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BRIEF COMMUNICATION

Photosynthetic characteristics in mulberry during water stress and rewatering

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Abstract

Three-month-old plants of mulberry (*Morus alba* L. cv. Kanva-2) were subjected to a drought stress by withholding water supply. As the leaf water potential (Ψ_w) was dropping progressively with the severity of treatment and increasing stress duration, the values of leaf area, dry mass accumulation, total chlorophyll (Chl) content, net photosynthetic rate (P_N), stomatal conductance (g_s), and transpiration rate (E) were declined. The photosystem 2 (PS2) photochemical efficiency significantly decreased only at a severe stress treatment. The intercellular CO_2 concentration (C_i) remained unaltered during a mild stress, yet it increased under moderate and severe stresses. The C_i/g_s ratio reflected the mesophyll efficiency during water stress. Rewatering of the plants led to an almost complete recovery of P_N, E, and g_s, indicating that a short-term stress brings about reversible effects only.

Additional key words: intercellular CO_2 concentration; leaf area, leaf dry mass; *Morus alba*; net photosynthetic rate; photosystem 2; relative water content; stomatal conductance; transpiration rate; water relations.

Photosynthesis belongs to the physiological and biochemical processes affected by water stress. Stomatal and/or non-stomatal components are involved in limiting P_N, depending on severity and persistance of the stress and also on the genetic response of plant species. Mulberry foliage is the best food for silkworm (*Bombyx mori* L.). This study was undertaken to extend the knowledge of stress physiology in mulberry.

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The mulberry (*M. alba* L. cv. Kanva-2) cuttings of equal length and diameter consisting of 3 to 4 active buds were planted in earthen pots containing 5 kg of air dried soil and farm yard manure in the ratio of 3:1. The pots were maintained in the department garden for three months under natural photoperiod by daily irrigation up to the field capacity. The three-month-old plants were divided into 4 sets. One set of pots received water to the field capacity daily and served as control. The remaining three sets received water daily to 75, 50, and 25 % of the field capacity and were characterized as mild, moderate and severe stresses, respectively. Duration of the stress treatment was 10 d, and the plants subjected to severe stress were assessed for recovery after 24 and 48 h of irrigating them to the field capacity.

The leaf Ψ_w, Chl content, and photosynthetic parameters were tested on the 5th and 10th d of the stress and measurements were taken in the 3rd leaf from the plant top, since it had the maximum P_N. The leaf Ψ_w was measured by a *PR55* psychrometer microvoltmeter (*Wescor*, Utah, U.S.A.). The leaf area was measured by a leaf area meter. The plant leaves were dried at 80 °C in a hot air oven until a constant mass formed. The Chl content was estimated spectrophotometrically as described by Arnon (1949), using 80 % acetone. The leaf relative water content (RWC) was determined according to Turner (1981). The 1,6-dichlorophenolindophenol (DCPIP) reduction in isolated chloroplasts was measured according to Sudhasundari and Raghavendra (1990). The P_N, g_s, C_i, and E were measured between 08:00 and 10:00 h by using a portable photosynthesis system, *LCA-3* (*ADC*, U.K.) with the aid of a Parkinson leaf chamber (6.2 cm^2) under the irradiance of 1100±100 µmol m^{-2} s^{-1} and temperature 32±2 °C.

With an increasing water stress, the leaf ψ_w and RWC values became significantly lower than those of the controls (Table 1). The values of RWC were fully regained after 24 h of rewatering. The leaf area declined with the declining leaf ψ_w (Table 1), and severe stress strongly affected the parameter (60 % inhibition). A decrease in the dry mass accumulation which could be attributed to both the reduced leaf area and P_N per leaf area unit was also observed (Table 1). The leaf extension was more sensitive to the declining leaf ψ_w than the P_N.

The leaf Chl content declined according to severity and duration of the stress (Table 1). Similar observations were made earlier in several plant species (*e.g.*, Hsiao 1973, Castrillo and Fernandez 1990, Nandwal *et al.* 1991, Castrillo and Trujillo 1994). The inhibitory effect of drought on Chl content may either be due to enhanced chlorophyllase activity (*e.g.*, Drążkiewicz 1994) or decreased synthesis. The total Chl content showed an upsurge on rewatering. A similar evidence was also presented by Nandwal *et al.* (1991) and Castrillo and Trujillo (1994).

Decreasing leaf ψ_w induced a decrease in P_N and g_s which led to a conclusion that stomatal closure was the major cause of reduced P_N under a water stress. Stomatal closure during a water stress without a change in mesophyll capacity should result in a lower C_i. However, in this study under moderate and severe stresses C_i remained higher than one would expect according to the decrease in P_N and g_s, which might indicate a decreased carboxylation efficiency (Kicheva *et al.* 1994). Water stress decreases the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Raggi 1992) and other Calvin cycle enzymes as well as inhibits the photosynthetic electron transport (Chaves 1991, Cornic *et al.* 1992, Kicheva *et al.* 1994). Upon rewatering, the C_i values attained the normal level.

The electron flow (DCPIP reduction) was significantly inhibited under a severe stress only on both days of sampling (Table 1). This PS2 inhibition during drought could be due to the loss of active PS2 centres (Meyer and de Kouchkovsky 1993) or protein alterations or conformational changes as a result of massive decrease of lipids induced by drought (Meyer *et al.* 1992). Upon rewatering, full recovery in the P_N and g_s was noticed (Table 1). During one day the P_N of rubber plants may increase and attain almost initial values (Ceulemans *et al.* 1983). Conversely, the P_N may not recover completely due to after-effects of stress on the stomata such as in egg plants (Behboudian 1977). Most likely the severity of water stress and drought tolerance capacity of given plant species or cultivar (cf. Lakshmi *et al.* 1996) are important for recovering the photosynthetic capacity.

Table 1. Effects of water stress on the mulberry leaf water potential (ψ_w) [-MPa], leaf relative water content (RWC) [%], leaf area [% of control], leaf dry mass accumulation [% of control], chlorophyll (Chl) content [g kg^{-1}(d.m.)], net photosynthetic rate (P_N) [μmol m^{-2} s^{-1}], stomatal conductance (g_s) [mmol m^{-2} s^{-1}], intercellular CO$_2$ concentration (C_i) [μmol mol^{-1}], C_i/g_s ratio, transpiration rate (E) [mmol m^{-2} s^{-1}], and photosystem 2 (PS2) activity (DCPIP reduction) [mmol kg^{-1}(Chl) s^{-1}]. Means, n = 6±SD.

Parameter	Day	Control	Mild	Moderate	Severe	Recovery	
						after 24 h	after 48 h
ψ_w	5	0.70±0.13	0.82±0.12	1.61±0.18	2.57±0.14	0.73±0.09	0.70±0.10
	10	0.71±0.11	0.84±0.14	2.08±0.17	3.13±0.17	0.81±0.06	0.70±0.08
WC	5	97	88	76	60	81	96
	10	96	84	70	42	73	92
Leaf area	5	100	98	92	83	--	--
	10	100	98	70	40	--	--
Dry mass	5	100	92	81	69	--	--
	10	100	87	72	52	--	--
Chl	5	5.28±1.2	4.49±1.0	3.73±0.96	2.48±0.67	5.72±0.93	5.68±1.3
	10	5.36±1.0	4.08±1.1	2.82±0.54	2.04±0.44	5.96±0.92	5.73±1.4
P_N	5	10.5±1.9	9.9±2.3	6.3±1.7	4.3±1.4	7.8+2.1	10.2+2.4
	10	10.6±2.4	9.8±2.6	4.6±1.2	2.1±0.8	5.6±1.4	9.9±2.0
g_s	5	0.88±0.16	0.75±0.11	0.54±0.10	0.22±0.10	0.56±0.14	0.87±0.12
	10	0.88±0.17	0.74±0.08	0.34±0.04	0.08±0.02	0.42±0.11	0.78±0.12
C_i	5	224±22	222±20	230±23	252±19	220±30	223±21
	10	223±26	222±20	238±16	264±23	226±24	224±18
C_i/g_s	5	2.5	2.9	4.2	11.4	3.9	2.5
	10	2.5	3.0	7.0	33.0	5.3	2.6
E	5	4.2±0.8	3.9±0.6	3.2±0.5	2.6±0.7	3.3±0.5	4.2±0.8
	10	4.3±0.6	3.7±0.5	2.8±0.4	1.4±0.3	2.7±0.5	4.2±0.4
PS2	5	74.2±6.7	73.3±6.4	73.3±6.7	38.3±3.1	54.7±3.9	72.2±6.1
	10	73.6±6.4	73.1±6.1	73.6±6.1	25.3±2.2	45.8±3.3	68.1±6.7

The P_N is under the control of both stomatal and mesophyll factors. The g_s can be directly measured, and the mesophyll factors can be estimated by the P_N/C_i curve. In this context, Seshashayee *et al.* (1996) report that the C_i/g_s ratio is as effective as that of the P_N/C_i curve in determining the involvement of mesophyll factors by stress. Therefore, the C_i/g_s can be used as a parameter to determine the non-stomatal limitations (mesophyll efficiency) to photosynthesis. In the present study, we adopted the C_i/g_s ratio as a reflection of mesophyll efficiency for carboxylation during water stress. The C_i/g_s ratio increased during stress particularly under severe and moderate stresses (10th d) (Table 1). Increase in the C_i/g_s reflected the decreased mesophyll efficiency and stood in evidence for the involvement of non-stomatal factors as constraints for photosynthesis. Upon rewatering, the C_i/g_s attained the control values.

The E declined correspondingly with the decline in P_N and g_s (Table 1). The reduction in water loss by stomatal behaviour is one of the adaptive responses maintaining a high water use efficiency as the drought develops. This, to an extent, ameliorates the stress onset and helps to maintain photosynthesis. During a severe stress, the mulberry stomata close to conserve water which is typical of most mesophytes. In addition to the stomatal behaviour, reduction of the water loss is also aided by the reduction of leaf area. An increased E was recorded upon rewatering.

The present study indicated that all the investigated parameters were affected during drought. The reduction in leaf biomass was due to reduced leaf extension as well as decreased P_N including a decline in the Chl content. Reduced P_N as a response to the decrease in the leaf ψ_w was modulated by stomatal and non-stomatal components, an effort largely determined by the stress severity. Although each parameter seemed to be affected during the drought, the plant could regain a full functional capacity after 48 h of rewatering, which showed the short-term recovery of structural and functional components of this process.

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