

Plant Water Relations as an Indicator of Drought Tolerance of *Senna* Species in the Egyptian Nubia

M.G. Sheded and U.A.A. Radwan

Department of Botany, Faculty of Science at Aswan, South Valley University, 81528, Aswan, Egypt

Abstract: Plant water relations of four *Senna* species were studied under imposed gradual soil moisture depletion in order to evaluate their drought tolerance. Leaf water potential at predawn, associated midday measurements and estimated diurnal range were recorded under different soil moisture contents, while transpiration rate was measured at different SMC and PPFD. Also stomatal frequency and pore length were calculated on the abaxial leaf surface. The current study revealed the high drought tolerance and stomatal control efficiency of *S. alexandrina* and also revealed the differences in plant water relations and their water requirements, which of great importance in the efforts of restoration and conservation of species of high economic value, which could be considered as a convenient plants for land reclamation in their natural habitats.

Key words: Water potential • soil moisture content • transpiration • photosynthesis • stomata

INTRODUCTION

Plants in hyper arid environmental conditions show different mechanisms to withstand drought and gradual water scarcity. Gradual soil moisture depletion in both field and natural ecosystems, which lead to sequence of events, starts with shoot growth cessation, followed by decreased transpiration rate, photosynthesis rates and finally leaf senescence at very low, available soil moisture content [1, 2]. The four genera of *Senna* L. (Leguminosae, Caesalpinoideae) were recognized in Egyptian Nubia [3-5] and well known for their medicinal uses [6]: *S. italica* Mill. and *S. alexandrina* Mill., which both considered as xerophytic plants with well known purgative effects in folk medicine, while *S. occidentalis* was recognized as weed assembled with crops in Upper Egypt [7]. On other hand, *S. didymbotyra* was recently recorded in the Egyptian flora rocky shorelines of some Nile islands at Aswan [8]. Their germination characteristics and seedling morphology were studied in Egyptian Nubia [9]. Also, their relative similarity and ecological diversity were studied by determination of amino acid and protein content [10]. The leaf water potential, transpiration rate and stomatal frequency were used as tools to characterize the response of plants to water stress and to evaluate their drought tolerance [11, 12]. Under the arid conditions, investigating the depletion mechanisms of indigenous species is greatly important to reveal their ecological

success, growth conditions and their suitability in reservation and restoration [13, 14].

Many authors used plant water potential measurements to qualify plants and their soil water status in different environmental conditions from hyper arid to semiarid regions [15-18]. Stomatal closer and stomatal control efficiency are very important factors in drought avoidance in most of desert plants [19]. The aim of the present study is to cover the gap in the knowledge regarding some mechanisms of above mentioned species to withstand drought and to evaluate the variations in response to drought stress tolerance, which of great importance in restoration and reservation of species of high economic value.

MATERIALS AND METHODS

Seeds of four *Senna* species were collected from Southeastern Egyptian Desert, in May 2006. *Senna alexandrina* and *Senna italica* were collected from western desert surrounding the South Valley University campus at Aswan, while *Senna occidentalis* was collected from Aswan Gardens and rudral areas. On the other hand, *Senna didymbotyra* was collected from western shores of Aswan reservoir.

Seeds were subjected to acid scarification by immersion in 95% H₂SO₄ for 10 minutes and then washed thoroughly with tap water [9]. Seeds were sown directly

into plastic pots of 30cm in diameter and 20 cm deep with four 1.5 mm-holes at the bottom. Soil used in experiment was clay: sand (1:2). Soil characteristics and water release curve was obtained using filter paper method for determination of field capacity and wilting points [20-21]. Two to three seeds were planted in each pot, after seedling establishment, seedling were thinned to one plant in each pot. Pots were watered with tap water with no addition of nutrient; watering regime was maintained at 9% by soil weight (field capacity). Plants were kept in room temperature ($25\text{C}\pm 5^\circ\text{C}$), PPFD ($400\text{-}700\mu\text{mol}^{-1}\text{s}^{-1}\text{m}^{-2}$) photoperiod of 12:12 L: D cycle and relative humidity ($20\pm 5\%$).

Homogenous 10-weeks-old plants were used in this experiment: the experimental design was completely randomized with one fixed factor which is gradual soil moisture depletion (short-term). Soil moisture was adjusted to 12% by weight of soil (3% above field capacity) and then allowed to gradual dryness to reach 1% by weight of soil (3% below wilting point). Pots were watered with tap water with no addition of nutrient confirmed by using of soil moisture meter (Model 5910AO); SOIL MOISTURE EQUIPMENT CORP, USA).

Six plants of the four *Senna* species were selected for leaf water potential measurements which were taken twice a day, at midday (1200-1430h) and then followed by predawn measurements (0300-0530h).

Measurements were taken at 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 and 1% of soil moisture content by weight at room temperature ($25\text{C}\pm 5^\circ\text{C}$). The lowest soil moisture (1%) was reached at a period varied between 15 to 20 days. Leaf water potential measurement were carried out using pressure Chamber (Soil Moisture Equipment corp, Model 3005) [22]; leaf water potential measurements were taken in one randomly selected plant from six randomly selected replicates in fully developed leaf [23]. The recovery from water stress during the day and in response to gradual soil moisture were obtained from plotting predawn and midday values and drawing 45 bisecting fine represents the boundary where both values were equal which considered as loss point or permanent wilting point of studied species [27, 28].

One set of six plants each of four *Senna* species were selected to measure transpiration rate of fully developed and expanded leaf (six measurements replicates were recorded at different soil moisture contents and PPFD ranged from 0 to $2000\mu\text{mol}^{-1}\text{s}^{-1}\text{m}^{-2}$, using Steady State Porometer (LI-COR, NE, USA), equipped with small aperture (1600-06) of total leaf area 0.60cm^{-2} . light source was (6V-30W) tungsten lamp, light intensity varied by power supply (Model Olympus, TGHM, Japan).

Fresh strips of abaxial leaf surfaces were collected from 3 individual from each *Senna* species and stained with 50% water soluble safranin for light microscopy examination [26]. Photographs were taken using Leitz Deplane photomicroscope and direct measurements using stage micrometer.

Differences of leaf water potentials in response to gradual soil moisture depletion and *Senna* species were examined using two-way analysis of variance, while water potential values of midday and predawn were subjected to paired t-test [24]. Differences of transpiration rates of *Senna* species were examined versus soil moisture depletion and PPFD using two-way analysis of variance. While numerical values of leaf water potential, stomatal frequency and pore length (l) are means \pm standard error. Stomatal diffusion resistance (r_s) of *Senna* species were calculated from the equation: $r_s = l / v \pi r^2 D$, where l , i , r and D are stomatal pore length, stomatal frequency, stomatal radius and diffusion coefficient, respectively (diffusion coefficient (D) = $24.2\text{mm}^2\text{s}^{-1}$ was derived from mutual diffusion coefficient of binary mixture containing air and water at 20°C) [11].

All above mentioned statistical data analysis were carried out using Minitab Statistical Program [25].

RESULTS

Leaf water potential of (Mean \pm SE) of *S. alexandrina*, *S. italica*, *S. occidentalis* and *S. didymbotrya* in response to gradual soil moisture depletion were shown in Table 1. In general, the four *Senna* species showed that mean predawn water potential was higher than associated mean midday values and larger negative leaf water potentials were attained at midday. The diurnal range varied from 0.29 ± 0.005 to $0.81\pm 0.01\text{MPa}$ in *S. alexandrina*, 0.20 ± 0.01 to $0.39\pm 0.008\text{MPa}$ in *S. italica*, from 0.10 ± 0.02 to $0.41\pm 0.04\text{MPa}$ in *S. occidentalis* and 0.03 ± 0.001 to $0.11\pm 0.01\text{MPa}$ in *S. didymbotrya*. From two-way analysis of variance, highly significance changes in predawn, midday and diurnal range values of leaf water potential were attributed to gradual soil moisture depletion, species difference and interaction between both factors. *S. alexandrina* showed a notable change in predawn and midday values of leaf water potentials in response to gradual soil moisture, as larger record at lowest soil moisture content (1%) was -1.99 ± 0.005 and $-2.8\pm 0.01\text{MPa}$ at predawn and midday respectively. The highest diurnal range 0.81 ± 0.01 and $0.80\pm 0.01\text{MPa}$ were recorded at 2 and 1% (SMC), respectively. On the other hand, *S. italica* and *S. occidentalis* gave the larger negative values of

Table 1: Leaf water potential (Mean±SE) of *S. alexandrina*, *S. italica*, *S. occidentalis* and *S. didymbotyra* in response to gradual soil moisture depletion

Species	Soil moisture content (% by wt.)	Water potentials (Mpa)		
		Predawn	Midday	Diurnal range
<i>S. alexandrina</i>	12	-0.63±0.01	-1.24±0.01	0.61±0.02
	11	-0.64±0.05	-1.19±0.01	0.54±0.01
	10	-0.71±0.05	-1.31±0.01	0.60±0.002
	9	-0.71±0.004	-1.40±0.001	0.69±0.004
	8	-0.80±0.01	-1.50±0.001	0.70±0.01
	7	-1.09±0.05	-1.80±0.004	0.71±0.03
	6	-1.51±0.01	-1.80±0.01	0.29±0.005
	5	-1.99±0.01	-2.39±0.01	0.41±0.003
	4	-1.99±0.005	-2.50±0.004	0.51±0.006
	3	-2.02±0.03	-2.61±0.01	0.59±0.02
	2	-1.99±0.006	-2.80±0.004	0.81±0.01
	1	-1.99±0.005	-2.80±0.01	0.80±0.01
<i>S. italica</i>	12	-0.63±0.01	-0.91±0.01	0.28±0.02
	11	-0.60±0.01	-0.99±0.005	0.39±0.01
	10	-0.60±0.005	-0.99±0.004	0.39±0.008
	9	-0.81±0.01	-1.10±0.001	0.30±0.01
	8	-0.80±0.01	-1.30±0.01	0.51±0.01
	7	-1.95±0.01	-1.50±0.01	0.31±0.01
	6	-1.39±0.01	-1.70±0.004	0.32±0.01
	5	-1.80±0.01	-2.10±0.004	0.31±0.01
	4	-1.91±0.003	-2.20±0.05	0.30±0.01
	3	-2.21±0.003	-2.41±0.002	0.20±0.01
	2	wilting		
	1			
<i>S. occidentalis</i>	12	-0.61±0.03	-0.69±0.004	0.10±0.02
	11	-0.61±0.03	-0.80±0.01	0.19±0.01
	10	-0.60±0.08	-0.80±0.01	0.20±0.001
	9	-0.64±0.02	-0.89±0.01	0.26±0.01
	8	-0.80±0.02	-0.99±0.01	0.19±0.004
	7	-0.81±0.03	-1.10±0.01	0.30±0.03
	6	-0.90±0.03	-1.21±0.003	0.30±0.01
	5	-1.60±0.03	-1.80±0.01	0.20±0.01
	4	-1.90±0.01	-2.30±0.01	0.40±0.01
	3	-1.99±0.02	-2.4±0.004	0.41±0.04
	2	wilting		
	1			
<i>S. didymbotyra</i>	12	-0.39±0.01	-0.47±0.01	0.08±0.01
	11	-0.45±0.01	-0.48±0.01	0.03±0.001
	10	-0.51±0.01	-0.56±0.01	0.05±0.01
	9	-0.60±0.01	-0.65±0.001	0.05±0.004
	8	-0.99±0.01	-1.10±0.001	0.11±0.01
	7	-1.10±0.01	-1.21±0.01	0.11±0.01
	6	-1.19±0.01	-1.30±0.003	0.10±0.01
	5	-1.30±0.01	-1.40±0.001	0.10±0.03
	4	-1.40±0.01	-1.49±0.01	0.10±0.01
	3	-1.41±0.01	-1.51±0.01	0.10±0.01
	2	-1.50±0.01	-1.60±0.003	0.11±0.01
	1	wilting		

predawn measurements of leaf water potential at 3% (SMC) were -2.21±0.003 and -1.99±0.02 MPa, respectively. While, diurnal range was 0.2±0.01 and 0.41±0.04MPa, respectively. Otherwise, plants of both species were wilted at 2% of soil moisture content. In *S. didymbotyra*, leaf water potential was the lowest in comparison with the other studied *Senna* species larger negative values of predawn (-1.5±0.01MPa), midday (-1.6±0.003MPa) and diurnal range of 0.11±0.01MPa, at 2% soil moisture content, reaching its wilting point at 1% of soil moisture content. The recoveries from water stress were shown in Fig. 1a-b, *S. alexandrina* (Fig. 1a) showed the highest water stress recovery where the bisecting line gave an equal boundary dividing the scattered predawn and midday values of leaf water potential. Otherwise, in *S. italica* (Fig. 1b), *S. occidentalis* (Fig. 1c) and *S. didymbotyra* (Fig. 1d), the 45° bisecting line gave the boundary which reveals that, both values of predawn and midday of leaf water potential where almost at nearby the 45° bisecting line (turgor loss point or permanent wilting point).

Transpiration rate of *Senna* species were shown in (Fig. 2a-b). From two-way analysis of variance, significant changes in transpiration rate of *Senna* species were attributed to soil moisture depletion, species differences and *PPFD* where, F=151.71; P<0.0001, respectively. On the other hand, only soil moisture depletion and species differences interacted to cause significant changes in transpiration rate, where, F=30.06 and P<0.0001. While, significant changes in transpiration rate in response to *SMC* and *PPFD* of each *Senna* species were shown in Fig. 2. a-b.

The highest transpiration rate (1.253µg cm⁻²s⁻¹) in *S. alexandrina* (Fig. 2a) at 0µmol⁻¹sm⁻² (*PPFD*) and 10% of soil moisture content and it tends to decrease gradually in response to increment in *PPFD* reaching its minimum rate (0.428µg s⁻¹m⁻²) at 2000 µmol s⁻¹m⁻² (*PPFD*). While, the lowest transpiration rate (0.006µg s⁻¹m⁻²) was recorded at 1000µmol s⁻¹m⁻² and 1% *SMC*. Similar pattern was recorded in *S. italica* (Fig. 2b), the maximum transpiration rate (1.107µg s⁻¹m⁻²) was recorded at 9% *SMC* and 0µ mol s⁻¹m⁻² (*PPFD*). While, minimum transpiration rate (0.026µg s⁻¹m⁻²) was recorded at 3% (*SMC*) and 2000 µmol s⁻¹m⁻² (*PPFD*). Maximum transpiration rate (1.016µg s⁻¹m⁻²) of *S. occidentalis* (Fig. 2c) was recorded at 0 µmol s⁻¹m⁻² (*PPFD*) and 9% (*SMC*) and minimum of 0.115µg s⁻¹m⁻²) at 3% (*SMC*) and 2000 µmol s⁻¹m⁻² (*PPFD*). Lowest transpiration rate was recorded in *S. didymbotyra* (Fig. 2d), where, maximum transpiration rate (0.949 µg s⁻¹m⁻²) was recorded at 10%

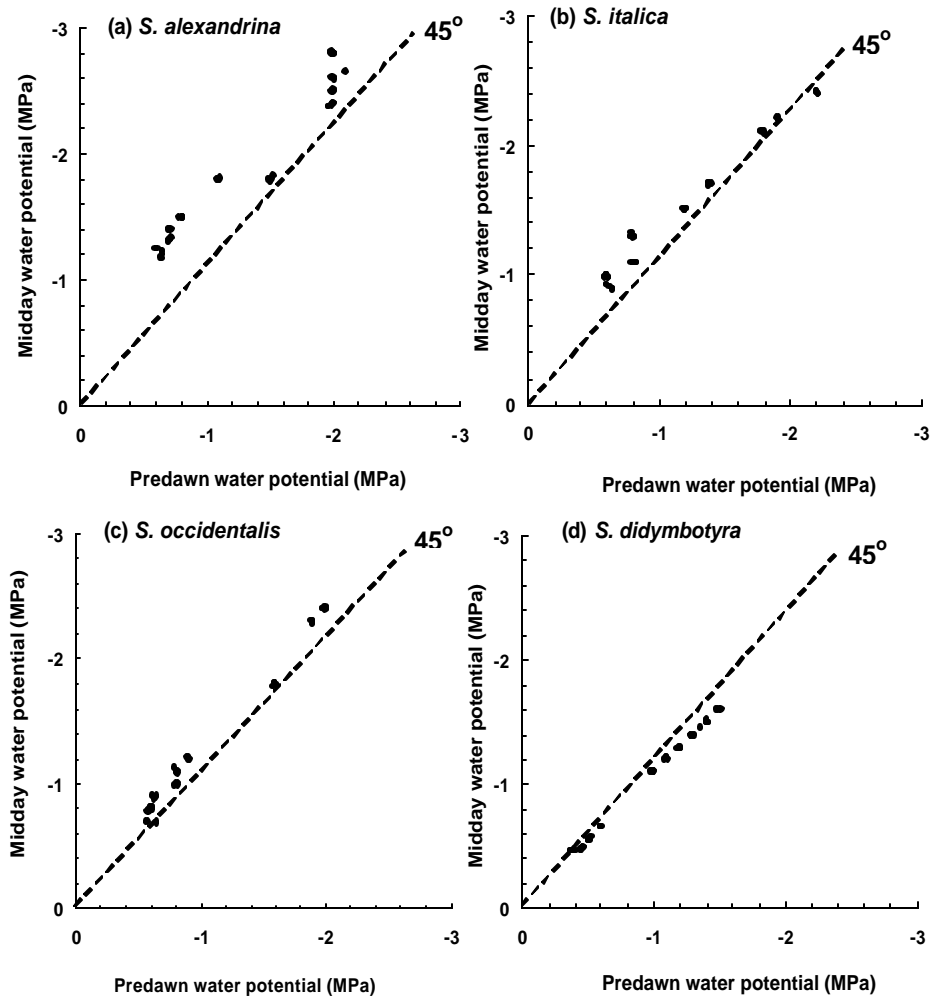


Fig. 1: Midday and predawn values of leaf water potential of (a) *S. alexandrina*, (b) *S. italica*, (c) *S. occidentalis* and (d) *S. didymbotyra* (the 45° bisecting line indicates the equal boundary between midday and predawn values)

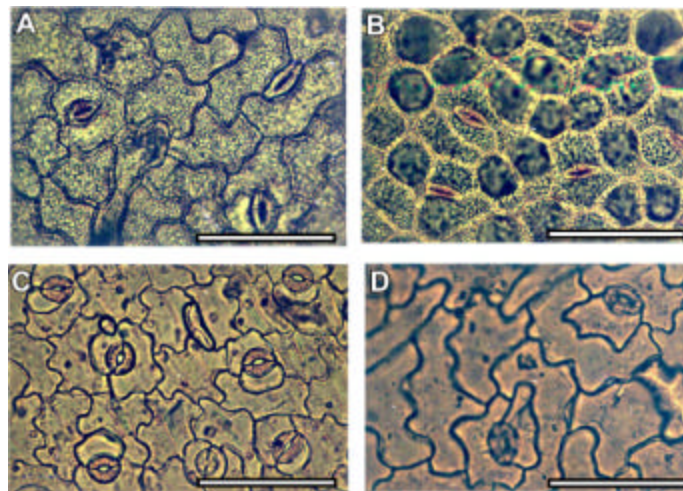


Plate 1: Stomatal frequency on abaxial leaf surface of (A) *S. alexandrina*, (B) *S. italica*, (C) *S. occidentalis* and (D) *S. didymbotyra* (white bar length = 100 μm)

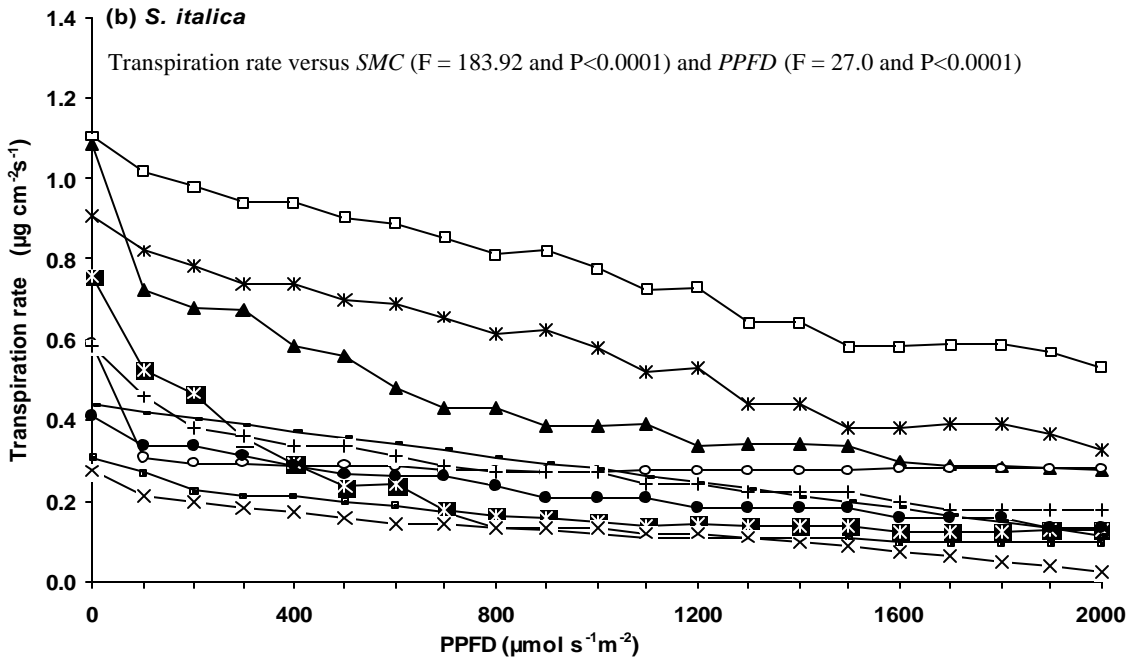
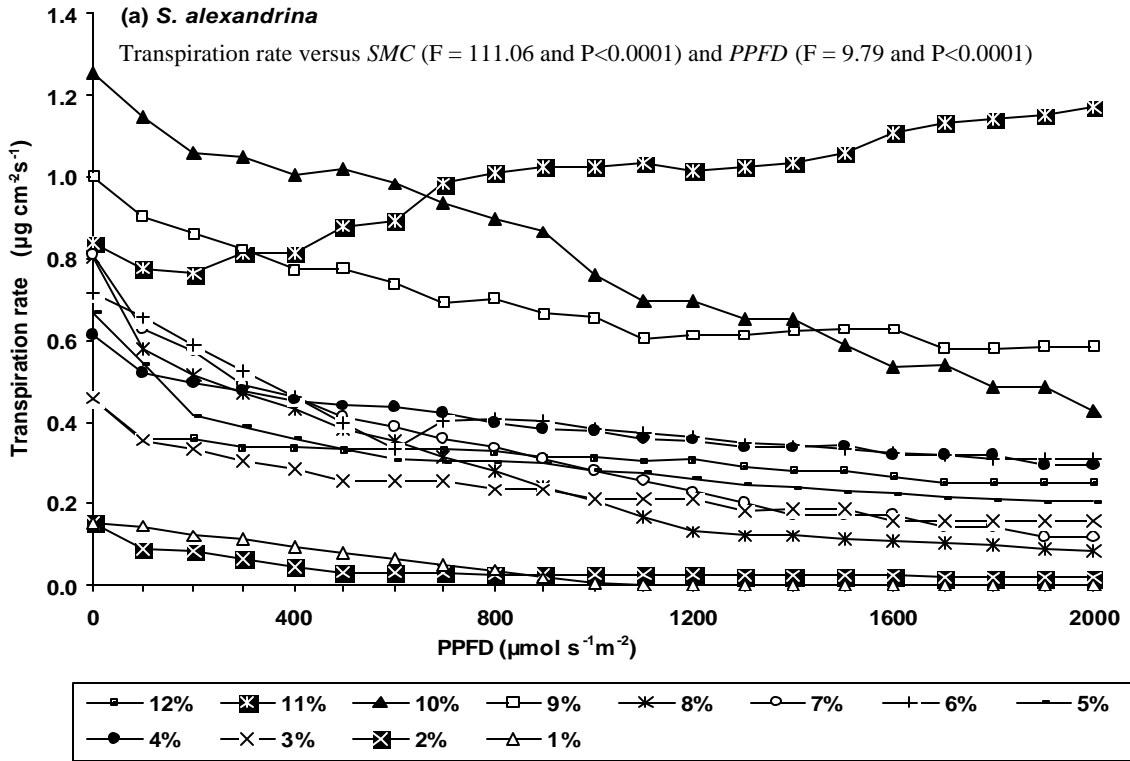


Fig. 2: Continued

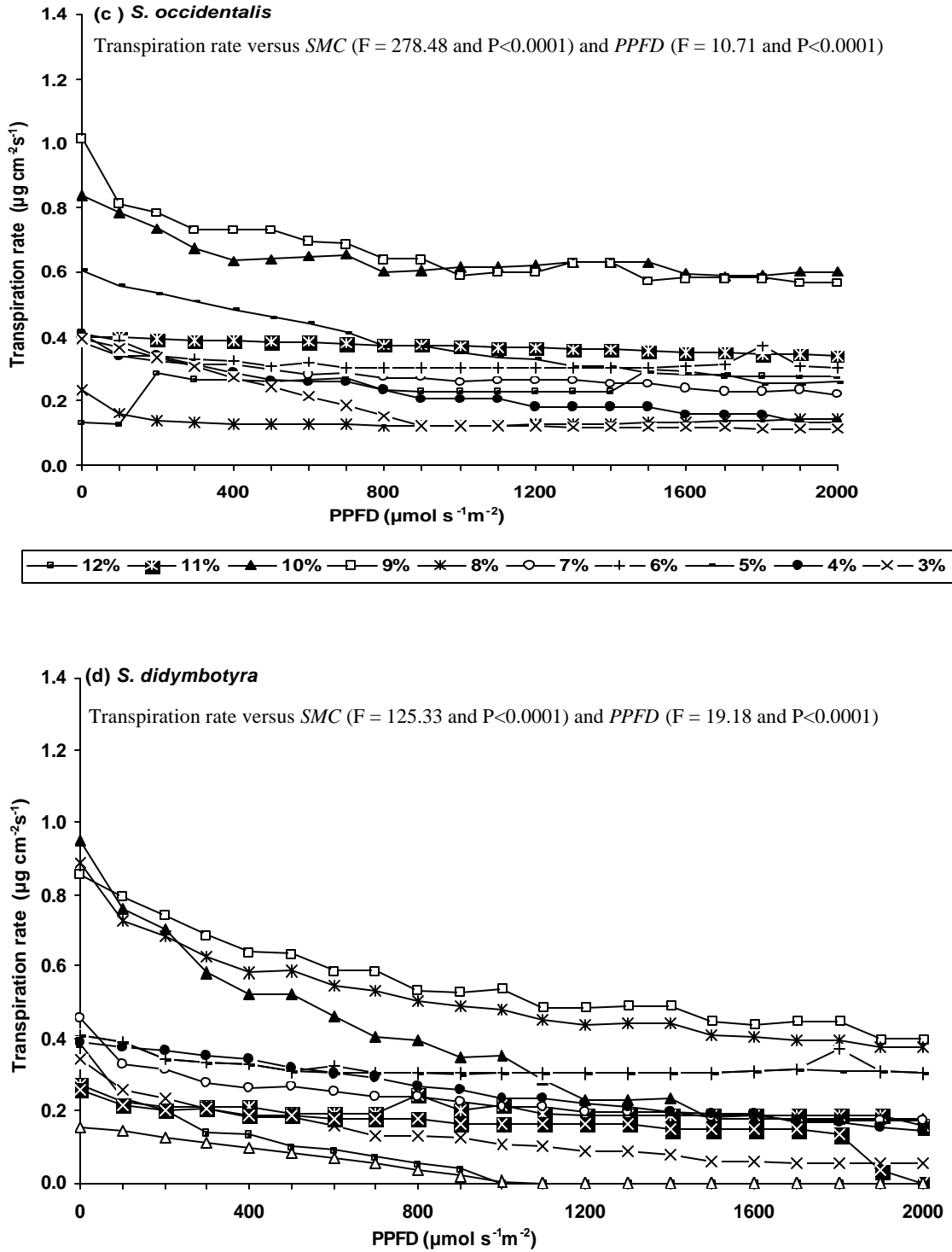


Fig. 2: Transpiration rate ($\mu\text{g cm}^{-2}\text{s}^{-1}$) of (a) *S. alexandrina*, (b) *S. italica*, (c) *S. occidentalis* and (d) *S. didymbotrya* under different soil moisture contents (% by weight) and at different photosynthetic photon flux densities (PPFD)

(SMC) and $0\ \mu\text{mol s}^{-1}\text{m}^{-2}$ (PPFD) and minimum of $0.034\ \mu\text{g s}^{-1}\text{m}^{-2}$ at $1900\ \mu\text{mol s}^{-1}\text{m}^{-2}$ and 2% (SMC). The single case for increment in transpiration rate was recorded in *S. alexandrina* (Fig. 2a) in response to increments in PPFD at 11% (SMC).

Plate 1A-D showed that the stomatal frequency was ranged from $87\pm 2\text{mm}^{-2}$ in *S. alexandrina* (Plate 1A) to $126\pm 1\text{mm}^{-2}$ in *S. italica* (Plate 1B), while, stomatal pore length (*l*) was almost the same ($20\pm 0.2\ \mu$) in both xerophytes species. On the other hand, in the two mesophytic *Senna* species (*S. occidentalis* and *S. didymbotyra*) stomatal frequencies were 148 ± 3 and $44\pm 1\text{mm}^{-2}$ respectively. Otherwise, stomatal pore length (*l*) was $10\pm 0.5\ \mu$ in both species, which gave more circular shape to the stomatal pore. Both *S. alexandrina* and *S. italica*, exhibited lower stomatal diffusion resistant (r_s) that ranged from 30 to 29sm^{-1} , respectively, which lead to higher stomatal conductance, transpiration rate and stomatal control efficiency. Otherwise, higher stomatal diffusion resistance was observed in *S. occidentalis* (50sm^{-1}) and in *S. didymbotyra* (120sm^{-1}), which lead to relatively low stomatal conductance, transpiration rate and water use efficiency.

DISCUSSION

Imposed water deficit (short-term) have different magnitude effect on *Senna* species. Mainly *S. alexandrina* showed high tolerance to the gradual drought treatment. The population of *S. alexandrina* in Southern Egyptian Desert can survive long rainless periods and can obtain available water from large soil volume by mean of extensive root system [29]. *S. alexandrina* as an evergreen sub shrub had maintained low midday leaf water potentials during whole range of soil moisture content. Savanna evergreen species growing in Africa had low midday leaf water potential and higher transpiration rate [30, 31]. In general, mean predawn water potential of all *Senna* species were higher than mean midday values as a result of increased internal resistance to sap flow while, larger negative values of leaf water potentials were observed at midday as the leaves in high demand for more water to maintain turgidity [17]. Some desert plants increasing transpiration and leaf water potentials rate in response to drought which considered as a mechanisms to withstand conditions of low water availability, as diurnal range between predawn and associated midday water potential tends to increase in response to gradual soil moisture depletion [19], which of high consistence with previous study on *Solenostemma*

arghel [18]. *S. alexandrina* maintained larger leaf water potential diurnal range in comparison with other of studies *Senna* species, which indicates its drought-adapted capability [32]. *S. italica* and *S. alexandrina* were recognized for their high germination percentage and fast radical growth at high temperature in comparison with *S. occidentalis* and *S. didymbotyra*. Also, the study on genus *Senna* in Egypt [10], based on variation in protein free amino acid and PAPD marker concluded that xerophytic *Senna* species (*S. alexandrina* and *S. italica*) exhibited higher content of amino acids and protein in comparison with mesophytic *Senna* species which of great importance in drought resistance.

In the current study, although *S. alexandrina* gave the highest transpiration rate but generally all *Senna* species tends to lower their transpiration rate in response to water deficit as a result of stomatal closure to tolerate low water potential [33]. Stomatal sensitivity to leaf water status leads to stomatal closure in response to decreased leaf water potential [34, 35]. Many authors agree with the fact that stomatal control efficiency and transpiration are positively correlated to hydraulic conductance of the soil-root-leaf pathway [36, 37]. Both soil water deficit and high radiation gave similar accumulative effect as they lowering leaf water potential and transpiration [38] and enhance plant tissue dehydration [39, 40]. Doubled stomatal pore length in xerophytic *Senna* species in comparison with mesophytic *Senna* species create a high capability in stomatal closure efficiency increase to soil water deficit and high irradiance as larger length of stomatal pore gave higher stomatal diffusion resistance [41]. On the other hand, circular stomatal pore and high stomatal frequency lead to lower stomatal diffusion resistance to water loss under the condition of high soil water deficit in mesophytic *Senna* species [11, 42]. Both *S. alexandrina* and *S. italica*, exhibited lower stomatal diffusion resistant which lead to higher stomatal conductance. Transpiration rate and stomatal control efficiency in comparison with higher stomatal diffusion resistance was observed in *S. occidentalis* and in *S. didymbotyra* which lead to low stomatal conductance, transpiration rate and water use efficiency [11]. The transpiration rate recovered before leaf water potential and leaf turgor potential during recovery between midday and predawn during drought stress which permits the plants to control water losses via transpiration to attain full turgor during this period [43].

In conclusion, plant water status of the studied *Senna* species revealed their responses to drought and optimum growth condition at different soil water

requirement through the knowledge of leaf water potential diurnal range as tool to assess the effect of water stress on the plants. In turn this can help in reservation and restoration of these species in their natural habitat as they consider as suitable indigenous candidates for land reclamation in such degraded areas. Further studies could be considered of great importance to comparative research about water potential of indigenous species in hyper arid environments.

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