

Tolerance Evaluation of *Hyoscyamus muticus* L. Seedlings to Radiative Heat Load and Gradual Soil Moisture Depletion

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Abstract: Transpiration rate, stomatal conductance and leaf temperature of three-months-old *Hyoscyamus muticus* seedlings were measured under different photosynthetic photon flux densities and gradual soil moisture depletion. Lowest transpiration rate ($0.57 \mu\text{gm m}^{-2}\text{s}^{-1}$) and stomatal conductance ($0.01 \mu\text{mol s}^{-2}\text{m}^{-2}$) were recorded at low level of soil moisture content (4%). While, leaf temperature and transpiration rate exhibited negative linear relationship under water deficit. Air-leaf temperature differential showed its maxima at high level of soil moisture content and PPFD. High leaf boundary layer conductance (0.115ms^{-1}) estimated from heat transfer properties revealed the great ability of *Hyoscyamus* leaves to withstand the high PPFD and drought stress. The main adaptive mechanisms for this ability are: lowering leaf temperature to minimize the effect of radiative heat load by mean of high boundary layer conductance to heat transfer, which provide resistance to water diffusion, easier heat convection from leaf to surrounding air and transpiration control.

Key words: Transpiration rate • stomatal conductance • boundary layer conductance • leaf temperature

INTRODUCTION

Hyoscyamus muticus L. is a Stout succulent perennial herb or shrub, over 1m, glabrous or pubescent; stem long, much branched in the upper part; lower leaves 8-12x4-9 cm, ovate to rectangular usually with several coarse teeth; petiole to 13cm, thick; upper leaves narrower, lanceolate, often entire; inflorescences elongating to 30cm or more [1]. It is known as Egyptian Henbane, which is widely grown in Egypt in different localities. The plant is fairly common and occurs in desert in patches occupying depression in sandy areas which receive run off water. However, it is not sufficient for industrialization [2]. It was the dominant plant on the shores of Lake Nasser, Southern Egyptian desert in the periods of high water level [3]. There is a large number of publications concentrated on its botanical ecological characteristics [4-8]. Many researchers contributed to isolation and identification of its alkaloidal and other contents [9-15]. Different tissue culture techniques were used to optimize *Hyoscyamus muticus* chemical constituents [16-24]. Vegetation arid zones is very poor, it is facing a complex of climatic and edaphic factors which represent unfavorable conditions for germination and life cycle of desert plants [5].

High irradiance and lack of moisture for prolong periods create a challenge for growth and establishment of desert plants [25]. Maximum diurnal seasonal averages of PPFD ($2400\text{-}2000 \mu\text{mol s}^{-2}\text{m}^{-2}$) and air temperature (43°C) were recorded in summer and around midday (1200-1600h) in the Southern Egyptian Desert [26]. In arid conditions, revealing the adaptation mechanisms of indigenous species is greatly important to understand their ecological success, growth conditions and suitability in restoration efforts [27, 28].

The survival of desert plants depend on their capacity to maintain a favorable balance between water uptake and water loss under conditions of severe climatic and atmospheric drought [29]. In desert plants an important physiological response of plants to soil drying is a decrease in leaf conductance to water [30]. Plants respond to drought by closing their stomata, reducing leaf transpiration and prevent development of excessive water deficits in their tissues [31].

One of the main concepts in adaptation mechanisms of desert plant is to withstand severe environmental condition is the energy exchange between leaves and interact with their physical environment through two main processes: 1. energy change by radiation absorption, heat transfer and reradiation and 2. mass exchange by

transpiration and photosynthesis [32]. Leaf temperature, which affects many physiological and ecological processes depend on the extent of radiative and diffusive coupling between leaf and the microclimate which determines the energy balance of leaf [33]. Diffusive and connective coupling of leaf depends on both stomatal and boundary conductance and their ratio which affect leaf conductance to water vapor [34-35].

The aim of the present research was to reveal the adaptive mechanisms of *Hyoscyamus* to withstand high irradiation and gradual soil moisture depletion stresses by measuring transpiration rate, stomatal and boundary layer conductances to determine the extent of *Hyoscyamus* leaves coupling to their physical environment under drought stress.

MATERIALS AND METHODS

Seeds of *Hyoscyamus* were collected from Southwestern Egyptian desert in October 2006. Seeds were soaked in water for 24 hours and rinsed thoroughly with water for few times [2], then sown directly into plastic pots of 30cm in diameter and 20cm deep with 1.5mm-holes at the bottom. Soil used in the experiment was clay:sand (1:3) and was kept moist above field capacity. Soil moisture characteristics and water release curve was obtained using the filter paper method [36, 37].

Two to three seeds were planted in each pot, seeds were covered with thin layer of soil (1-2 cm), pots were kept in controlled environmental conditions (temperature $25\pm 2^\circ\text{C}$, photoperiod of 18:6 L:D cycle at PPFD ranged from $400\text{-}700\mu\text{mol s}^{-1}\text{m}^{-2}$, as seed germination of *Hyoscyamus* was proved to stimulated by light [38].

Germination percentage (60%) occurred within 5-10 days, after establishment of seedlings, they were thinned to one plant in each pot. Pots were watered with tap water with no addition of nutrients, watering regime was maintained at 10% (1% above field capacity). Homogenous 8-weeks-old plants with maximum shoot length (23-25cm) were used in this experiment. The experimental design was completely randomized.

Gradual soil moisture depletion (short-term), soil moisture was adjusted to 1% above field capacity (10%) and then allowed to gradual dryness to reach 4% (wilting point based on soil water release curve), soil moisture was monitored gravimetrically.

Three sets of six plants each were selected to measure of transpiration rate, stomatal conductance and leaf temperature of fully developed and expanded leaf

(six measurements replicates were recorded at different soil moisture contents (10, 8, 6 and 4%) and PPFD ranged from 0 to $2400\mu\text{mol s}^{-1}\text{m}^{-2}$ using Steady State Porometer (LI-COR, NE, USA), equipped with broad leaf circular aperture cap (2cm^2). Light source was (6V-30W) tungsten lamp light intensity varied by power supply (Model Olympus, TGHM, Japan).

Boundary layer conductance (g_{aH}) of *Hyoscyamus* leaves obtained from heat transfer properties by using Steady State Porometer. Six measurements were taken on fully expanded leaves, evaporation was prevented by covering leaf surface with petroleum jelly to prevent transpiration [32]. The technique is to follow the time course of the change of 'leaf' temperature (T_l) when it is above air temperature (T_a). The value of (g_{aH}) was determined from the slop of a plot of time (s) against $\ln(\Delta T)$ from the following equation:

$$g_{\text{aH}} = \text{slop } \ln\Delta(T_l - T_a) / \ell c_p^* \rho_a^* / c_p \rho_a \quad (1)$$

Where ℓ , c_p^* , ρ_a^* , c_p and ρ_a are leaf thickness, leaf specific heat capacity, leaf density, 'air' specific heat capacity and dry air density at 20°C [39].

Significance of changes of transpiration rate, stomatal conductance and leaf temperature in response to gradual soil moisture depletion and PPFD differences were estimated from two way analysis of variance and Pearson Correlation were used to examine the correlation among factors and parameters.

All the above mentioned statistical data analysis were carried out using MINITAB statistical Program [40].

RESULTS

Transpiration rate of *Hyoscyamus* under imposed gradual soil moisture depletion and different PPFD at 25°C (Fig. 1) showed significant changes attributed to gradual soil moisture depletion, from Two-Way analysis of variance, $F = 40.62$ and $P < 0.0001$. Transpiration rate at highest level of soil moisture content (10%) tends to increase gradually in response to increment of PPFD ranged from $0.672400\mu\text{g cm}^{-2}\text{s}^{-2}$ at $0\mu\text{mol s}^{-1}\text{m}^{-2}$ (PPFD) to $0.98\mu\text{g cm}^{-2}\text{s}^{-1}$ at $1500\mu\text{mol s}^{-1}\text{m}^{-2}$. Maximum transpiration ($1.57\mu\text{g cm}^{-2}\text{s}^{-1}$) was observed at highest level of PPFD ($2400\mu\text{mol s}^{-1}\text{m}^{-2}$). Highest transpiration rate was observed when soil moisture was dropped to 8%. It ranged from $0.94\mu\text{g cm}^{-2}\text{s}^{-1}$ at $200\mu\text{mol s}^{-1}\text{m}^{-2}$ (PPFD) to maximum of $2.73\mu\text{g cm}^{-2}\text{s}^{-1}$ at $2400\mu\text{mol s}^{-1}\text{m}^{-2}$ (PPFD). On the other hand, as soil moisture decreased to 6% (2% above wilting point) transpiration rate showed

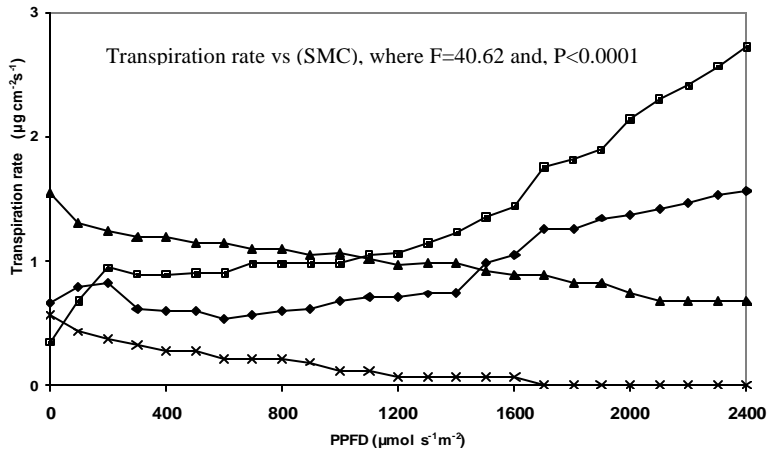


Fig. 1: Transpiration rate ($\mu\text{g cm}^{-2} \text{s}^{-1}$) of *Hyoscyamus muticus* under different PPFD densities ($\mu\text{mol s}^{-1} \text{m}^{-2}$) and gradual soil moisture depletion

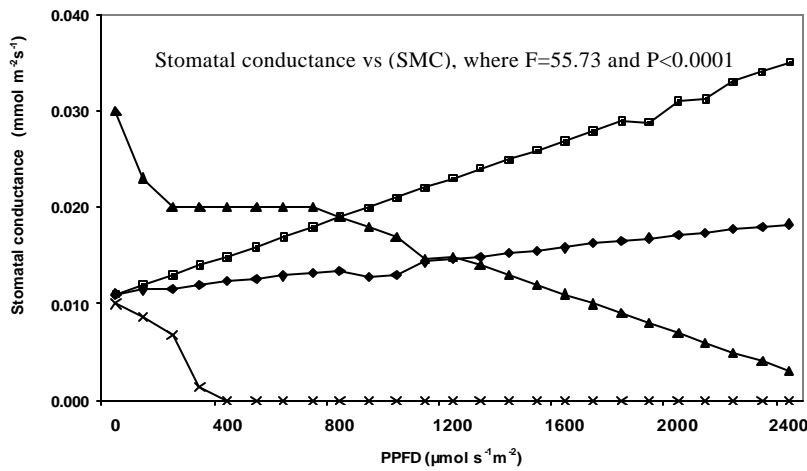


Fig. 2: Stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) of *Hyoscyamus muticus* under different PPFD densities ($\mu\text{mol s}^{-1} \text{m}^{-2}$) and gradual soil moisture depletion

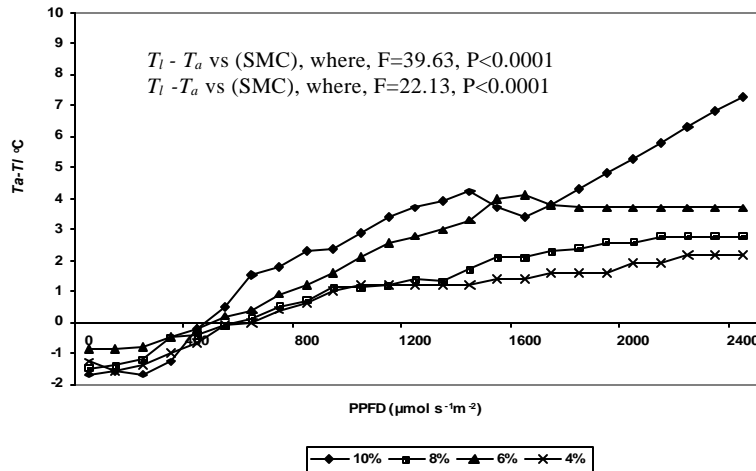


Fig. 3: Leaf-air temperature ($T_l - T_a$ °C) of *Hyoscyamus muticus* under different PPFD densities ($\mu\text{mol s}^{-1} \text{m}^{-2}$) and gradual soil moisture depletion

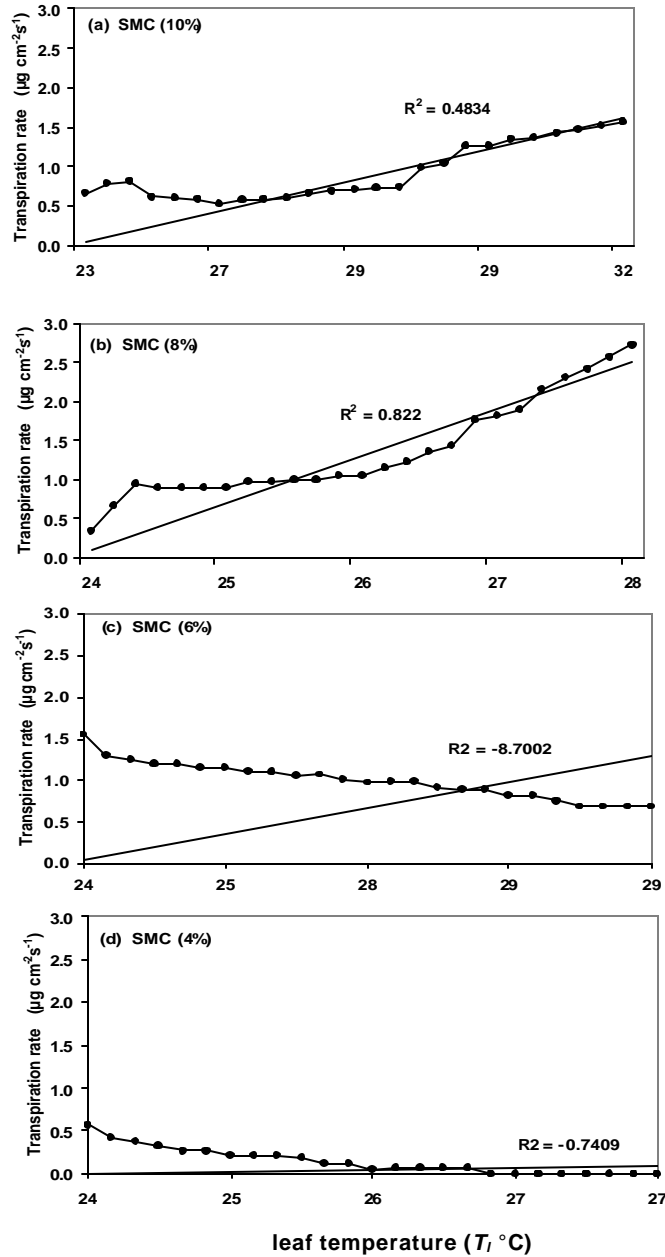


Fig. 4: Relationships between leaf temperature (T_l $^{\circ}\text{C}$) and transpiration rate ($\mu\text{g cm}^{-2} \text{s}^{-1}$) of *Hyoscyamus muticus* under different soil moisture content (a) 10%, (b) 8%, (c) 6% and (d) 4%

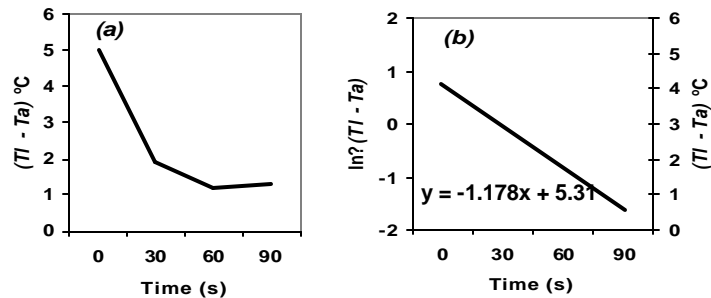


Fig. 5a-b: Estimation of Boundary layer conductance (g_{ah}) of *Hyoscyamus* leaf

different pattern. Maximum transpiration ($1.55\mu\text{g m}^{-2}\text{s}^{-1}$) at highest levels of PPFD range ($2100\text{-}2400\mu\text{mol s}^{-1}\text{m}^{-2}$). Lowest transpiration rate was observed at low level of soil moisture content (4%) which proved to be the wilting point from soil moisture release curve and visual biological wilting point. Transpiration rate ($0.57\mu\text{g m}^{-2}\text{s}^{-1}$) was observed at $0\mu\text{mol s}^{-1}\text{m}^{-2}$ and declined rapidly in response to increment of PPFD, reaching its lowest rate ($0\mu\text{g m}^{-2}\text{s}^{-1}$) at $1700\mu\text{mol s}^{-1}\text{m}^{-2}$ (PPFD).

Stomatal conductance of *Hyoscyamus* (Fig. 2) gave similar pattern to transpiration rate. From two-way analysis of variance significant changes in stomatal conductance was also attributed to gradual soil moisture depletion, where, $F=55.73$ and $P<0.0001$. At high level of soil moisture content (10%) stomatal conductance tends to increase gradually in response to increment in PPFD ranged from $0.01\text{mmol m}^{-2}\text{s}^{-1}$ at $0\mu\text{mol s}^{-1}\text{m}^{-2}$ (PPFD). As soil moisture content decreased (8%), highest stomatal conductance ($0.04\text{mmol m}^{-2}\text{s}^{-1}$) was recorded at highest level of PPFD ($2400\mu\text{mol s}^{-1}\text{m}^{-2}$). The imposed gradual soil moisture depletion affected the stomatal conductance, when soil moisture content reached 6%, highest stomatal conductance ($0.03\text{mmol m}^{-2}\text{s}^{-1}$) was recorded at $0\mu\text{mol m}^{-2}\text{s}^{-1}$ (PPFD), reaching its lowest values ($0.003\text{mmol m}^{-2}\text{s}^{-1}$) at highest level of PPFD ($2400\mu\text{mol s}^{-1}\text{m}^{-2}$). At lowest level of soil moisture content (4%), maximum stomatal conductance was $0.01\text{mmol m}^{-2}\text{s}^{-1}$ at $0\mu\text{mol s}^{-1}\text{m}^{-2}$ and decreased more rapidly to reach $0\text{mmol m}^{-2}\text{s}^{-1}$ at $400\mu\text{mol s}^{-1}\text{m}^{-2}$ (PPFD).

Concerning the air - leaf temperature ($T_l - T_a$ °C) highly significant differences (Fig. 3) were attributed to both gradual soil moisture depletion and PPFD, where, $F=39.63$, $P<0.0001$ and $F=22.13$; $P<0.0001$, respectively. In general, $T_l - T_a$ °C exhibited negative values at all soil moisture content levels at PPFD ranged from 0 to $500\mu\text{mol s}^{-1}\text{m}^{-2}$ and increased gradually to reach its maxima at highest PPFD level ($2400\mu\text{mol s}^{-1}\text{m}^{-2}$). The effect of leaf temperature (T_l °C) on transpiration rate (Fig. 4a-d) in response to gradual soil moisture depletion showed positive linear relationship under higher levels of soil moisture contents (10 and 8%) (Fig 4a and b), as the leaf temperature increased the transpiration increased. On the other hand, under low levels of soil moisture contents (6 and 4%) (Fig.4c and d), transpiration rate and T_l gave negative linear relationship.

From Pearson correlations coefficient, Photosynthetic Photon Flux Density (PPFD) was highly correlated with leaf temperature (T_l) and transpiration, while gradual soil moisture depletion gave high significant correlations with both transpiration rate and stomatal conductance and leaf

temperature (T_l). on the other hand, leaf temperature showed high significant correlation with transpiration rate and (PPFD) and high significant correlation was estimated between stomatal conductance and transpiration rate.

Boundary layer conductance (Fig. 5a-b) of *Hyoscyamus* leaf was calculated from heat transfer properties (Fig.5b) at 20°C , where $\text{slop} = -1.178$, leaf thickness (l) = 0.00155m , leaf density (ρ^*) = $3.467 \times 10^3\text{kg/m}^3 = 3467\text{kg/m}^3$, 'leaf' specific heat capacity (c_p^*) = $15239.56\text{Jkg}^{-1}\text{K}^{-1}$, 'air' specific heat capacity (c_p) = $1010.0\text{Jkg}^{-1}\text{K}^{-1}$, density of dry air (ρ_a) = 1.204Kg/m^3 , substituting values for ρ^* , c_p^* , l , ρ_a and c_p in Equation 1 gives:

$$g_{\text{air}} = 1.178 \times 0.00155 \times 15240 \times 3467 / 1010 \times 1.204 = 0.115 \text{ms}^{-1}.$$

DISCUSSION

Gradual soil moisture depletion has an adverse effect on both transpiration rate and stomatal conductance. *Hyoscyamus* is a sensitive plant to water as it rows more rapidly with irrigation [4]. One the most important physiological response of plant to soil drying is a decrease in leaf conductance to water to maintain to some extent tissue turgor [41]. Stomatal conductance of xerophytes is low in comparison with mesophytes [42]. Low leaf - air temperature ($T_l - T_a$) of *Hyoscyamus* under the effect of both drought and high irradiance, which lower T_l and in turn vapor pressure inside the leaf and tends to reduce water loss and conserve moisture as well as tendency to increase the ratio of photosynthesis [32]. This also, confirm the highly significant correlation among leaf temperature, transpiration and PPFD. Many authors were highlighted the morphological and anatomical characteristics of xerophytes which acting as adaptive mechanisms to withstand radiative heat load on leaf [43- 46].

The leaf-air temperature differential is related to leaf conductance and can be used as stress index [32]. Also, he found that, in *Calotropis procera* which grow in extreme arid conditions regions when water is available, the most productive strategy is to maximize latent heat loss by mean of low leaf conductance and minimize any heat gain from air by having large leaves with large boundary layer conductance, which of great similarity to *Hyoscyamus* adaptive mechanisms to avoid high irradiance heat and drought stresses [47].

Some morphological characteristics of *Hyoscyamus* could also play an important role in lowering leaf temperature such as leaf water content of its fleshy leaves

which is similar to *Encelia species* where leaf water content help in minimize leaf temperature in summer [48]. Another morphological character is alteration of *Hyoscyamus* leaf angle from horizontal to 70° could be expected to lower leaf temperature. Similar result where found in *Atriplex hymenelytra* which have lower leaf temperature by 2-3°C [49]. At low range of PPFD (0-300 $\mu\text{mol s}^{-1}\text{m}^{-2}$), T_l tends to be lower than T_a which prove the ability of *Hyoscyamus* to keep their leaves temperature lower than air temperature.

In conclusion, *Hyoscyamus* seedlings have an efficient energy budget by balancing energy absorbed to loss through the ability to lower leaf temperature which in turn decrease leaf absorption percentage and leaf conductance and transpiration. Decrease leaf temperature mean increase reradiation. Those three parameters known as leaf coupling factors [32].

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