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Alteration in yield, gas exchange and chlorophyll synthesis of ramie to progressive drought stress

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Abstract

Water-deficit is an important factor limiting the fiber yield, globally. In order to evaluate the effects of water stress on ramie (*Boehmeria nivea* L.), a pot experiment was carried out in a rain-protected wire-house at Chongqing, China. Ramie plants were subjected to 4 varying watering regimes (well-watered, mild, moderate and severe drought stress) at flourishing stage for 10, 15 and 20 days. After imposition of the treatments, the alteration in growth, yield, gas exchange and chlorophyll contents were assessed. Results indicated that all stress treatments promoted a substantial decrease in net photosynthetic rate (A), stomatal conductance (g_s) and transpiration rate (E). However, intercellular CO_2 concentration (Ci) initially decreased under mild and moderate stress and increased in severe drought stress while intrinsic water use efûciency (WUEi) changed in opposite direction. The variation in gas exchange attributes indicated the decreased photosynthetic activity under mild and moderate stress pertaining to stomatal mechanisms while under severe drought due to non-stomatal mechanisms. In addition, decreased photosynthetic activity inhibited the growth of plants and led to severe decline in raw fiber yield. A continuous reduction in plant height, stem diameter, leaf area and plant biomass was observed as well as bast thickness and bast fresh weight. Nonetheless, progressive water stress led to a continuous loss of photosynthetic pigment concentrations. In summary, water stress decreased chlorophyll synthesis, gas exchange and growth and yield of ramie plants, but the extent of these variations was closely related with the intensity, severity and duration of the drought event and their interaction.

Key words: Water stress, gas exchange, pigments, yield, ramie.

Introduction

Drought is one of the most important factors contributing to crop yield loss in regions where plants are often exposed to periods of water deficit. Drought-induced loss in crop yield perhaps exceeds the loss from all other stresses, as both severity and duration of stress are critical [1]. Drought-induced losses depend upon drought intensity and duration, with more severe drought stress causing more serious effects on plant [2]. Water stress results in stomatal closure and reduced transpiration rates, a decrease in photosynthesis and growth inhibition [3-5]. Water limitation mainly limits photosynthesis through stomatal closure and through metabolic impairment [6]. Both stomatal and non-stomatal factors contribute to the effects of water stress on photosynthesis [7]. Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing powers [8]. The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation [9].

Ramie (*Boehmeria nivea* L.), or 'Chinagrass', is a perennial herbaceous plant of the Urticaceae family. It is mainly cultivated in China and other adjoining Asian countries, but availability of soil water influences fiber yield of ramie, and seasonal drought are common in regions where the crop is produced [10]. Early studies revealed that the drought tolerant ramie genotypes have more fine hairs, thicker leaf cuticles, and adapted root system, as well as higher relative water content and cell membrane stability under drought stress [10]. However, there are not any reports pertaining to effect of drought stress in gas exchange and chlorophyll contents on ramie. The present work is unique and novel to the best of our knowledge because it would explore alteration on photosynthetic characteristics and fiber yield of ramie subjected to varying watering regimes. In order to improve the agricultural productivity it is imperative to ensure higher fiber yields against drought stress. Therefore, the present research was undertaken to explore alteration of hybrid ramie in growth, yield, gas exchange traits and chlorophyll contents subjected to drought stress.

Materials and Methods

Experimental details: Pot experiment was carried out in rain-protected wire-house at College of Agronomy and Biotechnology, Southwest University, Chongqing, China, during summer 2010. The experimental area lies between latitude 29°49'32"N, longitude 106°26'02"E and altitude 220 m. The hybrid seedlings (10 cm high) of ramie were provided by Dazhou Institute of Agricultural Sciences, Sichuan, China. On June 13, 2010 one young hybrid seedling (from the nursery beds in the fields) was transplanted into each plastic pot (32 cm in diameter, 28 cm in depth) filled with typical purple soil containing total nitrogen 1.38 g kg^{-1}, total phosphorus 0.70 g kg^{-1}, total potassium 20.90 g kg^{-1}, alkali-hydro nitrogen content 230.29 mg kg^{-1}, available phosphorus 87.66 mg kg^{-1}, available potassium 422.50 mg kg^{-1}, organic matter 9.00 g kg^{-1} and pH 6.73. Total weight of each pot was 14 kg after filling with soil and organic matter. Ramie plants were grown with normal water supply till the start of flourishing stage (about 45 days after transplanting), then the plastic pots were shifted to wire-house and arranged in a completely randomized design (CRD) with 20 pots per treatment and three replications of each experimental unit. All pots were grouped in four drought treatments as follows: (1) 75% soil field capacity (well-watered, CK), (2) 60% soil field capacity (mild drought stress), (3) 45% soil field capacity (moderate drought stress) and (4) 30% soil field capacity (severe drought stress).

The pots were weighed every evening to maintain the desired soil water levels by adding appropriate volume of water. After 20 days of imposition of different watering regimes all plants were rewatered with normal water supply till harvesting.

Leaf gas exchange measurement: Leaf gas exchange attributes were determined after 18 d of drought treatment and the 6-7th leaf from top following the method of Liu [11] were measured using a portable open-system infrared gas exchange analyzer based photosynthesis system (LI-6400, LI-COR, Lincoln, NE, USA) during 9:00–11:00 am. Fifteen leaves were selected for each treatment with the following adjustments: molar flow of air per unit leaf area 499.71 mmol lol^{-1} m^{-2} s^{-1}, water vapour pressure into leaf chamber was 3.7 mbar, PAR at leaf surface was up to 1000 mol m^{-2} s^{-1}, temperature of leaf ranged from 36.78 to 38.95°C, ambient temperature was 37.64 to 39.54°C, ambient CO_2 concentration 381.92 mol mol^{-1} and relative humidity (RH) was 40.82%. Intrinsic water use efficiency (WUEi) was calculated as ratio of photosynthetic rate (A) to stomatal conductance (g_s).

Estimation of leaf pigments: The ramie hybrid plants were sampled (6-7th leaf from top) after 10, 15 and 20 d of drought treatment to assess the chlorophyll contents. Chlorophyll a (Chl a), chlorophyll b (Chl b) and chlorophyll a+b (Chl a+b) were determined following the method of Arnon [12]. Of leaves 100 mg was cut into small pieces and placed in 15 ml centrifuge tube, along with 10 ml of miscible liquids by 95.5% acetone and absolute ethyl alcohol in 1:1 ratio. Then it was kept at dark place for 48 hours. The Chl a, Chl b and Chl a+b concentrations were measured using a UV-visible spectrophotometer and absorbance was measured at 663, 652 and 645 nm.

Growth and yield components: Leaf area of hybrid ramie plants was measured with a leaf area meter (LI-3100, LI-COR, Lincoln, NE, USA). At harvest 30 plants (10 plants from each replicate) per treatment were sampled randomly and leaf area, stem height, stem diameter, and shoot fresh weight were measured. Then fresh stem bast was stripped from plants and measured for bast thickness and bast fresh weight. Besides, fiber layer of fresh stem bast was separated and dried under sunlight and weighed to determine crude fiber yield. At last, the rest of the plants (except for fiber) include leaf, woody stem and residue of the fresh bast were oven dried for 72 h at 70°C to determine dry weight.

Statistical analysis: Data presented as the mean ± SE for each treatment, were tested with analysis of variance (one-way ANOVA), Newman-Keuls test, marked by letters, where the values sharing the different letters are significantly different at 5% level, using SPSS 16.0.

Results and Discussion

Growth and yield: The growth and yield attributes of hybrid ramie plants were considerably affected with progressive drought stress (Tables 1 and 2). Compared with the well-watered CK, leaf area, shoot fresh and dry weight/plant were all significantly declined by 9.67, 21.10 and 33.19%, 11.65, 29.61 and 32.04%, 13.37, 27.08 and 33.82% under water-deficit conditions, respectively, while moderate and severe drought caused considerable reduction by 7.73-13.99% in the plant height (Table 1).

Water stress largely hampered the growth which ultimately led to reduction in yield and yield traits. In comparison to the well-watered control, the bast stripping percentage continuously dropped under water stress, the stem diameter, bast thickness and bast fresh weight/plant slightly decreased by 6.63, 3.18 and 5.31% in mild drought stress but significantly decreased by 12.63-16.83%, 5.73-9.16% and 11.77-17.29% in moderate and severe stress. Raw fiber yield/plant substantially reduced by 16.65, 29.61 and 44.60%, even mild stress caused great loss of fiber yield (Table 2).

The above data showed though varying drought treatments noticeably impaired growth and yield related traits, the effect was more pronounced in moderate and severe stress plants than those in mild stress. Similar results were also reported in sweet sorghum[13] and common bean plants [14]. Water deficit resulted noticeable reduction in plant growth and yield parameters which might be attributed to damage of oxygen evolving complex of photosystem

Table 1. Influence on agronomic traits of ramie under different water regimes.

Treatments	Plant height (cm)	Leaf area/plant (cm^2)	No. of leaves/plant	Shoot fresh weight/plant (g)	Shoot dry weight/plant(g)
CK	79.61±0.94 a	910.70±13.16 a	19.65±0.69 a	103.55±2.09 a	20.64±0.30 a
MDS	77.45±1.38 ab	822.51±21.54 b	18.40±0.70 a	91.35±1.84 b	17.88±0.19 b
mDS	73.46±1.99 b	717.98±17.26 c	16.29±0.42 b	77.38±2.13 c	15.05±0.13 c
SDS	68.47±1.27 c	608.65±18.09 d	14.28±0.15 c	70.30±2.99 c	13.66±0.20 d

CK: well-watered; MDS: mild drought stress; mDS: moderate drought stress; SDS: severe drought stress. Values in the table are mean ± S.E (n = 3). Values followed by the same letter within columns don't differ significantly according to Newman–Keuls test (ρ<0.05).

Table 2. Influence on raw fiber yield and yield related traits of ramie under different water regimes.

Treatments	Stem Diameter (mm)	Bast thickness (mm)	Bast fresh weight/plant (g)	Bast stripping Percentage (%)	Raw fiber yield /plant (g)
CK	9.98±0.27 a	0.786±0.011 a	9.60±0.18 a	8.20±0.16 a	0.787±0.011 a
MDS	9.32±0.19 ab	0.761±0.005 ab	9.09±0.08 ab	7.15±0.31 b	0.656±0.023 b
mDS	8.72±0.26 bc	0.741±0.008 bc	8.47±0.14 b	6.30±0.32 b	0.554±0.018 c
SDS	8.30±0.21 c	0.714±0.011 c	7.94±0.15 c	6.25±0.29 b	0.436±0.015 d

CK: well-watered; MDS: mild drought stress; mDS: moderate drought stress; SDS: severe drought stress. Values in the table are mean ± S.E (n = 3). Values followed by the same letter within columns don't differ significantly according to Newman–Keuls test (ρ< 0.05).

II and its reaction centers [15] or by disrupting water relations and leaf gas exchange properties [9], thus photosynthesis was inhibited, which led to the decline in both fresh and dry weight of shoots and final harvestable yield.

Gas exchange: The field grown plants are extensively exposed to drought stress. Under drought stress usually a water deficit in plant tissues develops, thus leading to a significant inhibition of photosynthesis. The plants respond to water deficit by a rapid closure of stomata to avoid further loss of water through transpiration [16, 17]. Stomatal closure deprives the leaves of CO_2 and photosynthetic carbon assimilation is decreased in favor of photorespiration, which result in declined rate of photosynthesis.

In present study, varying drought stress treatments led to significant decline in net photosynthetic rate (A) by 13.95, 26.59 and 41.42%, stomatal conductance (g_s) by 29.46, 46.74 and 52.12%, transpiration rate (E) by 14.23, 30.48 and 41.3% compared with the control (Table 3). Decrease in intercellular CO_2 (Ci) (by 6.23 and 11.68%) showed a similar trend as A, g_s and E subjected to mild drought and moderate drought, whereas under severe stress it rapidly increased (Table 3). The observed gradual decline in A, g_s and Ci, indicated that mild drought and moderate drought caused stomatal inhibition with negative reflexes on the photosynthetic CO_2 uptake. However, under severe drought very low levels in A and g_s accompanied by a considerable increase in Ci observed, suggested non-stomatal limitations prevailed. Non-stomatal responses of carbon fixation such as PS_2 energy conversion and the dark reaction of Rubisco carbon fixation are resistant to water deficit [18, 19], therefore non-stomatal effects are usually considered to be more important during longer and more severe drought stress events. Limited leaf photosynthesis under drought stress conditions was caused by a combination of stomatal restriction and disruption of the mechanisms of photosynthetic carbon metabolism, leading to high Ci concentrations [20, 21]. Both stomatal and non-stomatal limitation was also detected for conifer and almond species subjected to more severe drought stress [7, 22]. Intrinsic water use efficiency (WUEi) increased under mild and moderate stress, but decreased under severe stress and the highest WUEi was recorded under moderate drought.

Photosynthetic pigments: The biosynthesis of photosynthetic pigments, including Chl a, Chl b and Chl a+b was noticeably reduced with the progression of drought stress. The greater the increase in drought stress, the lower the chlorophyll contents (Figs 1-3). It is obvious that substantially decreased or unchanged chlorophyll level during water stress depends on the intensity, severity and duration of drought. According to Agastian *et al.* [23], significant decrease in chlorophyll a, chlorophyll b and total chlorophyll concentrations under moisture stress amount due to water deficit and mainly because of the damage to chloroplasts

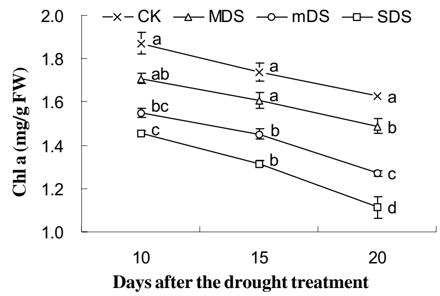

Figure 1. Influence of drought treatments on Chl a contents (Mean±SE, n=3) in ramie. Different letters denote significant differences among treatments according to Newman–Keuls test (ρ< 0.05). CK: well-watered; MDS: mild drought stress; mDS: moderate drought stress; SDS: severe drought stress.

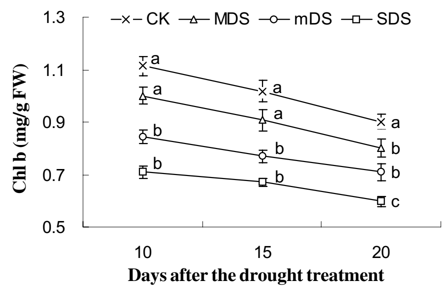

Figure 2. Influence of drought treatments on Chl b contents (Mean±SE, n=3) in ramie. Different letters denote significant differences among treatments according to Newman–Keuls test (ρ< 0.05). CK: well-watered; MDS: mild drought stress; mDS: moderate drought stress; SDS: severe drought stress.

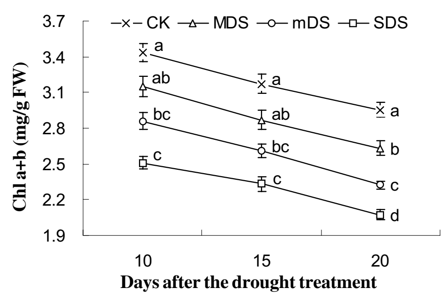

Figure 3. Influence of drought treatments on Chl a+b contents (Mean±SE, n=3) in ramie. Different letters denote significant differences among treatments according to Newman–Keuls test (ρ< 0.05). CK: well-watered; MDS: mild drought stress; mDS: moderate drought stress; SDS: severe drought stress.

Table 3. Influence on gas exchange traits and water use efûciency of ramie under different water regimes.

Treatments	Net photosynthesis (μmol m^{-2} s^{-1})	Transpiration rate (mmol m^{-2} s^{-1})	Stomatal conductance (μmol m^{-2} s^{-1})	Intrinsic water use efficiency (μmol $mmol^{-1}$)	Intercellular CO_2 (μmol mol^{-1})
CK	13.69±0.13 a	7.94±0.20 a	0.353±0.012 a	39.05±1.43 c	270.52±6.17 a
MDS	11.78±0.17 b	6.81±0.16 b	0.249±0.002 b	43.37±0.80 b	253.67±2.42 b
mDS	10.05±0.11 c	5.52±0.20 c	0.188±0.007 c	53.54±1.46 a	238.92±2.02 c
SDS	8.02±0.11 d	4.66±0.14 d	0.169±0.005 c	47.49±0.88 b	268.96±3.08 a

CK: well-watered; MDS: mild drought stress; mDS: moderate drought stress; SDS: severe drought stress. Values in the table are mean ± S.E (n = 3). Values followed by the same letter within columns don't differ significantly according to Newman–Keuls test (ρ< 0.05).

by active oxygen species. The photosynthetic pigments decreased to a significant level at higher water deficits were detected in sunflower plants, *Catharanthus roseus*, soybean, common bean and maize [9, 14, 24-27]. Low concentrations of chlorophyll contents are direct limitation of photosynthetic potential and hence primary production.

Conclusions

When subjected to drought stress, hybrid ramie plants responded through alteration in morphological and physiological processes, thus led to inhibition of growth and decrease of yield. However, trends and degree in change of morphological characteristics, gas exchange and photosynthetic pigments were highly varied due to the intensity, severity and duration of drought event and their interaction. To some extent, hybrid ramie has a certain tolerance to short time mild water stress. Different responses to different soil field capacity in hybrid ramie improved our understanding of the mechanisms that enable plants to survive under drought stress.

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