EFFECTS OF DROUGHT STRESS ON GROWTH AND PHYSIOLOGICAL CHARACTERISTICS OF ROSELLE (*Hibiscus sabdariffa* L.)

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ABSTRACT

Drought is the major abiotic stress which causes major losses to agriculture production. This study was conducted to determine the effect of drought stress on the growth and physiological characteristics of *Hibiscus sabdariffa*. The drought stress treatments were 33, 67 and 100% of the field capacity. Each treatment was replicated five times in a randomized complete block design. According to the results, drought stress had significant effects on the growth and physiological traits of *H. sabdariffa*. As the drought stress increased, the plant height, leaf area, specific leaf area, fresh and dry weight of shoot and root, photosynthetic rate, stomatal conductance, intercellular CO_2 concentration and the transpiration rate decreased. The root-shoot ratio was significantly increased in stressed plants. The changes in number of branches per plant and chlorophyll content were, however, not significant. These findings suggested that *H. sabdariffa* might be able to tolerate drought stress by increasing the root-shoot ratio and stability of chlorophyll content.

Keywords: Leaf area, root-shoot ratio, leaf gas exchange, chlorophyll content

INTRODUCTION

Drought stress is one of the major abiotic plant stresses that will increase due to global climate change, rise in temperature and fluctuating weather conditions (Hirt and Shinozaki 2004, Barnabas et al. 2008, Nelson et al. 2010). It is a major limiting factor for agricultural production by impairing growth and productivity of plants (Weckwerth 2011). Drought stress is usually marked by stomatal closure, loss of water content, reduced leaf and water potential, decreased cell elongation and loss of turgor (Jaleel et al. 2009). The development of optimal leaf area is important for photosynthesis. Leaf growth is generally decreased under drought stress, which leads to the reduction in leaf area. Another common adverse effect of water stress on plants is the reduction in fresh and dry biomass production (Farooq et al. 2009). The intensity of drought mainly depends upon the distribution and occurrence of rainfall, evaporation and water retention capacity of the soil (Wery et al. 1994).

The performance of photosynthesis has become a most informative physiological indicator because of its extreme sensitivity to the environmental stress; thus photosynthetic measurements by gas exchange and chlorophyll analysis have been extensively used in the field of plant response to different environmental stresses (Massacci et al. 2008; Sun et al. 2013). The main cause of the decline in photosynthetic rate is the CO_2 deficiency under drought stress conditions (Meyer and Genty 1998). The stomatal closure results in the decrease of intracellular CO_2 levels, which leads to over-reduction of electron transport chain components in water limited conditions. Therefore, the electrons are transferred to O_2 at photosystem I generating reactive oxygen species (Mahajan and Tuteja 2005). *Hibiscus cannabinus* responded to water stress with reduced stomatal conductance, leaf rolling and reduction in water potential (Ogbonnaya et al. 1998).

H. sabdariffa belongs to Malvaceae family and is successfully grown in tropical and subtropical climates (Mohamed et al. 2012). The calyx is a commercially important part of the *H. sabdariffa* commonly used in making jam, juice, jelly, gelatine, syrup, wine, ice cream, pudding, cake and

flavouring (Tsai and Huang 2004; Duangmal et al. 2008; Hussein et al. 2010). The calyx is also rich in secondary metabolites, which have medicinal properties (Hirunpanich et al. 2005; Olaleye Tolulope 2007). The calyces have large quantities of organic acids (citric, malic, oxalic, and tartaric acids) and vitamin C (Peng-Kong et al. 2002). Two anthocyanins namely cyanidin-3-sambubioside (gossypicyanin) and delphinidin-3-sambubioside (hibiscin) are dominant in calyces. Two minor anthocyanins, delphinidin- 3-glucoside and cyanidin-3-glucoside are also present (Wong et al. 2002, Amor and Allaf 2009; Cisse et al. 2011). Keeping in view the importance of *H. sabdariffa* plant, the present study was aimed to determine the effect of drought stress on the growth and physiological characteristics of *H. sabdariffa*.

MATERIALS AND METHODS

Plant material and experimental site

The present study was conducted under a rain shelter at field 15, Faculty of Agriculture, Universiti Putra Malaysia (2.9917° N, 101.7163° E) from July to November, 2014. The seeds of *H. sabdariffa* (var. UMKL-1) were purchased from the Department of Agriculture, Serdang, Selangor, Malaysia.

Crop establishment, treatments and experimental design

The seeds were sown in trays filled with organic matter in the nursery. After 14 days of sowing, the uniform seedlings of about 15 to 20 cm in height were selected and transplanted into polybags (16×18 cm) containing a mixture of topsoil, organic matter and sand (2:1:1). Drought stress treatments selected were based on a different percentage of field capacity (FC) which was determined by the gravimetric method following the methodology described by Souza et al. (2000), which consisted of the difference between the wet soil after saturation and free drainage and the weight of the dry soil. Two drought levels, i.e. 33 and 67% along with 100% FC (control) were maintained throughout the experiment. Drought stress was imposed at one week after transplanting (WAT). The crop was supplied with fertilizers, NPK (15:15:15) 581mg per polybag at 14, 28 and 42 days after transplanting (DAT), NPK (12:12:17:12+TE) 1358mg per polybag at 56, 71, 84, 98 and 112 DAT. The experiment was carried out in a randomized complete block design with five replications and each replication consisted of six plants. The data were collected at three weeks interval (data not shown). The shown data was recorded at 18 weeks after treatments (WAT).

Determination of growth traits

The growth parameters such as plant height (cm), shoot fresh weight (g), shoot dry weight (g), root fresh weight (g), root dry weight (g), root-shoot ratio, total leaf area (cm^2) and specific leaf area (cm^2g^{-1}) were recorded. Plant height was measured from the base to the shoot tip of the plant using a measuring tape. The number of branches was determined by counting the primary branches. The plants were uprooted carefully at 18 WAT and separated into the shoot and root. Then the roots were washed with tap water and rinsed with distilled water in order to remove soil particles. Fresh and dry weights of the shoot and root were weighed with an electronic balance (Sartorius A and D FX200iWP, Germany). Later, the shoots and roots were oven dried at 60 ℃ for 72 h until constant weight was achieved. The root-shoot ratio was computed on dry weight basis. The total leaf area per plant (TLA) was measured with an automatic area meter (LI-3000, Li-Cor Inc., Nebraska, USA). Specific leaf area (leaf area per leaf dry matter) was calculated according to Li et al. (2011).

Determination of leaf gas exchange

Photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$), stomatal conductance (mol $m^{-2}s^{-1}$), intercellular CO_2 concentration (µmol CO_2 mol^{-1}) and transpiration rate (mmol $m^{-2}s^{-1}$) were determined by using a portable photosynthesis system (Li 6400, Li-Cor, USA). The measurements used optimal conditions set at 400 µmol mol^{-1} CO_2, 30°C cuvette temperature, and 60% relative humidity with air flow rate set

at 500 cm^3 min^{-1}. The measurements were done between 09:00 to 11:00 am using the fully expanded young leaf at 18 WAT.

Determination of chlorophyll content

Chlorophyll content (mg cm^{-2}) was determined according to the method of Coombs et al. (1985). Four leaf discs of 4 cm^2 were taken from the middle portion of young and fully developed leaf which were then transferred into a plastic vial containing 20 ml of 80% acetone. Immediately the vial was corked airtight and kept in the dark for 72 h at room temperature until all the pigments were extracted from the leaf discs. The absorbance values of the solution of each sample at 647 and 664 nm were measured using a spectrophotometer (**UV-3101PC** UV-VIS-NIR, Shimadzu, Japan). Chlorophyll *a*, chlorophyll *b* and total chlorophyll contents were calculated as follows:

Chlorophyll a content (mg cm^{-2} fresh leaf) = 13.19 (A_{664}) - 2.57 (A_{647})
Chlorophyll b content (mg cm^{-2} fresh leaf) = 22.1 (A_{647}) - 5.26 (A_{664})
Total Chlorophyll content (mg cm^{-2} fresh leaf) = 3.5 (chl *a* + chl *b*)/4
Where, A_{647} and A_{664} represent absorbance of the solution at 647 and 664 nm, respectively, while 13.19, 2.57, 22.1 and 5.26 are the absorption coefficients, 3.5 was total volume used in the analysis taken from the original solution (ml) and 4 was the total discs area (cm^2).

Statistical analysis

The collected data were subjected to analysis of variance and the entire means were evaluated by using Least Significant Difference (LSD) test at $p < 0.05$. All analyses were carried out using SAS statistical software. Correlation analysis by means of Pearson's Correlation Matrix was performed to establish the relationship between growth and physiological parameters.

RESULTS AND DISCUSSION

Effects of drought stress on growth traits

Results showing various growth traits are given in Table 1. Plant height was not significantly different at 67% FC and 100% FC. However, 33% FC significantly reduced plant height by 18.33% as compared to 100% FC (Control). These results are supported by Moeini Alishah et al. (2006) and Bahreininejad et al. (2013). The reduction in plant height under water stress may be attributed to the reduced turgor pressure and cell enlargement (Shao et al. 2008). The number of branches did not differ in stressed and control plants. Khalil and Abdel-Kader (2011) also reported that there was no effect on number of branches of *H. sabdariffa* which was due to different soil moisture levels. The maximum and minimum shoot fresh and dry weights were recorded in 100% and 33% FC respectively. At 33% FC, there were reduced shoot fresh by 16.53% and dry weight by 37.51% compared to control. The 33% FC level caused a decrease in the root fresh (29.58%) and dry weight (20.45 %) compared to control. Moreover, root-shoot ratio was higher in stressed plants than control plants. Alaei (2013) reported fresh and dry weights of root and shoot decreased while root-shoot ratio increased in *Dracocephalum moldavica* due to decreased amount of irrigation water. The increase in the root-shoot ratio under drought conditions is a mechanism to explore more soil volume in order to absorb water from deeper soil layer, which is not available for less developed roots (Matsui and Singh 2003). The highest TLA was found in the control plants, followed by the plants under 67% FC, while TLA of plants grown in 33% FC had the least leaf area, suggesting that severe drought stress caused the decreased leaf area. The reduced leaf area is part of adaptive mechanism in drought stress condition (Liu and Stutzel 2004). Similar variation in response to water stress was reported by Khalil and Abdel-Kader (2011). They confirmed the ability of water stress in reducing leaf area in *H. sabdariffa*. Specific leaf area (SLA), an indicator of leaf thickness, has often been observed to be declined under drought stress conditions (Marcelis et al. 1998; Monti et al. 2005). Decreases in SLA occur in response to drought stress as a result of a reduced transpiration leaf area, allowing increased resistance to drought conditions (Chaves et al. 2003). Results of the current study showed that SLA was decreased by an average value of 27.06% at 33% FC compared to 100% FC. Meanwhile, no

significant difference was observed at 67% and 100% FC. Xu and Zhou (2008) reported the reduction in the SLA of plants grown under stress environment was due to the limited availability of assimilates.

Table 1. Effects of drought stress on growth traits in *H. sabdariffa*

Drought stress treatments (% FC)	Plant height (cm)	Number of branches Plant^{-1}	Shoot Fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Root-shoot ratio	Total Leaf area (cm^2)	Specific leaf area (cm^{2}g^{-1})
100	110.20a	8.40a	269.51a	44.46a	61.83a	10.07a	0.23b	3477.51a	253.38a
67	104.20a	8.60a	240.91b	35.63b	56.20b	8.78b	0.25b	3151.19b	238.04a
33	90.00b	8.20a	224.97c	27.78c	43.54c	8.01c	0.29a	2054.00c	184.18b

Means for each treatment with same letter within column are not significantly different by LSD at 0.05 (n=5).

Effects of drought stress on chlorophyll content

Chlorophyll content is one of the main factors influencing the photosynthetic capacity. The present study found that chlorophyll content (*a*, *b*, *a+b*) was not affected under drought stress treatments as compared to the control plants (Table 2). The stable chlorophyll content during drought stress is a desirable trait viewed as one of the criteria to decide regarding tolerance (Sairam 1994; Long and Bernacchi 2003). Evans and Al-Hamdani (2015) reported that chlorophyll *a* and chlorophyll *b* in *H. sabdariffa* showed no significant difference at the various drought stress treatments.

Table 2. Effects of drought stress on chlorophyll a, chlorophyll b and total chlorophyll content in *H. sabdariffa.*

Drought stress treatments (% FC)	Chlorophyll content (mg cm^{-2})		
	Chl *a*	Chl *b*	Chl *a+b*
67	19.56a	19.96a	34.68a
33	19.92a	20.18a	35.08a
100	19.04a	19.83a	34.02a

Means for each treatment with same letter within column are not significantly different by LSD at 0.05 (n=5).

Effects of drought stress on leaf gas exchange parameters

Photosynthesis is one of the main physiological processes affected by drought stress. In this study, photosynthetic rate (A) was declined with increasing drought stress. The 33% and 67% FC plants induced reductions in A by 30 and 12.28% respectively, as compared to the control plants (Figure 1A). Several studies have shown that drought stress usually decreases photosynthetic rate (Fang et al. 2010; Evans and Al-Hamdani 2015; Yuan et al. 2016). In most plant species, water limitation leads to a decrease in photosynthetic rate as a result of stomatal closure (Lenzi et al. 2009). The first response of the plants is the closure of stomata under drought stress condition. The major role of stomata in plants includes the uptake of CO_2 for photosynthesis and controlling detrimental water loss through transpiration. Results showed that drought stress significantly affected stomatal conductance (gs). The stomatal conductance was lowest at 33% FC followed by 67% FC (Figure 1B). Decreased stomatal conductance is an indication of stomata closure to avoid further dehydration of leaf under drought stress (Farooq et al. 2009). Moreover, the intercellular CO_2 concentration (Ci) was also significantly higher in control plants compared to drought treated plants (Figure 1C). Transpiration is an important trait for measurement of drought tolerance and is widely influenced by environmental stress conditions. The transpiration rate (E) was significantly affected by drought stress in this study. E value was 5.06 mmol $m^{-2}s^{-1}$ for 100% FC plants while it declined to the minimum average value of 3.06 mmol $m^{-2}s^{-1}$ (39.52%) for 33% FC plants (Figure 1D). The decrease in transpiration rate suggested

water conservation by reduced water loss through stomata. These results are supported by Jones et al. (1985) and Stanton and Mickelbart (2014) who reported that stomatal closure led to reduced transpiration.

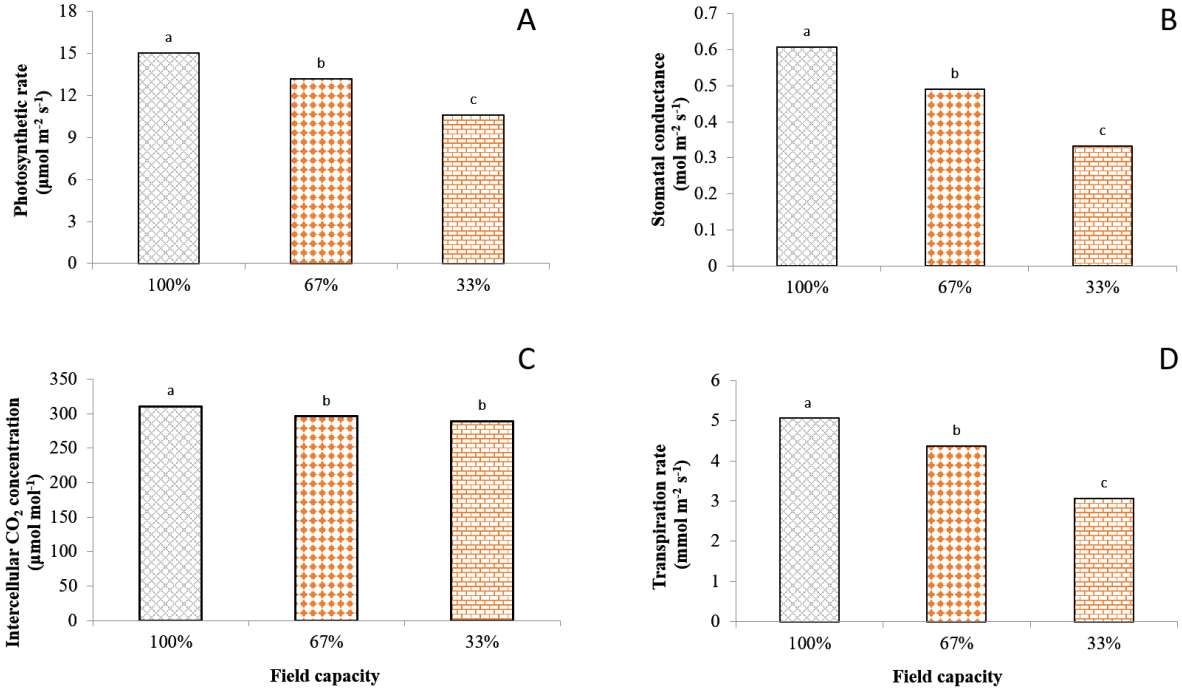

Figure 1. Effects of drought stress on photosynthetic rate (A), stomatal conductance (B), intercellular CO_2 concentration (C) and transpiration rate (D) in *H. sabdariffa*.

Correlation between growth and physiological parameters

The results of the correlation analysis showed that growth and physiological parameters under drought stress condition had significant correlation (Table 3). There were positive and significant correlations among plant height and TLA, SLA, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, root-shoot ratio, A, gs, Ci and E. These results suggested that an increase in physiological parameters could simultaneously increase growth traits under drought stress conditions. Jatoi et al. (2011) reported that stomatal conductance was significantly correlated with leaf area in water deficit condition. A significant strong and positive correlation between photosynthetic rate and transpiration was found in this study. Tiwari et al. (2013) found a strong and positive correlation between photosynthesis rate and transpiration rate (r= 0.999) in water stressed plants.

CONCLUSION

Our findings showed that drought stress negatively affects the growth and physiological parameters of *H. sabdariffa*. Drought stress reduced TLA, SLA, fresh and dry weights of shoot and root but increased root-shoot ratio. The reduction in leaf area was considered as an avoidance mechanism to minimise the evaporative surface area. Drought stress reduced E as a response to the reduction in gs. The reduction in gs also reduced A. This study also showed that chlorophyll content was not affected under drought stress. Drought tolerance of *H. sabdariffa* was found to be associated with a higher reduction in TLA and SLA, a better adjustment of A and E, increasing root-shoot ratio and stability of chlorophyll.

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Table 2. Correlation coefficients for growth and physiological traits of *H. sabdariffa.*

	PH	TLA	SLA	SFW	SDW	RFW	RDW	RSR	A	gs	Ci	E
PH	--											
TLA	0.78**	--										
SLA	0.65**	0.96**	--									
SFW	0.67**	0.82**	0.77**	--								
SDW	0.77**	0.90**	0.86**	0.89**	--							
RFW	0.69**	0.86**	0.78**	0.76**	0.75**	--						
RDW	0.83**	0.83**	0.77**	0.84**	0.93**	0.75**	--					
RSR	0.69**	0.89**	0.85**	0.81**	0.94**	0.74**	0.77**	--				
A	0.87**	0.90**	0.79**	0.90**	0.90**	0.84**	0.87**	0.84**	--			
gs	0.83**	0.89**	0.80**	0.87**	0.85**	0.86**	0.87**	0.76**	0.92**	--		
Ci	0.59*	0.76**	0.69**	0.84**	0.79**	0.74**	0.75**	0.73**	0.79**	0.81**	--	
E	0.85**	0.93**	0.82**	0.88**	0.89**	0.88**	0.84**	0.88**	0.97**	0.92**	0.80**	--

PH, Plant height; TLA, Total leaf area; SLA, Specific leaf area; SFW, Shoot fresh weight; SDW, Shoot dry weight; RFW, Root fresh weight; RDW, Root dry weight; A, Photosynthesis rate; gs, Stomatal conductance; Ci, Intercellular CO_2 concentration; E, Transpiration rate.
*, $P < 0.05$; **, $P < 0.01$.

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