechanism 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 or 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 treatment, 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 stress interaction had no significant effect on measured parameters. Water stress significantly reduced stomatal conductance, while nitrogen had no significant effect on it. The well watered treatment 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 of its stomata and employing other morphological strategies like reducing plant growth.

Keywords: allowable depletion, growth response, nitrogen, *Pelargonium sidoides*, stomata, water stress

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 plants and therefore this study focused on these two production factors. Exchange of water and carbon dioxide (CO_2) 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 CO_2 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*., 2008); 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 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 improve 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 colour in the leaves, decreased leaf area and intensity of photosynthesis, leading to reduced photosynthate production and thus lowers yields (Alva, *et al*., 2006, Bojović and Marković, 2009). Over application 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 deficit 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 (25º59'S; 28º35'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 as 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 colour) at ARC-Roodeplaat VOPI. Root cuttings were made from the mother plants in January 2012.

Rooted cuttings of *P. sidoides* (four 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 m^2 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. 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 seven months after planting to give the plants enough time to establish.

A base application of potassium (K) and phosphorus (P) were applied five days after planting, based on soil nutrient status (Table 1 & 2) and estimated nutrient requirements of rose-scented geranium (Araya *et al.*, 2006), since there was no recommendation available for *P. sidoides*. K was applied as potassium chloride (50% K) at the rate of 110 $kg \cdot K \cdot ha^{-1}$ and P was applied as single-super phosphate (11% P) at the rate of 30 $kg \cdot P \cdot ha^{-1}$, as a once off to boost the plants.

The nitrogen treatments were at different levels, where N was applied at the following rates: 0, 50, 100 and 150 $kg \cdot N \cdot ha^{-1}$. The N source used was Limestone Ammonium Nitrate (LAN, 28% N). The fertilizer was applied in two split applications of 50% each, with the first N application eight weeks after planting and the second application four months after planting.

2.3. Data collection and statistical analysis

Leaf area index (LAI) was measured non-destructively, using a LAI 2200 plant canopy analyzer (Li-Cor Bioscience, USA). The instrument uses measurements made above and below the canopy to calculate light interception at five zenith angles, from which LAI is computed using a model of radiative transfer in vegetative canopies. One above canopy reading and four below canopy readings were taken using the 270° view cap; and this was replicated two times in each plot. Plant height (cm) was measured and the number of leaves were counted manually. LAI and plant height measurements were taken on a monthly basis after treatment implementation. After harvesting, the total leaf area per plant was measured with a leaf area meter (Li-3100 leaf area meter, Li-Cor Inc., Lincoln, USA).

Stomatal conductance, which is a measure of the rate of passage of carbon dioxide (CO_2) or water vapour through the stomata of a leaf, was measured using the SC-1 Leaf porometer (Decagon Devices, USA), on a monthly basis. Stomatal conductance is described as a function of the density, size, and degree of opening of stomata. The measurements were taken on the abaxial (bottom) side of a matured fully expanded leaf, during midday when the environmental factors were at their peak.

Data was subjected to analysis of variance (ANOVA) using *GenStat®* version 11.1 (Payne *et al*., 2008). Treatment means were separated using Fisher's protected T-test least significant differences (LSD) at 5% level of significance (Snedecor and Cochran, 1980).

2.4 Scanning Electron Microscope (SEM)

Three leaf samples were collected and observed under an electron microscope to observe the stomata, following the method described by Motsa (2006) and Eiasu *et al*. (2012). The samples comprised of young, mature and old leaves. Samples (10 mm x 10 mm) were cut from each leaf sample and fixed in glutaraldehyde (3% w/v) immediately after cutting from the plant. They were then rinsed thoroughly with a phosphate buffer (0.1 M, pH 7.0) for 15 minutes, and repeated three times. Thereafter the samples were dehydrated in ethanol series (30 – 100% w/v) and then dried in a critical point drying apparatus (Bio-Rad E300, Watford, England). The dried samples were mounted on copper stubs and coated with gold in a vacuum coating unit (Polaron E5200C, Watford, England). The samples were then observed under a JSM 840 scanning electron microscope (JEOL, Tokyo, Japan) at 2000X magnification.

3. Results and discussion

3.1. Growth response

Nitrogen and water level interaction had no significant effect on the growth parameters measured. Table 4 shows the average LAI, plant height and number of leaves per plant (means across treatments) taken from seven months after planting until 11 months after planting, which was four months after water treatment application. Average LAI, plant height and number of leaves per plant decreased over the growing period across all water treatments (Table 4). The results in Figure 1, further shows that the LAI for the well watered treatment (30% ADL) dropped slightly after one month of water treatment application, but thereafter there were no further significant reductions. The well watered treatment always had a significantly higher LAI throughout the growing period (Figure 1). Though the severely stressed treatment (70% ADL) had the lowest LAI throughout, it was not significantly different from the moderately stressed treatment (50% ADL). A number of studies on different crops have also reported a decline in LAI values due to water stress. In their studies, Eiasu *et al*. (2008, 2009) also found that the LAI of rose-scented geranium was negatively affected by water stress, with a significant decline in LAI between the well watered and the water stressed treatments. Laurie *et al*. (2009) found a large reduction in LAI, of about 64 - 80%, due to reduced irrigation on different sweet potato varieties.

Plant height was also significantly reduced by water stress, as shown in Figure 2. The well watered treatment had a significantly higher plant height but there were no significant differences observed between the moderately and severely stressed treatments. Mabhaudhi *et al*. (2011) reported a marginal decrease in plant height of bambara landraces under rainfed conditions when compared to irrigated conditions. This could be due to the lower amount of rain received by crops under rainfed conditions. Alishah *et al*. (2006) found that in basil an increase in water stress levels resulted in a decrease in plant height. Similar results were also reported for *Jatropha curcas* (Hedayati *et al*., 2013).

Within the second month of water treatment application (8 months after planting - MAP) there were no significant differences in number of leaves across all the treatments (Figure 3). However after three and four months of water treatment application (9 and 10 MAP, respectively) the severely stressed treatment (70% ADL) had a significantly lower number of leaves, while there were no significant differences between the well watered treatment and the moderately stressed treatments. No significant differences were observed again at four months after water treatment application between all the treatments. The sudden increase in number of leaves, 11 month after planting, was due to the fact that the data was taken a week after irrigation of all water treatments, resulting in plant recovery. Since most of these new leaves were small, the mean LAI did not increase (Figure 1). Significant reductions in the number of leaves due to water stress were also reported in other crops such as parsley (Petropoulos *et al*., 2008), basil (Alishah *et al*., 2006) and common beans (Ghanbari *et al*., 2013). According to Munné-Bosch and Alegre (2004) leaf senescence is an adaptive strategy that contributes to plant survival under stress, including water stress. Decreasing of canopy leaf area through reduced growth, is also another strategy to minimize water loss under water stress conditions (Chaves *et al*., 2003) Leaf senescence may be sequential, starting gradually from oldest to youngest leaves, and depending on the duration and severity of stress, may allow young leaves to grow once stressful conditions have passed (Munné-Bosch and Alegre, 2004). A decrease in total leaf surface due to water stress induced senescence leading to leaf abscission, was reported on *Cichorium intybus* (Vandoorne *et al*., 2012).

Water stress also had a significant effect on leaf area, as indicated in Table 5. There was a declining trend in leaf area with an increase in water stress. The well watered treatment had a significantly higher leaf area compared to the severely stressed treatment. The moderately stressed treatment showed no significant difference in leaf area when compared to both the well watered treatment and severely stressed treatment, respectively. Similar results were reported by Liu *et al*. (2006) on potatoes, where the full irrigation treatment (irrigating to compensate for the full evapotranspiration water loss) had a significantly higher leaf area compared to the deficit irrigation and partial root drying treatments, with no significant differences observed between the two stress treatments. Karkanis *et al*., 2011, found the lowest leaf area of velvetleaf plant for the water stressed treatment (50% field capacity refill) with the highest leaf area in the well watered control (100% field capacity refill). In another study on vegetable amaranth, Liu and Stützel (2002) reported a significantly lower leaf area for water stressed plants, where irrigation was withheld for a certain period, compared to the control which was irrigated to 90% of the water holding capacity of the soil, for all genotypes studied.

Although nitrogen application had no significant effect on the other parameters, it had an effect on number of leaves at harvest. Figure 4 shows that nitrogen at the rate of 100 kg · N · ha^{-1} had a significantly higher number of leaves, than the other treatments. Hussain *et al*. (2006) reported that the maximum number of branches per plant was found with the application of 90 kg · N · ha^{-1}, on asparagus. Zhu *et al*. (2009) found that the application of medium amounts of N and P fertilizer on *Bupleuri radix*, either alone or in combination, increased shoot growth amongst other parameters. Araya *et al*. (2006) reported that application of organic N at the rate of 100 kg · ha^{-1} increased fresh herbage and oil yield of rose scented geranium, compared to the control in the first harvest. In the second harvest both inorganic and organic N at the rate of 100 kg · ha^{-1} increased fresh herbage and oil yield over the control (Araya *et al*., 2006). Number of leaves has direct relationship with essential oil yield.

3.2. Stomatal conductance

The average stomatal conductance of *P. sidoides* as affected by water stress at different times over the growth period is shown in Figure 5. Stomatal conductance of the well watered treatment (30% ADL) was always significantly higher than that of the stressed treatments, while the 50% and 70% ADL treatments did not differ significantly from each other in most cases. Nitrogen did not show a significant effect on stomatal conductance. Green and Mitchell (1992) also reported a lack of N-related difference in stomatal response to water stress of loblolly pine seedlings.

The stomatal conductance results, when observed across water stress treatments (Table 5), showed that it decreased with an increase in the stress. The well watered treatment had a significantly higher stomatal conductance compared to the other two water treatments. The moderately stressed treatment (50% ADL) also had a significantly higher stomatal conductance than the severely stressed treatment (70% ADL). The results on stomatal conductance in this study are consistent with results of work done on other crops. Eiasu *et al*. (2012) found that rose-scented geranium plants (*Pelargonium* spp) exposed to water stress had a lower stomatal conductance compared to those irrigated more often. Karkanis *et al*. (2011) reported that water stress reduced stomatal conductance of velvetleaf by 37 – 89%. All the species studied by Galméz *et al*. (2007) showed a progressive decline in stomatal conductance as water stress intensified.

The increase in stomatal conductance observed in month 10 and 11, could have been due to a decrease in vapor pressure deficits (VPD) in March and April 2013 (Table 3). Increases in VPD between leaf and air results in partial closure of the stomata, thus decreasing stomatal conductance so as to prevent excessive dehydration and physiological damage (Oren *et al*., 1999, Ocheltree *et al*., 2014). Sweet pepper plants grown under low VPD consistently maintained a higher stomatal conductance compared to plants grown at ambient and high level VPD (Zabri and Burrage, 1998). Similar results were reported by Comstock and Ehleringer (1993) in their study on common beans; and Dai *et al*. (1992) on castor bean.

The higher stomatal conductance observed at the well watered treatment was the result of fully open stomata on both the abaxial and adaxial side of the leaves (Figure 6). The stomata on the moderately stressed samples were opened on the abaxial side and partially closed on the adaxial side of the leaf sample. The lowest stomatal conductance on the severely stressed plants was due to the stomata that were partially closed on the abaxial side to fully closed on the adaxial side, as was observed on the leaf samples.

Though stomatal regulation in response to water stress has been a controversial issue for long, it has been recognized that stomatal closure results in a limiting resistance, controlling the flow of water through the plant (Comstock and Mencuccini, 1998). The mentioned study suggested a simple threshold model where stomatal closure is triggered as leaf water potential reaches a critical stress level. Stomatal closure is thus amongst the earliest responses to water stress, protecting the plants from extensive water loss (Chaves *et al*., 2003).

3.3 Fresh root yield

Fresh root yield (kg · plant^{-1}) followed the same trend as LAI, where water stress significantly reduced the yield (Table 5). The well watered treatment had a significantly higher root fresh yield than the water stressed treatments for both parameters and there was no significant difference between the water stressed treatments. Vandoorne *et al*. (2012), in their study on root chicory, found that the mean root fresh yield was lowered by water stress, with a decrease of more than 50%, compared to the control. Fresh root yield of plain leafed and turnip rooted parsley exposed to higher water stress was also significantly reduced (Petropoulos *et al*. 2008). Similarly, Darwish *et al*. (2006) reported that severe deficit irrigation (60% ET) led to a 21% loss in potato fresh yield, due to lowered tuber dry matter production and average mass of the commercial tubers.

4. Conclusions

There was no significant interaction effect between nitrogen and water level for all the parameters measured in this study. Water stress significantly decreased stomatal conductance. Closing of the stomata is a physiological mechanism employed by plants to cope with water stress. However, because stomata are the pathway for water and CO_2 exchange with the atmosphere, this mechanism has a negative effect on photosynthesis, and therefore on plant growth and yield. Microscopic observations confirmed that *P. sidoides*, like most other plant species, respond to water stress by closing their stomata. It was also observed that water stress resulted in closing of the stomata on the adaxial side of the leaves first, followed by closing of those on the abaxial side.

Morphologically, plants respond to water stress by leaf senescence, smaller canopy and smaller leaves, amongst others. *P. sidoides* showed similar response with reductions in LAI, plant height and leaf area per plant. These observed morphological responses and reduced fresh root yield were probably the result of reduced photosynthetic rate, since CO_2 uptake was decreased by closing of the stomata.

Nitrogen had a significant effect on number of leaves per plant, but not on leaf area, which means that although more leaves were stimulated, they were not bigger in size and therefore did not result in higher LAI. The study presented the first results on response of *P. sidoides* to water stress and nitrogen levels, which could be important in the establishment of nitrogen and water management guidelines for cultivation of *P. sidoides*.

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Table 1. Physical properties of the experimental site soil.

Soil Depth (cm)	*Sand				*Silt		*Clay	*PWP	*FC	BD
	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)	4.7	17.2	26.2	11.9	10.9	10.4	16.5	10.3	19.9	1.59
Sub soil (20–40)	3.6	15.9	23.2	12.1	7.2	13.8	22.1	12.9	25.5	1.56

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

Table 2. Chemical properties of the experimental site soil.

Soil depth (cm)	Fe	Mn	Cu	Zn	Ca	Mg	Na	P	K	Total N	pH H_2O
	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

Table 3. Summary of weather data collected during the experiment period.

Month (2013)	Temperature (°C)		Wind speed (ms)	Relative humidity (%)		VPD (kPa)	Rainfall (mm)
	Average						Total
	max	min		max	min		
Jan	30.73	16.85	0.90	87.45	34.61	1.28	90.3

Feb	32.06	15.77	0.81	88.80	27.89	1.40	35.0
Mar	29.53	14.43	0.75	89.78	30.54	1.11	75.9
Apr	26.10	9.21	0.67	91.70	32.39	0.89	98.2
May	24.83	4.97	0.59	89.73	23.43	0.87	0.55

*Max: maximum, min: minimum, VPD: vapor pressure deficit

Table 4. Averages of plant height, number of leaves and leaf area index of *P. sidoides* over the growth period.

Growth period (MAP*)	LAI (m^2 leaf area/m^2 ground area)	Plant height (cm)	Number of leaves/plant
7	1.40^{a}	-	-
8	1.18^{b}	17.4^{a}	173^{a}
9	1.08^{bc}	17.8^{a}	162^{b}
10	0.95^{cd}	16.5^{b}	153^{c}
11	0.88^{d}	14.3^{c}	123^{d}
LSD $_{0.05}$	0.13	0.65	9.07

*MAP = month after planting. Values with different letters are significantly different from each other. Values represent means across all water treatments. LSD: least significant differences.

Table 5. Average leaf area index, leaf area per plant, stomatal conductance and fresh root yield per plant of *P. sidoides* in response to water stress.

Treatments ADL (%)	Mean LAI m^2 leaf area · m^{-2} ground area	Mean leaf area $cm^2 \cdot plant^{-1}$	Mean conductance mmol m^{-2} s^{-1}	Fresh root yield (kg · $plant^{-1}$)
30	1.328^{a}	899.6^{a}	100.5^{a}	0.30^{a}

50	1.009^{b}	707.9^{ab}	49.04^{b}	0.22^{b}
70	0.934^{b}	617.3^{b}	36.44^{c}	0.21^{b}
$LSD_{0.05}$	0.11	204	9.99	0.04

*LSD: least significant difference, ADL: allowable depletion level.

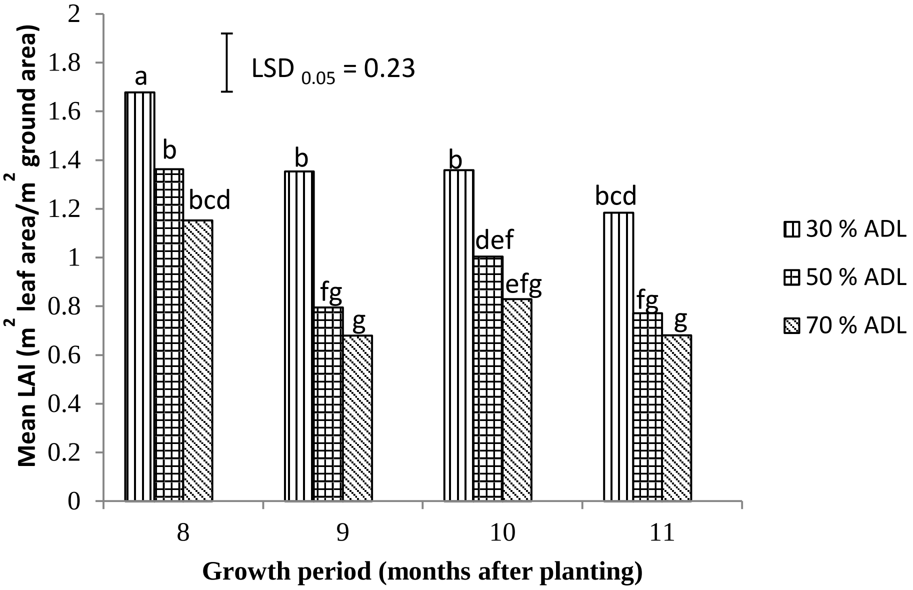


Fig. 1. Leaf area index of *P. sidoides* in response to water treatment, over the growing period. LSD: least significant difference, ADL: allowable depletion level.

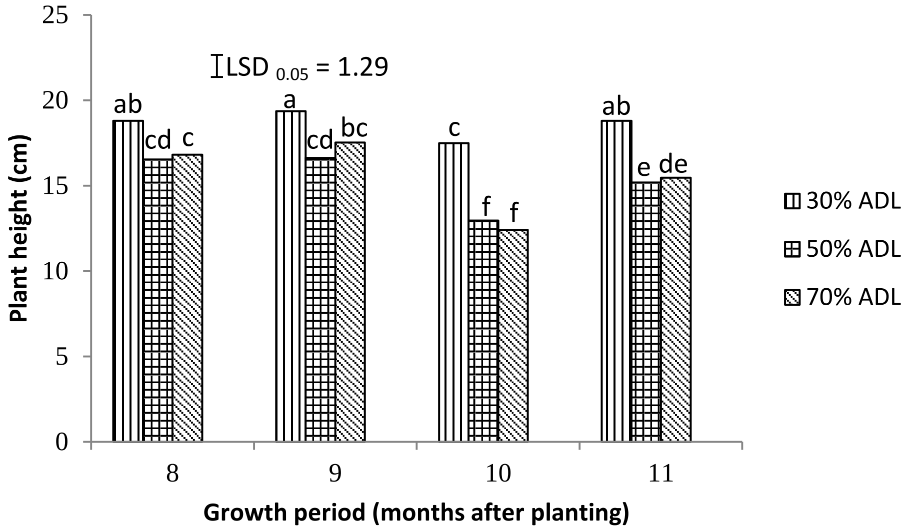


Fig. 2. Plant height of *P. sidoides* over the growing period in response to water treatment. LSD: least significant difference, ADL: allowable depletion level.

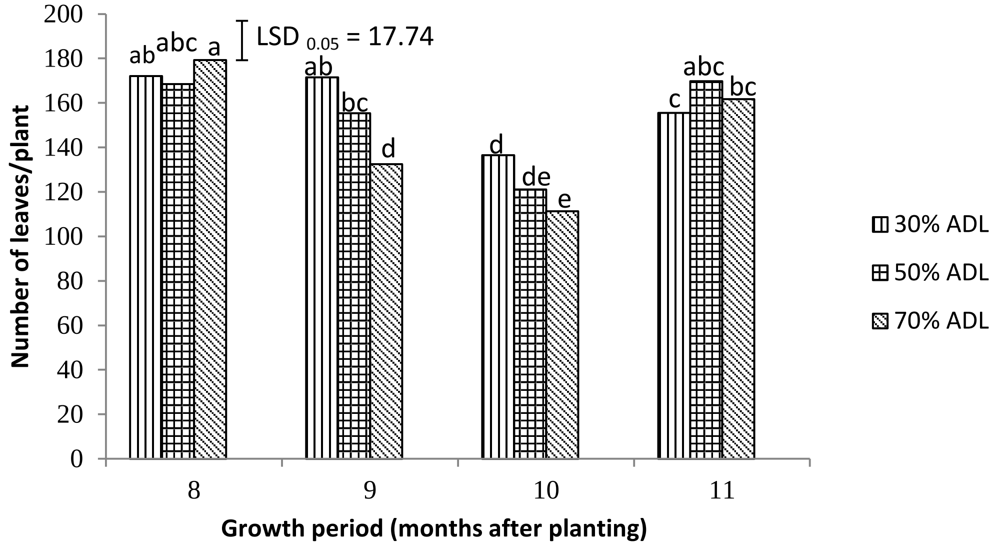


Fig. 3. Number of leaves of *P. sidoides* over the growing period, in response to water treatment. LSD: least significant difference, ADL: allowable depletion level.

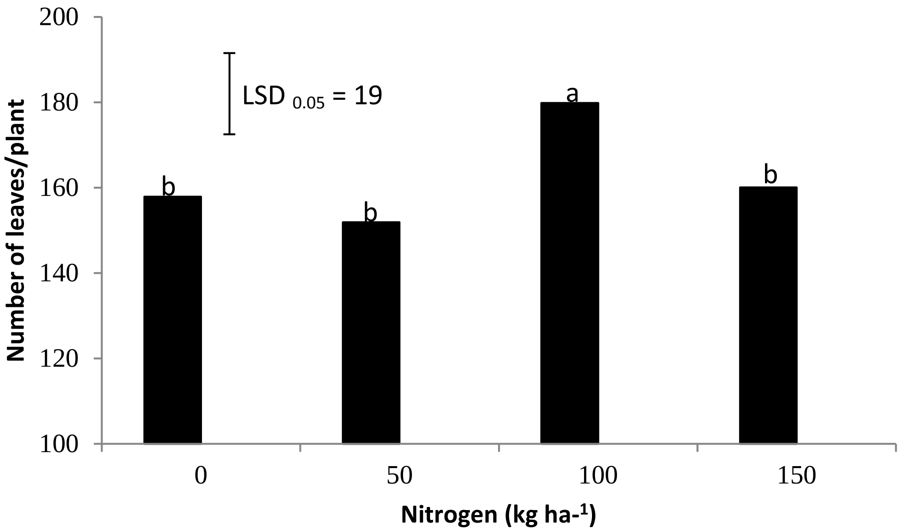


Fig. 4. Average number of leaves per plant in response to nitrogen level, at harvesting. LSD: least significant difference.

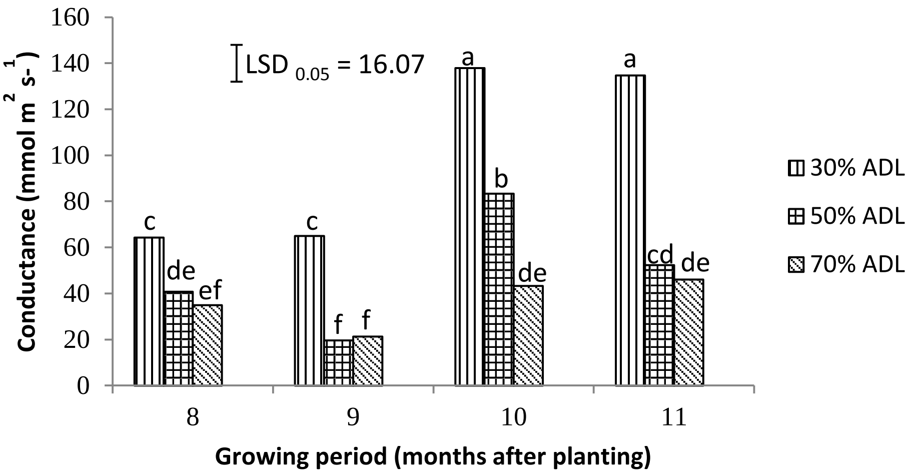
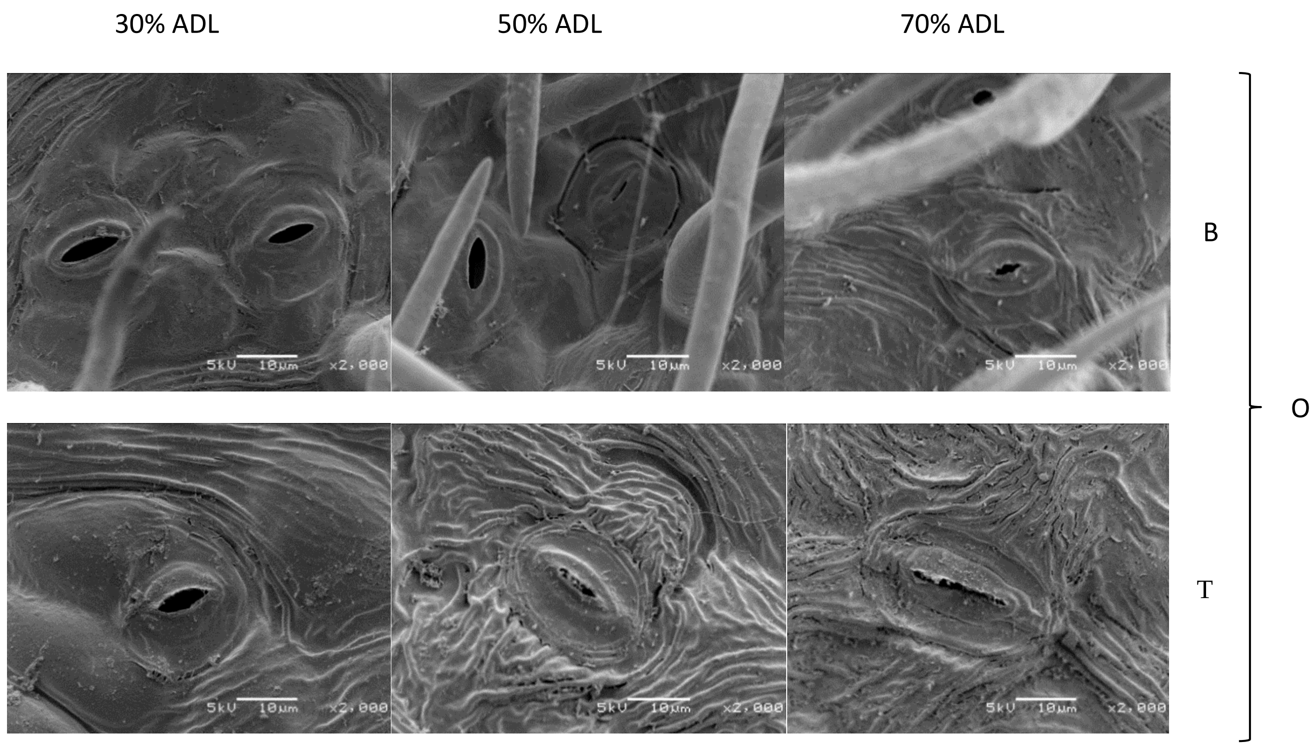


Fig. 5. Average stomatal conductance of *P. sidoides* at different times over the growing period, in response to water treatments**.** LSD**:** least significant difference, ADL: allowable depletion level.


30% ADL
50% ADL
70% ADL
B
T
O

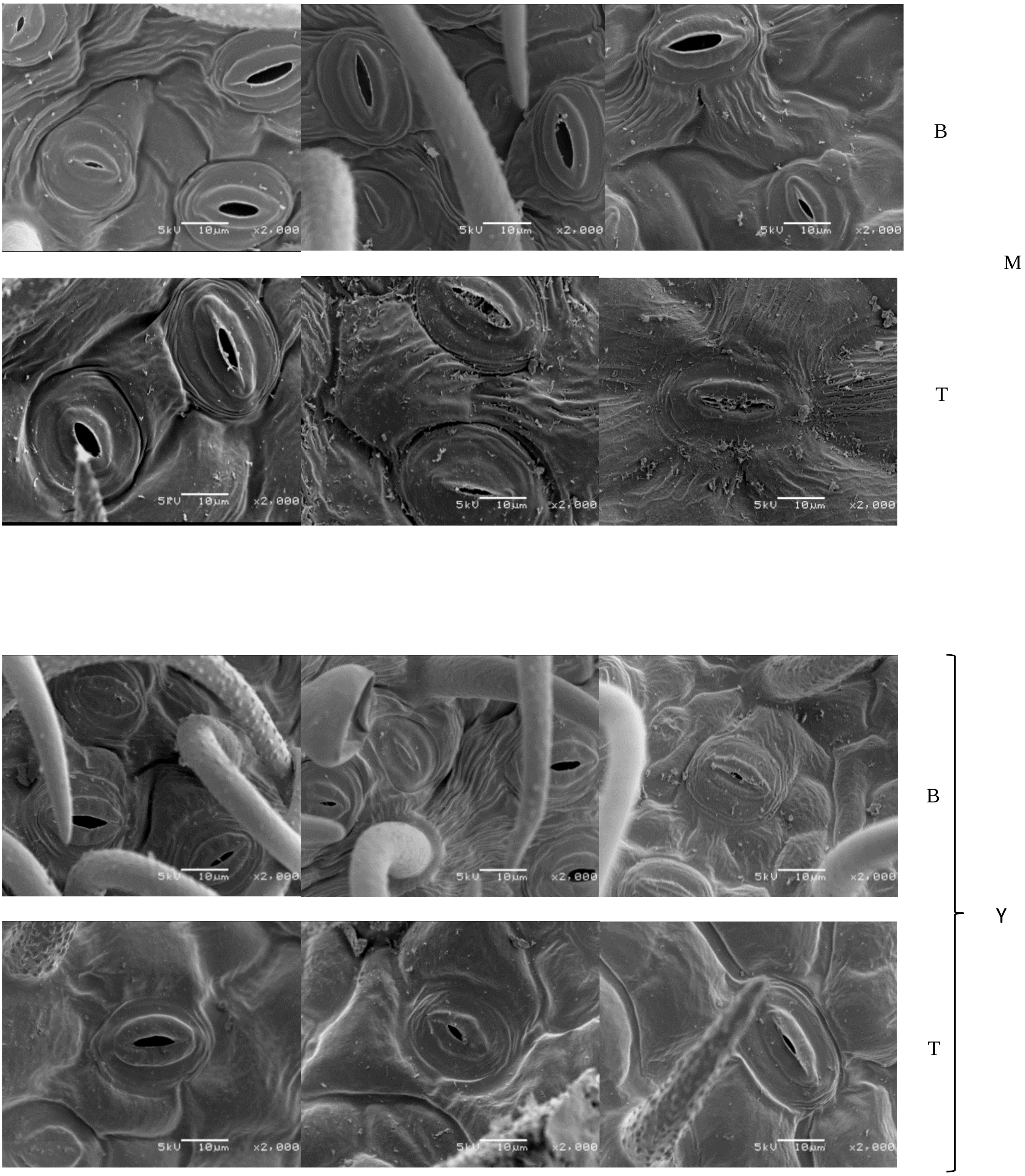


Fig. 6. *P. sidoides* leaf samples showing stomatal pores as observed under an electron microscope. O = old leaves, M = mature leaves and Y = young leaves. B = abaxial side and T = adaxial side.