PHOTOSYNTHETICA 36 (4): 611-616, 1999

BRIEF COMMUNICATION

Photosynthesis parameters in two cultivars of mulberry differing in salt tolerance

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Abstract

Three-month-old mulberry (*Morus alba* L.) cultivars (salt tolerant cv. S1 and salt sensitive cv. ATP) were subjected to different concentrations of NaCl for 12 d. Leaf area, dry mass accumulation, total chlorophyll (Chl) content, net CO_2 assimilation rate (P_N), stomatal conductance (g_s), and transpiration rate (E) declined, and intercellular CO_2 concentration (C_i) increased. The changes in these parameters were dependent on stress severity and duration, and differed between the two cultivars. The tolerant cultivar showed a lesser reduction in P_N and g_s coupled with a better C_i and water use efficiency (WUE) than the sensitive cultivar.

Additional key words: chlorophylls; intercellular CO_2 concentration; leaf area; leaf dry mass; *Morus alba;* net photosynthetic rate; salt stress; stomatal conductance; transpiration; water use efficiency.

Salinity mainly occurs in arid and semiarid conditions where the precipitation is not enough to leach the excess soluble salts from the root zone. It is one of the main environmental constraints which limits photosynthesis and consequently productivity in crop plants. Better understanding of the mechanism that enables plants to adapt to water deficit or salinity stress and maintain growth will ultimately help in the selection of stress tolerant cultivars for exploiting saline soils. In the present study an

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Received 17 June 1999, *accepted* 30 August 1999.

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Abbreviations: C_i - intercellular CO_2 concentration; E - transpiration; g_s - stomatal conductance; P_N net photosynthetic rate; WUE - water use efficiency.

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Acknowledgements: This study was supported by a research grant (F3-9/93 SR-II) to C. Sudhakar by the University Grants Commission, India which is gratefully acknowledged. K.V.M. is grateful to the Council of Scientific and Industrial Research, India for the JRF award. Authors are thankful to the authorities of RSRS, Anantapur for providing plant material.

attempt is made to assess the tolerance potentials in two mulberry cultivars with different sensitivity to salt stress based on P_N and associated parameters.

Mulberry (*Morus alba* L.) cultivars S1 and ATP were procured from the Regional Sericultural Research Station (CSB), Anantapur, India. The cuttings of approximately equal length and diameter, with 3 to 4 active buds were prepared and immediately planted in earthen pots containing 8 kg of red loamy soil and farm yard manure (3 : 1 ratio). The pots were watered daily and were kept in the Botanical Garden under natural photoperiod of 12-13 h and temperature of 32±4 °C. Three-month-old plants were subjected to salt stress induced by a range of NaCl concentrations [0 (control), 0.5, 1.0, and 1.5 %]. The electrical conductivity (EC) of soil saturation extract was 1.7, 2.0, 4.0, and 6.9 mS cm^{-1}, respectively. Care was taken to avoid drainage of solution during the treatment by giving water slightly less than field capacity. EC of soil extract was monitored and adjusted on alternate days.

The measurements were taken in the 3rd leaf from the plant top, since it had the maximum P_N. The leaf area was measured by a leaf area meter. For determination of dry mass the leaves were dried at 80 °C in a hot air oven until a constant mass was formed. The Chl content was estimated spectrophotometrically as described by Arnon (1949), using 80 % acetone extracts. P_N, g_s, C_i, and E were recorded between 08:00 and 10:00 h on the 4th, 8th, and 12th d after stress induction, by using a portable photosynthetic system, *LCA-3* (*ADC*, England) with the aid of a *Parkinson* leaf chamber (6.2 cm^2) under the irradiance of 1100±100 μmol m^{-2} s^{-1} and at temperature of 32±2 °C, an ambient CO_2 concentration of 335-340 μmol mol^{-1}, and RH 70 %.

Under salt stress a decrease in the dry mass accumulation which could be attributed to both the reduced leaf area, P_N, and Chl content was observed (Table 2). A decline in P_N under salt stress was observed in both the genotypes (Table 1). This effect was gradual and remarkable at higher NaCl concentrations combined with the increase in stress duration. The P_N decreased less in the cultivar S1 than in ATP; it was reduced by 35 and 14 % on the 12th d of exposure to 1.5 % NaCl concentration in S1 and ATP, respectively.

High salinity drastically decreased g_s with the increasing stress duration in both cultivars. Further, even low concentration of NaCl (0.5 %) significantly inhibited g_s in both cultivars. However, the degree of inhibition of g_s was higher in ATP than in S1.

The C_i values were almost unaltered under low concentrations of NaCl in both the cultivars. However, they were significantly increased under severe salt stress in both the cultivars. Nevertheless, the increase was greater in ATP compared to S1.

Transpiration rate (E) showed a similar pattern as P_N under 0.5 % NaCl in S1, however, P_N decreased more than E in ATP. The decrease in E with increasing external NaCl concentration was similar in both cultivars. Water use efficiency (WUE) determined as P_N/E increased under NaCl treatment in both cultivars, but better WUE values were found in the cultivar S1.

Dry mass accumulation was decreased in salt stressed plants of both genotypes compared to controls. These results agree with earlier reports (Heuer and Nadler 1998). The magnitude of decline in leaf dry mass accumulation depended on severity and duration of stress and inhibition in dry mass yield was relatively less in S1 than

Table 1. 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 [mmol m^{-2} s^{-1}], transpiration rate, E [mol m^{-2} s^{-1}], and water use efficiency, WUE [mmol mol^{-1}] in control and NaCl stressed (0.5, 1.0, 1.5 %) cultivar S1 and ATP of mulberry. The mean values represented by the same letter in a row for each cultivar are not significantly different at $p<0.05$ according to Duncan's multiple range test. Figures in parentheses represent per cent of control.

Treatment/ days	cv. S1 control	0.5 %	1.0 %	1.5 %	cv. ATP control	0.5 %	1.0 %	1.5 %
P_N								
4	17.4 c	15.4 b	13.1 ab	10.9 a	14.6 c	11.5 b	7.2 a	6.0 a
	(100)	(82)	(75)	(63)	(100)	(79)	(49)	(41)
8	17.5 d	14.3 c	12.2 b	9.8 a	14.8 d	8.2 c	5.1 b	3.1 a
	(100)	(82)	(70)	(59)	(100)	(55)	(35)	(21)
12	17.8 d	12.8 c	11.0 b	6.2 a	14.9 d	5.2 c	2.9 b	2.1 a
	(100)	(72)	(62)	(35)	(100)	(35)	(20)	(14)
g_s								
4	1.98 d	1.05 c	0.82 b	0.65a	1.09 d	0.77 c	0.43 b	0.32 a
	(100)	(53)	(41)	(33)	(100)	(71)	(39)	(29)
8	2.0 d	1.23 c	0.79 b	0.59 a	1.16 d	0.72 c	0.34 b	0.21 a
	(100)	(62)	(40)	(30)	(100)	(63)	(29)	(18)
12	2.4 d	1.28 c	0.7 b	0.52 a	1.19 d	0.65 c	0.28 b	0.12 a
	(100)	(53)	(35)	(22)	(100)	(55)	(24)	(10)
C_i								
4	255 a	249 a	248 a	258 a	230 a	242 a	245 a	258 b
	(100)	(93)	(93)	(101)	(100)	(105)	(106)	(112)
8	252 a	258 a	266 a	286 b	238 a	255 b	263 b	275 b
	(100)	(102)	(109)	(113)	(100)	(107)	(110)	(115)
12	225 a	259 a	260 a	291 b	235 a	263 b	270 b	290 c
	(100)	(115)	(115)	(129)	(100)	(112)	(114)	(123)
E								
4	5.3 d	4.7 c	4.0 b	3.3 a	4.3 d	3.5 c	2.2 b	1.8 a
	(100)	(89)	(75)	(62)	(100)	(81)	(51)	(42)
8	5.3d	4.4 c	3.8 b	2.9a	4.4d	2.6 c	1.7 b	1.3 a
	(100)	(83)	(72)	(55)	(100)	(59)	(39)	(30)
12	5.4 d	3.9 c	3.4 b	1.9 a	4.3 c	1.8 b	1.2 a	1.1 a
	(100)	(72)	(63)	(35)	(100)	(42)	(29)	(25)
WUE								
4	3.28	3.27	3.27	3.30	3.39	3.28	3.28	3.30
8	3.30	3.25	3.21	3.37	3.36	3.15	3.00	2.38
12	3.29	3.28	3.28	3.26	3.46	2.88	2.41	1.90

in ATP (Table 2). The dry mass decrease as a result of stress is attributed to the altered carbon and nitrogen metabolism, which are responsible for total biomass production (Kluge 1976). Decrease in dry mass accumulation of leaves also attributed to decreased rates of reduced leaf area, photosynthesis, and Chl content (Ramanjulu *et al.* 1994, 1998) which is in agreement with our results.

The leaf Chl content declined according to severity and duration of the stress (Table 1). Decrease in Chl content was attributed to suppression of specific enzyme

Table 2. Leaf area [cm^2], leaf dry mass accumulation [g per plant], chlorophyll (Chl) content [g kg^{-1}(d.m.)] in control and stressed cultivars S1 and ATP of mulberry. For further explanations see Table 1.

	Day	cv. S1 control	0.5 %	1.0 %	1.5 %	cv. ATP control	0.5 %	1.0 %	1.5 %
Leaf area	4	108b	103a	104a	104a	69a	66b	70a	65a
		(100)	(95)	(96)	(96)	(100)	(96)	(101)	(94)
	8	114b	104a	106a	105a	72b	68b	70.5b	65a
		(100)	(91)	(93)	(92)	(100)	(94)	(98)	(90)
	12	117b	105a	108a	104a	79b	71ab	70a	65a
		(100)	(90)	(92)	(89)	(100)	(90)	(90)	(82)
Dry mass	4	3.11b	3.03b	2.84ab	2.60a	3.56c	3.40c	3.01b	2.50a
		(100)	(97)	(91)	(84)	(100)	(96)	(85)	(70)
	8	3.84c	3.71c	3.12b	2.32a	4.48c	4.06c	3.21b	2.14a
		(100)	(97)	(81)	(60)	(100)	(91)	(72)	(48)
	12	4.55c	4.46c	3.49b	2.82a	5.31d	4.36c	3.01b	2.29a
		(100)	(98)	(77)	(62)	(100)	(82)	(57)	(43)
Chl	4	1.51b	1.34a	1.26a	1.20a	1.41c	1.20b	1.04a	0.96a
		(100)	(89)	(83)	(79)	(100)	(84)	(73)	(68)
	8	1.56c	1.23b	1.10b	1.04a	1.42c	1.08b	0.98b	0.77a
		(100)	(78)	(70)	(66)	(100)	(76)	(69)	(54)
	12	1.57c	1.09b	0.96b	0.80a	1.42d	0.91c	0.80b	0.57a
		(100)	(69)	(61)	(51)	(100)	(64)	(56)	(40)

responsible for synthesis of Chl and to disarrangement of pigment-protein complexes, and disruption of fine structure of chloroplasts was reported (Levitt 1980). The reduction in Chl content was also related to the enhanced activity of chlorophyllase or decreased synthesis (Rao and Rao 1981, Drążkiewicz 1994).

The decline in P_N under NaCl stress is partly attributed to a reduced g_s (Nagy and Galiba 1995, Lakshmi *et al.* 1996), partly to a decline in Chl content (Kolchevskiĭ *et al.* 1995). Simultaneously, the reduction in P_N is associated with inhibition of non-stomatal components by salt stress (Plaut *et al.* 1990, Everard *et al.* 1994, Sreenivasulu Reddy *et al.* 1998). However, the extent to which stomatal closure affects photosynthetic capacity is indicated by the magnitude of reduction in C_i (Berry and Downton 1982). A decrease in g_s for water vapour is an almost universal response to increasing salinity (Brugnoli and Lauteri 1991) such as are decreases in P_N and E (Sharma and Hall 1989). High NaCl concentration resulted in increased C_i in both the cultivars; this may indicate a decreased carboxylation capacity. The salt stress affects the capacity for fixing CO_2, since it inhibits the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase in the C_3 plants and phosphoenoipyruvate carboxylase and NADP-dependent malate dehydrogenase in the C_4 plants (Stiborová *et al.* 1987). However, the degree of decline in C_i was relatively less in cultivar S1 under NaCl stress conditions, reflecting a better maintenance of carboxylation. In the present study, P_N at low concentration of NaCl was probably mediated through

stomatal closure while both the biochemical processes and stomatal components were affected under more severe stress (Table 1).

NaCl salinity decreases E in both the cultivars when compared with controls. This was positively correlated with WUE as a consequence of salinity (Plaut *et al.* 1990, Everard *et al.* 1994). Reduced carbon assimilation under salinity was associated with an increase in WUE, which was due to a drastic decrease in water loss rates than in carbon gain.

The present study indicates that all the investigated parameters were affected during salt stress. The reduction in leaf biomass was due to reduced leaf extension as well as in decreased P_N including a decline in the Chl content. The salt tolerance of S1 could be ascertained from the present study, based on relatively lesser decrease in P_N and g_s coupled with better C_i and WUE.

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