Drought stress influences leaf water content, photosynthesis, and water-use efficiency of *Hibiscus rosa-sinensis* at three potassium concentrations

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Abstract

The influence of drought stress (DS) upon whole-plant water content, water relations, photosynthesis, and water-use efficiency of *Hibiscus rosa-sinensis* cv. Leprechaun (*Hibiscus*) plants at three levels of potassium (K) nutritional status were determined after a 21-d gradually imposed DS treatment. Compared to K-deficient plants, adequate K supply improved the leaf water content (LWC) and leaf water relations of *Hibiscus* by decreasing the Ψ_π, and generally sustained rates of net photosynthesis (P_N) and transpiration (E), and stomatal conductance (g_s), both in DS and non-DS plants. In K-deficient *Hibiscus*, LWC, turgor potential (Ψ_P), and P_N, E, and g_s as well as instantaneous water-use efficiency, WUE (P_N/E) were consistently lower, compared to K-sufficient plants. Carbon isotope discrimination (Δ) was lower (*i.e.* long-term WUE was greatest) in DS than non-DS plants, but K had no effect on Δ during the 21-d drought treatment period under glasshouse conditions. However, the trend in the Δ value of DS plants suggests that Δ could be a useful index of the response of *Hibiscus* to DS under glasshouse growing conditions. Thus the incorporation of a properly controlled fertilization regime involving sufficient levels of K can improve the acclimation of P_N to low Ψ_{leaf}, increase P_N/E of *Hibiscus*, and may have potential benefit for other woody plants species.

Additional key words: carbon isotope discrimination; Chinese hibiscus; stomatal conductance, transpiration.

Introduction

In commercial nursery production systems frequent and severe drought stress (DS) of container grown plants can reduce crop quality, delay marketing, and consequently profitability. Despite numerous studies evaluating the effect of K nutrition on the water relations of crop plants, the range of tissue K at which plant water content and CO_2 assimilation is optimized in container grown woody plants under DS conditions has not been clearly defined. High osmotic potential in the stele of roots is essential for turgor-pressure-driven solute transport in the xylem and for the water balance of plants. Potassium is the main inorganic solute that plays a major role in these processes, as well as the extension and various movements of individual cells in certain tissues (Marschner 1995). Improvements in the water relations, water-use efficiency (WUE), and growth of *Salvia splendens* under drought conditions (Eakes *et al*. 1991), as well as increased leaf area and total tuber dry mass of potato (Cao and Tibbits 1991) have been attributed to adequate supply of K. In other studies, transpiration rate (E) in barley was reduced at higher tissue K (Andersen *et al.* 1992a,b). We reported that during drought treatment, tissue K content within the sufficiency range (15–30 g kg^{-1}; Mills and Jones 1996), increased growth, leaf K and micronutrient contents, as well as root survivability of *Hibiscus* plants (Egilla *et al.* 2001). Adequate K can enhance the total dry mass (DM) accumulation in alfalfa (Peoples and Koch 1979), while K starvation reduced plant dry mass (DM) of tomato (Del Amor and Marcelis 2004). This increased DM accumulation might be attributable to the lower sensitivity of K-sufficient plants to DS (Lindhauer 1985), which may be related to the role of K^+ in stomatal regulation (the major

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Abbreviations: DM = dry mass; DS = drought stressed, non-DS = non-drought stressed; E = transpiration rate; g_s = stomatal conductance; K_0 = 0 mM K, $K_{2.5}$ = 2.5 mM K, K_{10} = 10 mM K; LWC = leaf water content; P_N = net photosynthetic rate; WUE = water use efficiency; Δ = stable carbon isotope discrimination; Ψ_l = leaf water potential; Ψ_P = leaf turgor potential; Ψ_π = leaf osmotic potential.

mechanism controlling the water regime of higher plants), or the stimulating effect of the higher K^+ content in the stroma and correspondingly higher rates of photosynthesis (Marschner 1995).

In C_3 plants, stable carbon isotope discrimination (Δ) has been used to assess genotypic variation in WUE and physiological responses to environmental factors (Hubick *et al.* 1986, Martin and Thorstenson 1988, Johnson *et al.* 1990). The natural $^{13}C/^{12}C$ ratio of C_3 plant tissue is related quantitatively to the ratio of intercellular CO_2 to atmospheric CO_2 (C_i/C_a) contents, a parameter which reflects a balance between assimilation of CO_2 by photosynthetic activity and the supply of CO_2 through stomatal diffusion (Farquhar *et al.* 1989). The integration of current knowledge of fertilizer management and environmental factors, as they affect rates of physiological processes, can lead to better control of propagation practices for high quality and cost-efficient production of container-grown woody species. The objective of this study was to determine the effect of deficient and sufficient levels of K supply on tissue water content, CO_2 assimilation, and WUE of drought stressed *Hibiscus* in a container production system.

Materials and methods

Plants: *Hibiscus rosa-sinensis* L. cv. Leprechaun plants were grown from rooted stem cuttings in 100 % fine textured loamy sand under glasshouse, and drought and K treatments were imposed under the same day/night temperature, ambient humidity, and irradiance as previously described (Egilla *et al.* 2001). The K treatment involved fertilization every 2 d with Hoagland's nutrient solution (Arnon and Hoagland 1940) modified to supply K at 0 mM (K_0), 2.5 mM ($K_{2.5}$), and 10.0 mM (K_{10}) as K_2SO_4, under two irrigation regimes DS and non-DS. All plants received adequate irrigation until 54 d after transplanting (d 0 of drought treatment) at all three K levels.

Photosynthesis measurements: The net photosynthetic rate (P_N) of two leaf sub-samples (the third and fourth fully expanded leaves from the shoot apex) of three plants at each of the three K treatments within the two irrigation regimes were measured, using a *LI-6200* portable gas exchange system (*LI-COR*, Lincoln, NE, USA). All measurements were conducted at midday (11:30 to 12:30 h). The same leaves on which gas exchange measurement was performed were subsequently excised for Ψ_l measurement. Thus, to avoid plant stress that may be caused by continuous leaf removal, the gas exchange measurements were made from different plants within each treatment on the different d of measurement [d 0 (before drought-stress treatment was initiated), and on d 14 and 21 after the initiation of drought treatment].

Mean VPD in the leaf chamber was 1.22, 1.39, and 1.43 kPa, while average leaf temperature (T_l) was 33.10, 34.60, and 32.34 °C, and air temperature (T_a) was 32.71, 32.04, and 29.65 °C on d 0, 14, and 21, respectively. All gas exchange measurements were carried out under an artificial radiation source: *1000/BU-1000W Metal Halide* (USA) with PFF in excess of 1 000 µmol m^{-2} s^{-1}. The saturation irradiance of well-watered *Hibiscus* plants not deficient in mineral nutrients was 900 µmol m^{-2} s^{-1}. Plants were allowed to acclimate to the higher irradiance under the artificial radiation source for at least 30 min before all the measurements, which were conducted in a well-ventilated area of the glasshouse. Infra-red radiation was filtered by passing the light through a plexiglas filter containing non-circulating water 7.5 cm deep.

Plant water relations: Leaf water potential was measured with a portable pressure chamber (Scholander *et al.* 1965), using the techniques of Ritchie and Hinckley (1975). Leaves were enclosed in a polyethylene bag immediately after gas exchange measurements, excised, and rapidly transferred to a pressure chamber to avoid excessive water loss during Ψ_l determination (Turner 1988). Leaf disks (0.45 cm^2) were taken immediately after Ψ_l measurements from the same leaves, and frozen quickly in watertight vials using liquid nitrogen at –198 °C. Leaves were later transferred to *C52* sample chambers connected to a *WesCor PR-55* microvoltmeter (*WesCor*, Logan, UT, USA), and allowed to thaw at room temperature for the psychrometric determination of Ψ_π The Ψ_π of the leaf sap was measured after equilibration for 120 min in the *C52* sample chamber. Subsequently, pressure potential (Ψ_P) was estimated as the difference between Ψ_l and Ψ_π (Slavík 1974) from samples harvested on d 0, 14, and 21, respectively.

Leaf water content (LWC) was calculated as LWC = TM – DM; where TM and DM represent turgid mass and dry mass of the fresh leaf, respectively (Turner 1981).

Leaf carbon isotope discrimination (Δ): The two leaves (the third and fourth fully expanded leaf from the shoot apex) sampled for photosynthesis measurement between 11:30 to 12:30 h on d 0, 14, and 21 were also used for determination of Δ. Since these leaves were formed during the treatment period, their Δ value was more representative of CO_2 fixed during the 21-d drought treatment than leaves with an earlier ontogeny. The leaf sub-samples taken during gas exchange measurement from individual plants of every treatment were dried in a forced draft oven at 70 °C for 72 h. Dried leaf samples from individual plants were ground separately in a *Cyclone Sample Mill* (*U-D Corp.*, Boulder, CO, USA) to pass a 0.4 mm screen, combusted to CO_2 and analyzed for $\delta^{13}C$ as described by Boutton (1991). All $\delta^{13}C$ values were expressed relative to the international PDB standard in units of per mil [‰]. The $\delta^{13}C$ values were determined with an overall precision (machine error plus sample preparation error of ≤0.1 ‰±1 standard deviation. Carbon isotope discrimination values were calculated according to the equation:

$$\delta = [(\delta^{13}C_a - \delta^{13}C_p) \div (1 + \delta^{13}C_p/1\,000)],$$

where $\delta^{13}C_a$ is the $\delta^{13}C$ value of atmospheric CO_2 (–8 ‰, Mook *et al.* 1983), and $\delta^{13}C_p$ is the $\delta^{13}C$ value of the plant sample.

Experimental design consisted of a 2×3 factorial design with two irrigation regimes (DS and non-DS) and three K treatments: 0, 2.5, and 10.0 mM K. A completely randomized design (CRD) was employed, and all data was analyzed by performing ANOVA using the General Linear Model (GLM) procedure (*SAS Institute* 1999). Treatment effects were determined by using ANOVA and regression analysis, and orthogonal contrasts were used for comparison of treatment main effects.

Results and discussion

Plant water relations: Data for non-DS plants were similar to data on d 0, and are reported only where they are significantly different from that obtained on d 0. DS reduced Ψ_l at all K concentrations ($p<0.0001$), from d 0 to 21. Leaf water potential decreased to a minimum value of –1.61 MPa at K_{10}, while DS significantly lowered Ψ_π by –0.11, –0.36, and –0.19 MPa at K_0, $K_{2.5}$, and K_{10}, respectively ($p<0.0491$), from d 0 to 21, as well as leaf Ψ_P ($p\leq0.0001$), but neither K effect nor day×K interaction was significant for Ψ_l, Ψ_π, and Ψ_P. The trend for Ψ_P/Ψ_l was similar to that of Ψ_l and Ψ_P.

Since variations in plant water loss due to K-influenced plant size, leaf number, and leaf area differences were controlled by adding a percentage of the total daily water transpired back to individual plants during the DS period, the change in Ψ_l among the K treatments was uniform in DS plants (Table 1). Although, compared to K_0, the contribution of K^+ to turgor maintenance was not statistically significant under the conditions of this experiment (Table 1), Ψ_P values indicate that turgor pressure was slightly higher at $K_{2.5}$ and K_{10}. The additional increase in K^+ of the cell solute might have contributed to the higher LWC observed at $K_{2.5}$ and K_{10} compared to K_0 (Fig. 1*A,B*), both under DS and non-DS conditions. However, during the 21-d DS period, there was no statistical difference between $K_{2.5}$ and K_{10}, and percent LWC at K_0 remained constant but approximately 10 to 20 % lower than values at $K_{2.5}$ and K_{10} (Fig. 1*B*). This significantly lower LWC at K_0 compared to $K_{2.5}$ and K_{10} even in non-DS plants indicates that K supply enhanced the tissue water retention of *Hibiscus* during the 21-d DS period, and may have enhanced shoot growth both under non-DS and DS (Egilla *et al.* 2001). Potassium ions (K^+), charge-balanced by inorganic and organic anions, make a major contribution to the cell sap Ψ_π of most cultivated crops (Hsiao and Läuchli 1986). Increased osmotic adjustment and cellular Ψ_P in DS *Salvia splendens* was attributed to K nutritional status (Eakes *et al.* 1991), while high K application increased both Ψ_P and leaf cell size in *Phaseolus vulgaris* (Mengel and Arneke 1982).

Table 1. Effects of K and drought stress on midday leaf water relations of *Hibiscus rosa-sinensis* L. cv. Leprechaun during a 21-d drought stress period. Means of 2 leaves from 3 plants per K treatment ± S.E., $n = 6$. Ψ_l = leaf water potential, Ψ_π = leaf osmotic potential, Ψ_P = leaf pressure potential [MPa]. D_1 = day 0, D_3 = day 21; K_0 = 0.0 mM K, $K_{2.5}$ = 2.5 mM K, K_{10} = 10 mM K.

Time [d]	K supply	Ψ_l	$\Psi\pi$	Ψ_P	Ψ_P/Ψ_l
0	K_0	–0.62±0.08	–1.99±0.04	1.37±0.09	2.50±0.44
	$K_{2.5}$	–0.43±0.04	–1.87±0.09	1.44±0.10	3.65±0.63
	K_{10}	–0.48±0.05	–2.18±0.22	1.70±0.22	3.90±0.74
21	K_0	–1.50±0.04	–2.10±0.14	0.59±0.13	0.40±0.08
	$K_{2.5}$	–1.52±0.02	–2.23±0.08	0.71±0.09	0.47±0.06
	K_{10}	–1.61±0.04	–2.37±0.11	0.76±0.13	0.48±0.09
Source	df	Main Treatment Effect Prob > F			
Day	2	<0.0001	0.0491	<0.0001	<0.0001
K	2	NS	NS	NS	NS
Day × K	4	NS	NS	NS	NS
D_1K_0 *vs.* D_3K_0		<0.0001	NS	<0.0002	0.0287
$D_1K_{2.5}$ *vs.* $D_3K_{2.5}$		<0.0001	0.0584	<0.0032	<0.0028
D_1K_{10} *vs.* D_3K_{10}		<0.0001	NS	<0.0006	<0.0016

There was no statistically significant change in leaf TM/DM (a measure of water content on DM basis) among the K treatments during the 21-d DS period. However, the consistently greater value of leaf TM/DM at $K_{2.5}$ and K_{10} compared to K_0 in DS plants (Fig. 1*C*) before d 21, and at K_{10} on d 21 suggest that there was a greater K-induced increase in cell volume per unit mass of cell wall material produced at $K_{2.5}$ and K_{10}, thus contributing to the higher LWC observed in *Hibiscus*. The TM/DM ratio may give some indication of accumulation of solutes (Turner 1987).

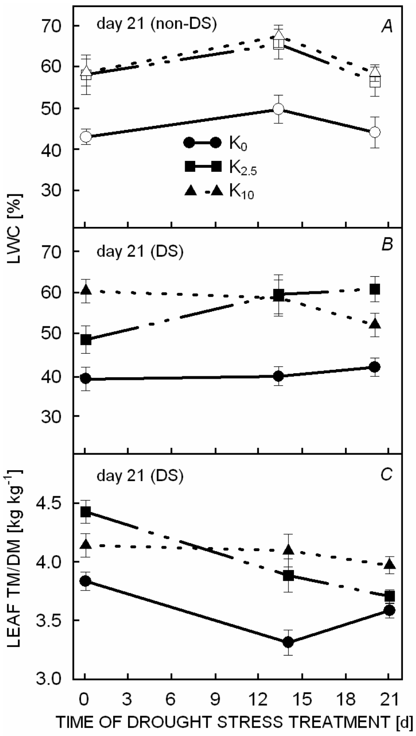

Fig. 1. Changes in leaf water content (LWC) [%] of (*A*) non-drought stressed (non-DS), (*B*) drought stressed (DS), and (*C*) leaf turgid mass (TM) to dry mass (DM) ratio at three K concentrations of *Hibiscus rosa-sinensis* over a 21-d treatment period.

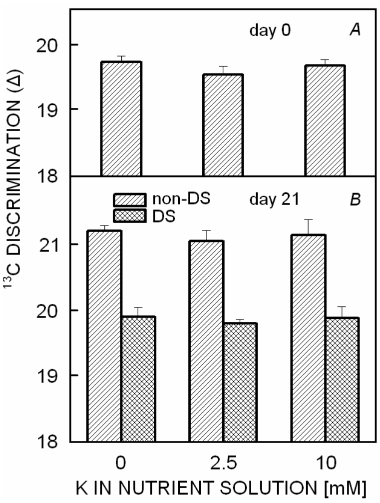

Fig. 2. Long-term water-use efficiency (Δ) of drought stressed (DS) and non-drought stressed (non-DS) *Hibiscus rosa-sinensis* at three K concentrations (*A*) before DS (d 0) and (*B*) after 21 d of growth under glasshouse conditions.

$\boldsymbol{P_N}$, $\boldsymbol{E}$, and $\boldsymbol{g_s}$: DS treatment caused a statistically significant reduction in P_N, E, and g_s ($p<0.0001$) from d 0 to 21 (Table 2). Before DS, P_N, E, g_s, and P_N/E were highest at $K_{2.5}$ and K_{10} than at K_0, suggesting that K supply at 2.5 mM K and 10 mM K normalized those gas exchange parameters, even under non-DS conditions. The higher P_N, E, g_s, and consequently P_N/E at $K_{2.5}$ corresponded to the higher Ψ_l and Ψ_π at this K supply and indicated that P_N, E, and g_s, were optimum at 2.5 mM K under non-DS conditions. The lower P_N at K_0 compared to $K_{2.5}$ and K_{10} before DS (Table 2) shows that sufficient contents of tissue K were required for optimum P_N in *Hibiscus*. Similarly, in hydroponically grown alfalfa P_N was reduced at lower (0 and 0.6 mM) compared to higher (4.8 mM) K (Peoples and Koch 1979). K can ameliorate the effect of drought stress in non-woody plant species and monocots (Gupta *et al.* 1989) and increase xylem sap flow in tomato (Del Amor and Marcelis 2004). The positive effect of K supply on P_N of *Hibiscus* was reduced by DS at –1.61 MPa Ψ_l. On d-21, P_N/E at K_0 was 37 and 43 % lower than values at $K_{2.5}$ and K_{10}, respectively. Increase in P_N/E due to K supply was statistically significant ($p\leq0.0216$), and P_N/E was significantly increased at K_{10} ($p\leq0.0477$) in DS plants, indicating that instantaneous WUE in *Hibiscus* can be improved at higher K supply. Duration of DS (day)×K interaction was significant for g_s and P_N/E (*i.e.* the effect of K on g_s and P_N/E varied with days of DS treatment). The lower P_N/E of K-deficient *Hibiscus* was apparently due to a greater reduction in P_N than E by stomatal closure. The rate of P_N *versus* E may differ under stress because, although water loss and CO_2 uptake are linked by the stomata, their pathways are different within the leaf, and their fluxes are therefore subjected to different constraints. Consequently, stomatal closure will not affect the two processes to the same degree (Cornish and Radin 1990).

Effects of K and DS on Δ: Leaf K content had no effect on long-term WUE (estimated by Δ) either on d 0 or 21 (Fig. 2*A,B*). Thus, the positive response of P_N/E to K is in contrast to the trend observed with Δ. Similarly, Syvertsen *et al.* (1997) found no correlation between the nitrogen nutritional status of citrus trees and Δ. Long-term WUE decreased (Δ increased) significantly ($p<0.01$) from d 0 to 21 regardless of K supply in non-DS, but not DS plants. This decrease in long-term WUE was ≈8.0 % at all K concentrations (Fig. 2*A,B*). However, DS treatment caused Δ to decrease (long-term WUE increased) compared to non-DS plants on d 21 by ≈7.0 %, at K_0, and ≈6.0 % at $K_{2.5}$ and K_{10}, respectively (Fig. 2*B*).

Despite the tendency for Δ in non-DS plants to increase (*i.e.* decrease in long-term WUE) under the greenhouse growing conditions in this experiment, DS plants maintained consistently lower Δ values compared to non-DS plants, regardless of K supply (Fig. 2*B*). This observation is consistent with the expected response of Δ to water deficit stress, and can be attributed to the stomatal

Table 2. Effects of K and drought stress on midday leaf water relations of *Hibiscus rosa-sinensis* L. cv. Leprechaun during a 21-d drought stress period. Means of 2 leaves from 3 plants per K treatment ± S.E., $n = 6$. P_N = net photosynthetic rate [µmol m^{-2} s^{-1}], E = transpiration rate [mmol m^{-2} s^{-1}], g_s = stomatal conductance [mol m^{-2} s^{-1}]. D_1 = day 0, D_3 = day 21; K_0 = 0.0 mM K, $K_{2.5}$ = 2.5 mM K, K_{10} = 10 mM K.

Time [d]	K supply	P_N	E	g_s	P_N/E
0	K_0	17.95±0.63	8.32±0.38	0.86±0.07	2.17±0.07
	$K_{2.5}$	22.69±0.47	9.23±0.35	1.17±0.07	2.47±0.05
	K_{10}	21.21±0.88	9.15±0.39	0.99±0.05	2.34±0.05
21	K_0	5.39±0.64	2.98±0.41	0.17±0.03	1.92±0.22
	$K_{2.5}$	8.27±1.28	3.13±0.39	0.18±0.01	2.64±0.23
	K_{10}	6.04±0.99	1.98±0.23	0.16±0.03	2.95±0.17
Source	df	Main Treatment Effect Prob > F			
Day	2	<0.0001	0.0001	0.0001	NS
K	2	0.0239	NS	0.0418	0.0216
Day × K	4	NS	NS	0.0492	0.0347
D_1K_0 *vs.* D_3K_0		<0.0001	<0.0001	<0.0001	<0.0001
$D_1K_{2.5}$ *vs.* $D_3K_{2.5}$		<0.0001	<0.0001	<0.0001	<0.0001
D_1K_{10} *vs.* D_3K_{10}		<0.0001	<0.0001	<0.0001	<0.0001

regulation of gas exchange during DS. Discrimination between ^{12}C and ^{13}C during photosynthesis is greatest when g_s is high. When stomata are partially or completely closed, nearly all of the CO_2 inside the leaf reacts with ribulose-bisphosphate (RuBP) carboxylase, and there is little fractionation of the isotope. It is for this reason that the isotopic ratio of plant tissue is directly related to the average g_s during its growth (Farquhar *et al.* 1989), providing a long-term index of WUE.

The decrease in long-term WUE observed in non-DS plants during the 21-d treatment could be attributed to the relatively more humid glasshouse environment, which would produce a canopy effect similar to those for large crop canopies on the field (Farquhar *et al.* 1988, 1989). Thus, the boundary layer that forms within the plant canopy under the glasshouse can make E from leaves within the canopy less dependent upon g_s than that observed for single leaves inside the leaf chamber during gas exchange measurements. Similar to the data obtained for *Hibiscus*, Condon *et al.* (1987) observed a trend in which the DM/E and Δ of well-watered wheat plants were positively correlated, and a tendency for Δ to increase with above ground DM and with g_s under glasshouse growing conditions. Despite the response of Δ observed under greenhouse growing conditions, data obtained for plant water retention, P_N, E, g_s, and P_N/E in this study indicate that the incorporation of a properly controlled fertilization regime involving sufficient levels of K can improve the acclimation of P_N to low Ψ_l, increase P_N/E of *Hibiscus*, and may be of potential benefit to other woody plant species.

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