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Photosynthesis and the enzymes of photosynthetic carbon reduction cycle in mulberry during water stress and recovery

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Abstract

Net photosynthetic rate (P_N), stomatal conductance (g_S), transpiration rate (E), intercellular CO_2 concentration (C_i), leaf water potential (Ψ_w), leaf area, chlorophyll (Chl) content, and the activities of photosynthetic carbon reduction cycle (PCR) enzymes in two mulberry (*Morus alba* L.) cultivars (drought tolerant Anantha and drought sensitive M-5) were studied during water stress and recovery. During water stress, P_N, g_S, and E declined whereas C_i increased. P_N, g_S, and E were less affected in Anantha than in M-5, which indicates tolerance nature of Anantha over M-5. Activities of ribulose-5-phosphate kinase, NAD- and NADP-glyceraldehyde-3-phosphate dehydrogenases, and 3-phosphoglycerate kinase decreased with increasing stress in both the cultivars. The enzyme activities less affected in tolerant (Anantha) than in sensitive cultivar (M-5) were restored after re-watering to almost initial values in both the cultivars. Re-watering 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 effect in these two cultivars of mulberry.

Additional key words: chlorophyll content; intercellular CO_2 concentration; leaf area; *Morus alba*; net photosynthetic rate; PCR cycle enzymes; water use efficiency.

Introduction

Water availability is a major limitation in crop production. Several reports indicated that reduction in plant growth due to stress is often associated with decrease in photosynthetic activities (Hsiao *et al.* 1976, Greenway and Munns 1980). Effects of water and salt stresses have been examined in various stress-sensitive and tolerant crop plants (Cheeseman 1988), including mulberry (Ramanjulu *et al.* 1998, Giridara Kumar *et al.* 1999, Agastian *et al.* 2000), but mechanisms of inhibition of photosynthesis by water stress remain poorly defined in this plant species. Finding efficient methods of identifying resistant genotypes and understanding their mechanisms to tolerate periods of water deficit have been goals of plant physiologists and breeders. In the present study we tried to assess the tolerance potentials based on photosynthetic rate and associated parameters in two cultivars differing in sensitivity to water stress.

Materials and methods

The mulberry (*Morus alba* L.) cultivars were classified as drought tolerant cv. Anantha and drought sensitive cv. M-5. The cuttings 12 to 15 cm long, of 8 to 10 mm diameter, with 3 to 4 active buds were planted in earthen pots containing 5 kg of air-dried red loamy soil and farmyard manure in 3 : 1 proportion. Pots were maintained and watered daily for 75 d under natural photoperiod in the botanical garden. Pots of each cultivar were then

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Abbreviations: C_i – intercellular CO_2 concentration; E – transpiration rate; g_S – stomatal conductance; NAD-Gly-3-P-DH – NAD-glyceraldehyde-3-phosphate-dehydrogenase; NADP-Gly-3-P-DH – NADP-glyceraldehyde-3-phosphate-dehydrogenase; P_N – net photosynthetic rate; WUE – water use efficiency; Ψ_w – leaf water potential.
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divided into 4 sets and arranged in the complete randomised block design. 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 % field capacity and were characterised as mild, moderate, and severe stress, respectively. Experimental values were collected on day 10 after stress induction. Plants subjected to severe stress were assessed for recovery 48 h after re-watering them to field capacity. The measurements were taken in the 3rd leaf from the plant top, since it had the maximum P_N, g_S, C_i, and E. These values were recorded at 08:00 and 10:00 h using a portable photosynthesis system *LCA-3* (*ADC*, Hoddesdon, UK) with the aid of *Parkinson* leaf chamber (6.2 cm^2) under the irradiance of 1 100±100 μmol m^{-2} s^{-1}, temperature 32±2 °C, ambient CO_2 concentration of 335-340 μmol mol^{-1}, and relative humidity of 70 %. The leaf area was measured with Portable Laser Area Meter *CI-203* (Washington, USA) and leaf Ψ_w was measured by using a *PR55* psychrometer with the aid of microvoltmeter (*Wescor*, Utah, USA). The chlorophyll content was estimated spectrophotometrically as described by Arnon (1949), using 80 % acetone extracts.

The activities of photosynthetic enzymes were determined by adopting the method of Veeranjaneyulu (1978). The leaves were extracted (0.5 g cm^{-3}) by grinding with 50 mM Tris-HCl buffer (pH 7.8) that contained 5 mM dithiothreitol, 2 mM $MgCl_2$, and 10 mM 2-mer-capto-ethanol. The extract filtered through 4-layered mus-lin cloth was centrifuged at 20 000×*g* for 15 min at 4 °C. The supernatant was passed through a *Sephadex G-50* column pre-equilibrated with the same extraction buffer. Collected active fractions were used for the enzyme assay.

Enzyme activities were measured spectrophotometrically at 340 nm by using UV-visible spectrophotometer (*Shimadzu-1601*, Tokyo, Japan), by following the oxidation of NAD(P)H or reduction of NAD(P). The assay procedures used for NADP-glyceraldehyde-3-P-dehydrogenase (NADP-Gly-3-P-DH, EC 1.2.1.12), NAD-glyceraldehyde-3-P-dehydrogenase (NAD-Gly-3-P-DH, EC 1.2.1.13), 3-phosphoglycerate kinase (EC 2.7.2.3), and ribulose 5-P kinase (EC 2.7.1.19) were described in Sheoran *et al.* (1990).

Results

P_N decreased with increase in water stress in both the cultivars at all stress regimes (Table 1). Further, the decrease in P_N was larger in M-5 than in Anantha. g_S values decreased with increasing stress in both cultivars, being 28 and 23 % in Anantha and M-5, respectively. After re-watering, g_S values were restored to 83 and 75 % in Anantha and M-5, respectively. The decrease in E with increase in stress was also observed in both cultivars (Table 1), being greater in M-5 than in Anantha. Better water use efficiency values was found in Anantha compared to M-5 (Table 1). Under water stress C_i increased slightly in both cultivars and after re-watering, C_i attained almost normal values.

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 [μmol mol^{-1}], transpiration rate, E [mmol m^{-2} s^{-1}], and water use efficiency, WUE [mmol(CO_2) mol^{-1}(H_2O)] in two cultivars of mulberry during water stress and recovery. Means±SD, *n* = 6. Numbers in parentheses represent per cent control.

Parameter	Cv.	Control	Mild	Moderate	Severe	Recovery after 48 h
P_N	Anantha	15.36±1.40	13.01±1.00 (84.70)	8.53±2.40 (55.53)	4.06±1.10 (26.43)	14.54±1.70 (94.66)
	M-5	13.40±2.70	10.26±0.90 (76.56)	6.44±1.80 (48.05)	2.08±1.50 (15.52)	11.96±2.10 (89.25)
g_S	Anantha	0.84±0.03	0.66±0.14 (78.57)	0.46±0.13 (54.76)	0.24±0.06 (28.57)	0.70±0.08 (83.33)
	M-5	0.69±0.07	0.52±0.02 (77.61)	0.31±0.09 (44.92)	0.16±0.03 (23.18)	0.52±0.11 (75.36)
C_i	Anantha	245±31	255±27 (104.08)	268±16 (108.16)	297±20 (121.22)	240±21(97.95)
	M-5	256±24	272±30 (106.25)	288±18 (112.5)	321±23 (125.39)	236±19 (92.18)
E	Anantha	6.17±0.60	5.85±0.70 (94.81)	3.78±0.40 (61.78)	2.21±0.90 (35.81)	5.66±0.50 (91.73)
	M-5	5.83±0.30	4.50±0.60 (77.81)	3.25±0.40 (55.74)	1.67±0.50 (28.64)	4.92±0.90 (84.39)
WUE	Anantha	2.48	2.27	2.25	1.83	2.56
	M-5	2.29	2.10	1.98	1.24	2.33

Increase in water stress caused lowering of leaf water potentials compared to controls in both cultivars. Further, the degree of decrease was larger in Anantha than in M-5 in all stress regimes studied (Table 2). Leaf area and total Chl content decreased in both cultivars at all stress regimes (Table 2). After re-watering of the plants, leaf area was restored to 73 and 56 % and Chl content to 94 and 86 % in Anantha and M-5, respectively.

NADP-Gly-3-P-DH and NAD-Gly-P-DH activities decreased during all stress regimes. A greater inhibition

Table 2. Effect of water stress on mulberry leaf water potential, Ψ_w [–MPa], leaf area [cm^2], chlorophyll (Chl) content [g kg^{-1} (d.m.)], and activities of NADP-Gly-3-P-DH, NAD-Gly-3-P-DH, 3-PGA kinase, and ribulose-5-P kinase [mmol kg^{-1}(protein) s^{-1}]. For further explanations see Table 1.

Parameter	Cv.	Control	Mild	Moderate	Severe	Recovery after 48 h
Leaf Ψ_w	Anantha	0.91±0.11	1.36±0.14	2.24±0.09	2.92±0.17	0.99±0.13
	M-5	2.96±0.11	0.92±0.16	0.71±18	3.25±0.07	0.83±0.09
Leaf area	Anantha	95.81±2.10	92.11±1.71 (96.13)	72.43±2.73 (75.59)	62.33±1.92 (65.05)	70.11±2.64 (73.17)
	M-5	70.18±1.34	65.93±2.11 (93.94)	45.54±1.40 (64.89)	36.20±1.20 (51.58)	39.31±1.33 (56.01)
Chl	Anantha	6.04±1.14	4.82±0.91 (79.80)	3.14±0.61 (51.98)	2.32±0.48 (38.41)	5.71±0.56 (94.53)
	M-5	5.31±0.72	3.70±0.31 (69.67)	2.62±0.91 (49.34)	1.81±0.64 (34.08)	4.59±0.39 (86.44)
NADP-Gly-3-P-DH	Anantha	7.66±0.12	6.54±0.09 (85.37)	4.82±0.72 (62.92)	3.14±0.19 (40.99)	6.87±0.23 (89.68)
	M-5	6.98±0.62	5.13±0.31 (73.49)	4.00±0.14 (57.30)	2.61±0.41 (37.39)	6.01±0.09 (86.10)
NAD-Gly-3-P-DH	Anantha	8.92±0.14	7.18±0.62 (80.49)	5.24±0.19 (58.74)	3.47±0.18 (38.90)	7.69±0.21 (86.21)
	M-5	8.10±0.19	6.11±0.21 (75.43)	3.89±0.32 (48.02)	2.60±0.14 (32.09)	6.71±0.32 (82.83)
3-PGA-kinase	Anantha	28.31±0.41	25.48±0.20 (93.53)	21.43±0.17 (75.69)	13.87±0.36 (48.99)	27.04±0.12 (95.51)
	M-5	27.84±0.22	24.08±0.31 (86.49)	18.30±0.96 (65.73)	8.34±0.16 (29.95)	23.32±0.22 (83.76)
Ribulose-5-P kinase	Anantha	30.46±1.14	28.32±1.37 (92.97)	22.37± 0.98 (73.44)	16.12±1.10 (52.92)	28.97±2.10 (95.10)
	M-5	29.31±1.71	25.61±2.31 (87.37)	17.34±0.87 (59.16)	7.61±0.42 (25.96)	25.12±1.70 (85.70)

in enzyme activity was noticed in M-5 under severe stress (Table 2). 3-PGA kinase and ribulose-5-P kinase enzyme activities were significantly inhibited under severe stress in both cultivars. After re-watering the plants the enzyme activities were restored almost to initial values.

Discussion

The decrease of P_N under water stress was accompanied by decrease in carboxylation efficiency in both mulberry cultivars. Similar reports under water and salt stress conditions were already observed (Renou *et al.* 1990, Ramanjulu *et al.* 1998, Giridara Kumar *et al.* 1999, Barathi *et al.* 2001). A decrease in P_N without a corresponding decline in C_i has usually been interpreted as a documentation of non-stomatal effects of water stress on photosynthesis. Genotypic variation in P_N of several stressed crop plants was evident from the studies of Kicheva *et al.* (1994), including mulberry (Ramanjulu *et al.* 1998, Giridara Kumar *et al.* 1999).

The C_i values were almost unaltered under mild stress and only slightly increased under moderate stress. However, they significantly increased under severe stress in both the cultivars. The occurrence of high C_i values at reduced g_S under water stress indicated non-stomatal limitation to photosynthesis. Severe stress treatment resulted in increased C_i in both cultivars; this may indicate a decreased carboxylation efficiency (Kicheva *et al.* 1994).

Under water stress, *E* declined more than P_N in both cultivars. This was correlated with increased WUE as a consequence of stress (Everard *et al.* 1994). In the present study, Ψ_w decreased with increase in stress intensity and duration. A similar decline in Ψ_w as a result of drought stress was reported by several investigators (Irigoyen *et al.* 1992, Lawlor *et al.* 1993, Savé 1995, Ramanjulu *et al.* 1998, Heuer and Nadler 1998). In the present study Anantha showed a smaller decrease in Ψ_w than M-5. Thus the maintenance of better Ψ_w would aid in conferring drought resistance to plants.

Per cent leaf area retention is the widely adopted approach to assess the relative stress tolerance in plants (Gopalakrishna *et al.* 2001). We found a lesser rate of decrease in leaf growth under stress in cv. Anantha compared to M-5. After re-watering of the plants the functional (green) leaf area retention was larger in Anantha than in M-5.

Water stress inhibits some enzymes of Chl biosynthesis, particularly, 5-aminolevulinic acid synthetase (Bharadwaj and Singhal 1981). Therefore, the lower amount of Chl under water stress may be due to decreased synthesis. However, activity of many hydrolases including that of chlorophyllase was also increased under water stress (Hsiao 1973).

In general, up- or down-regulation of enzymes in response to stress regimes has been demonstrated in many crop plants (Kandpal *et al.* 1981, Ramanjulu *et al.* 2000), including mulberry (Ramanjulu and Sudhakar 1997, Sudhakar *et al.* 2001). In our study the photosynthetic enzyme activities were altered under stress and greatly differed between the two cultivars.

The supply of RuBP to carboxylation sites of RuBPC is the most important process in determining the carboxylation rate and synthesis of 3-PGA. Since, synthesis of RuBP depends on supply of ATP and NADPH (activities of photophosphorylation and electron transport, respectively), and on the enzymes of PCR cycle, decreased ATP due to inhibition of photophosphorylation was observed under water stress (Lawlor and Uprety 1993, Dua *et al.* 1994). The decrease in photophosphorylation will particularly slow the rates of 3-PGA kinase and ribulose 5-P-kinase reactions that could be due to the high sensitivity of 3-P kinase and ribulose-5-P kinase to water stress. This indicates that general decrease in the activity of enzymes under long term or severe stress be either due to decreased contents of enzymes caused by reduction in protein synthesis (Lawlor and Uprety 1993) or altered concentration of cellular metabolites brought about by osmotic dehydration (Leegood *et al.* 1985). In the present study activities of NADP-Gly-3-P-DH and NAD-Gly-3-P-DH decreased under moderate and severe stress conditions in both cultivars.

We found that all studied parameters were affected during drought. The relative drought tolerance of Anantha was evident from lesser reduction in P_N, g_S, Ψ_w, and better WUE. Further, better maintenance of enzyme activities in Anantha during stress supports its drought tolerance over M-5. The plants could regain almost full functional capacity after 48 h of re-watering, which showed the short-term recovery of structural and functional components of this process.

References

Agastian, P., Kingsley, S.J., Vivekanandan, M.: Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes. – Photosynthetica **38**: 287-290, 2000.

Arnon, D.I.: Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. – Plant Physiol. **24**: 1-15, 1949.

Barathi, P., Sundar, D., Ramachandra Reddy, A.: Changes in mulberry leaf metabolism in response to water stress. – Biol. Plant. **44**: 83-87, 2001.

Bhardwaj, R., Singhal, G.S.: Effect of water stress on photochemical activity of chloroplasts during greening of etiolated barley seedlings. – Plant Cell Physiol. **22**: 155-162, 1981.

Cheeseman, J.M.: Mechanisms of salinity tolerance in plants. – Plant Physiol. **87**: 547-550, 1988.

Dua, A., Talwar, G., Singal, H.R., Singh, R.: CO_2 exchange, primary photochemical reactions and enzymes of photosynthetic carbon reduction cycle in *Brassica* pods during water stress and recovery. – Photosynthetica **30**: 261-268, 1994.

Everard, J.D., Gucci, R., Kann, S.C., Flore, J.A., Loescher, W.H.: Gas exchange and carbon partitioning in the leaves of celery (*Apium graveolens* L.) at various levels of root zone salinity. – Plant Physiol. **106**: 281-292, 1994.

Gopalakrishna, R., Ganesh Kumar, Krishna Prasad, B.T., Mathew, M.K., Udaykumar, M.: A stress-responsive gene from groundnut, Gdi-15, is homologous to flavinol 3-0-glucosyl transferase involved in anthocyanin biosynthesis. – Biochem. biophys. Res. Commun. **284**: 574-579, 2001.

Greenway, H., Munns, R.: Mechanism of salt tolerance in non halophytes. – Annu. Rev. Plant Physiol. **31**: 367-379, 1980.

Giridara Kumar, S., Lakshmi, A., Madhusudhan, K.V., Ramanjulu, S., Sudhakar, C.: Photosynthesis parameters in two cultivars of mulberry differing in salt tolerance. – Photosynthetica **36**: 611-616, 1999.

Heuer, B., Nadler, A.: Physiological response of potato plants to soil salinity and water deficit. – Plant Sci. **137**: 43-51, 1998.

Hsiao, T.C.: Plant responses to water stress. – Annu. Rev. Plant Physiol. **24**: 519-570, 1973.

Hsiao, T.C., Acevedo, E., Fereres, E., Henderson, D.W.: Water stress, growth and osmotic adjustment. – Phil. Trans. roy. Soc. London **B 273**: 479-500, 1976.

Irigoyen, J.J., Emerich, D.W., Sánchez-Díaz, M.: Alfalfa leaf senescence induced by drought stress: photosynthesis, hydrogen peroxide metabolism, lipid peroxidation and ethylene evolution. – Physiol. Plant. **84**: 67-72, 1992.

Kandpal, R.P., Vaidyanathan, C.S., Udaykumar, M., Krishnasastry, K.S., Appaji Rao, N.: Alteration in the activities of the enzymes of proline metabolism in ragi (*Eleusine coracana*) leaves during water stress. – J. Biosci. **3**: 361-369, 1981.

Kicheva, M.I., Tsonev, T.D., Popova, L.P.: Stomatal and nonstomatal limitations to photosynthesis in two wheat cultivars subjected to water stress. – Photosynthetica **30**: 107-116, 1994.

Lawlor, D.W., Uprety, D.C.: Effects of water stress on photosynthesis of crops and the biochemical mechanism. – In: Abrol, Y.P., Mohanty, P., Govindjee (ed.): Photosynthesis: Photoreactions to Plant Productivity. Pp. 419-449. Kluwer Academic Publ., Dordrecht – Boston – London 1993.

Leegood, R.C., Walker, D.A., Foyer, C.H.: Regulation of the Benson-Calvin cycle. – In: Barber, J., Baker, N.R. (ed.): Photosynthetic Mechanisms and the Environment. Pp. 189-258. Elsevier, Amsterdam – New York – Oxford 1985.

Ramanjulu, S., Sreenivasalu, N., Giridhara Kumar, S., Sudhakar, C.: Photosynthetic characteristics in mulberry during water stress and rewatering. – Photosynthetica **35**: 259-263, 1998.

Ramanjulu, S., Sudhakar, C.: Drought tolerance is partly related to amino acid accumulation and ammonia assimilation: A comparative study in two mulberry genotypes differing in drought sensitivity. – J. Plant Physiol. **150**: 345-350, 1997.

Ramanjulu, S., Sudhakar, C.: Proline metabolism during dehydration in two mulberry genotypes with contrasting drought tolerance. – J. Plant Physiol. **157**: 81-85, 2000.

Renou, J.-L., Gerbaud, A., Just, D., André, M.: Differing substomatal and chloroplastic CO_2 concentrations in water-stressed wheat. – Planta **182**: 415-419, 1990.

Savé, R., Biel, C., Domingo, R., Ruiz-Sánchez, M.C., Torrecillas, A.: Some physiological and morphological characteristics of citrus plants for drought resistance. – Plant Sci. **110**: 167-172, 1995.

Sheoran, I.S., Singal, H.R., Singh, R.: Effect of cadmium and nickel on photosynthesis and enzymes of the photosynthetic carbon reduction cycle in pigeonpea (*Cajanus cajan* L.). – Photosynth. Res. **23**: 345-351, 1990.

Sudhakar, C., Lakshmi, A., Giridarakumar, S.: Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. – Plant Sci. **161**: 613-619, 2001.

Veeranjaneyulu, K.: Mechanism of Heavy Metal Tolerance in Plants. – Ph.D. Thesis. Sri Venkateswara University, Tirupati 1978.