

BRIEF COMMUNICATION

Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes

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Abstract

Mulberry genotypes were subjected to salinity ($0-12 \text{ mS cm}^{-1}$) in pot culture experiment. Chlorophyll and total carotenoid contents were reduced considerably by salinity. At low salinity, photosynthetic CO_2 uptake increased over the control, but it decreased at higher salinity. Contents of soluble proteins, free amino acids, soluble sugars, sucrose, starch, and phenols increased at salinity of $1-2 \text{ mS cm}^{-1}$ and decreased at higher salinity ($8-12 \text{ mS cm}^{-1}$). Glycine betaine accumulated more than proline, the maximum accumulation of both was at salinity of $2-4 \text{ mS cm}^{-1}$. Among the genotypes studied, BC2-59 followed by S-30 showed better salinity tolerance than M-5.

Additional key words: carotenoids; chlorophyll; glycine betaine; *Morus alba*; phenols; proline; proteins; saccharides; starch.

Soil salinity is one of the most important negative factors in agriculture that leads to severe crop loss every year. Under salinity, the reduction of growth is often accompanied by lower photosynthetic rate due to lower stomatal conductance as well as lower capacity of the plant to CO_2 fixation (Stiborová *et al.* 1987). In the present study, mulberry genotypes were exposed to various concentrations of artificial saline water and effects on chloroplast pigments, net photosynthetic CO_2 uptake (P_N), and contents of various organic compounds including osmolytes such as proline and glycine betaine were studied.

Mulberry (*Morus alba* L.) genotypes BC2-59 and S-30 were chosen as test plants together with M-5, a commonly cultivated mulberry cultivar in Tamilnadu, India. BC2-59 and S-30 showed tolerance to both coastal salinity and coastal drought (water stress) (Agastian 1997). Cuttings of BC2-59, S-30, and M-5 with 3 nodes were planted in the earthen pots containing sandy loam red soil [$\text{pH } 8.000 \pm 0.005$; electrical conductivity (EC) $0.21 \pm 0.01 \text{ mS cm}^{-1}$]. Irrigation with available water [$\text{pH } 8.30 \pm 0.02$, EC $0.07 \pm 0.01 \text{ mS cm}^{-1}$] was used upto 30 d for establishment. Artificial saline water was prepared using NaCl , Na_2SO_4 , and CaCl_2 by keeping ratios of

$\text{Na}^+ : \text{Ca}^{2+}$ and $\text{Cl}^- : \text{SO}_4^{2-}$ as 4 : 1 (meq. basis) in different salinities [0 (control), 1, 2, 4, 8, 12 mS cm^{-1}]. Plants were irrigated to the maximum capacity of the pots twice in a week. After 60 d of plantation, P_N was measured using Portable Infra-Red Gas Analyser (LI-COR model LI 6252). Later the plants were uprooted and shade dried until complete loss of water (~ 2 d), powdered, and used for biochemical analyses. Chlorophylls (Arnon 1949), carotenoids (Goodwin 1954), total soluble proteins (Lowry *et al.* 1951), total free amino acids (Troll and Canan 1953), total soluble sugars (Dubois *et al.* 1956), total soluble sucrose (Van Handel 1968), total starch (McCready *et al.* 1950), total phenols (Swain and Hillis 1959), total free proline (Bates *et al.* 1973), and glycine betaine (Storey and Wyn Jones 1977) were estimated. The values presented are the means of three different experiments.

Chlorophyll (Chl) *a*, Chl *b*, and total carotenoids (Car) were affected differently by salinity, but in general their contents decreased with salinity (Table 1). Among the genotypes, M-5 was affected more severely by salinity followed by the S-30 genotype. BC2-59 showed lesser reduction in Chl *a* and *b* contents. P_N was higher in BC2-59 than in the other two genotypes. At lower salinities

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(1-2 mS cm⁻¹) P_N was higher compared to the control irrespective of genotype. Higher salinities inhibited P_N also in the promising tolerant genotypes BC2-59 and S-30.

The contents of soluble proteins, free amino acids, soluble sugars, sucrose, starch, and phenols increased at low salinity (1-2, in certain cases upto 4 mS cm⁻¹) and decreased at higher salinity (8 and 12 mS cm⁻¹) irrespective of genotype. But the magnitude of decrease was higher in M-5 (free amino acids, soluble sugars, sucrose, and phenols) followed by S-30, and only marginal reduction was found in BC2-59. However, the contents of soluble proteins and starch showed marginal decrease in M-5, followed by BC2-59 and S-30. Osmoprotectants (proline and glycine betaine) accumulated under stress (Table 2). There was significant increase in content of osmoprotectants under lower salinity (1-4 mS cm⁻¹) and their contents decreased at high salinity (8-12 mS cm⁻¹). Trend of proline accumulation was similar to that of glycine betaine, but its content was higher. Both proline and glycine betaine accumulated more in BC2-59 than in S-30 and M-5.

Generally, the genotype BC2-59 was more salt tolerant than its counterparts (S-30 and M-5). The

significant increase in chloroplast pigment contents and the uninhibited P_N upto 8 mS cm⁻¹ was observed in BC2-59. The increase in contents of chloroplast pigments in mulberry in response to salinity was found by Anas and Vivekanandan (1994). Cultivar specific responses in Chl content were observed in rice and wheat (Peiris and Ranasinghe 1993). The contents of soluble proteins, free amino acids, soluble sugars, sucrose, starch, and phenols increased under lower salinities and decreased at high salinity (12 mS cm⁻¹). Similarly, Ramanjulu *et al.* (1993) reported that K content decreased, while the contents of amino acids, proline, and Na⁺ increased in NaCl-treated mulberry. Accumulation of proline, glycine betaine, and sucrose in leaves of salt-stressed mulberry genotypes were similar to the findings of Flowers *et al.* (1977). Stewart and Hanson (1980) suggest that glycine betaine contributes to osmotic adjustment in the leaves in response to water stress; this was also seen in our experiment. Our results are also in agreement with those of Ramanjulu *et al.* (1998a, b) who determined photosynthetic characteristics of mulberry under water stress. In conclusion, genotype BC2-59 was found most suitable for saline land cultivation among the three genotypes.

Table 1. Chloroplast pigments (chlorophyll - Chl, total carotenoids - Car) [g kg⁻¹(d.m.)] and net photosynthetic rate (P_N) [$\mu\text{mol}(\text{CO}_2 \text{ m}^{-2} \text{ s}^{-1})$] of mulberry genotypes subjected to salinity [mS cm⁻¹]. Means of three different experiments \pm SE; $p < 0.05$.

Genotype	Salinity	Chl <i>a</i>	Chl <i>b</i>	Chl <i>a/b</i>	Car	P_N
M-5	0	1.84 \pm 0.03	0.86 \pm 0.01	2.14 \pm 0.04	0.59 \pm 0.01	150.00 \pm 7.50
	1	1.78 \pm 0.03	0.83 \pm 0.02	2.14 \pm 0.04	0.60 \pm 0.01	190.00 \pm 9.50
	2	1.59 \pm 0.03	0.73 \pm 0.01	2.18 \pm 0.04	0.47 \pm 0.01	190.00 \pm 8.56
	4	1.22 \pm 0.02	0.56 \pm 0.01	2.17 \pm 0.04	0.36 \pm 0.01	146.00 \pm 7.30
	8	0.72 \pm 0.01	0.31 \pm 0.01	2.32 \pm 0.05	0.25 \pm 0.01	143.00 \pm 7.15
	12	0.56 \pm 0.01	0.23 \pm 0.01	2.43 \pm 0.05	0.18 \pm 0.01	136.00 \pm 6.80
BC2-59	0	2.70 \pm 0.05	1.24 \pm 0.03	2.18 \pm 0.05	0.87 \pm 0.02	246.00 \pm 12.30
	1	3.17 \pm 0.07	1.46 \pm 0.04	2.17 \pm 0.05	0.92 \pm 0.02	333.00 \pm 11.10
	2	2.41 \pm 0.06	1.08 \pm 0.02	2.23 \pm 0.05	0.76 \pm 0.02	299.00 \pm 14.95
	4	3.01 \pm 0.08	1.40 \pm 0.04	2.15 \pm 0.05	0.90 \pm 0.02	297.00 \pm 14.80
	8	2.58 \pm 0.07	1.31 \pm 0.03	1.97 \pm 0.04	0.91 \pm 0.02	254.00 \pm 8.46
	12	2.25 \pm 0.06	1.14 \pm 0.03	1.97 \pm 0.03	0.82 \pm 0.02	186.00 \pm 5.31
S-30	0	3.10 \pm 0.08	1.30 \pm 0.02	2.38 \pm 0.03	0.89 \pm 0.04	236.00 \pm 7.86
	1	2.44 \pm 0.04	1.00 \pm 0.02	2.44 \pm 0.04	0.79 \pm 0.03	301.00 \pm 8.60
	2	2.09 \pm 0.03	1.02 \pm 0.03	2.04 \pm 0.02	0.80 \pm 0.04	257.00 \pm 7.34
	4	2.92 \pm 0.07	1.24 \pm 0.04	2.35 \pm 0.03	0.87 \pm 0.04	235.00 \pm 6.71
	8	2.21 \pm 0.05	0.92 \pm 0.02	2.51 \pm 0.05	0.73 \pm 0.02	204.00 \pm 5.82
	12	2.10 \pm 0.04	0.88 \pm 0.02	2.28 \pm 0.03	0.72 \pm 0.02	180.00 \pm 5.14

Table 2. Analysis of leaves [$\text{g kg}^{-1}(\text{d.m.})$] of mulberry genotypes subjected to salinity [mS cm^{-1}]. Means of three different experiments $\pm \text{SE}$; $p < 0.05$.

Genotype	Salinity	Soluble proteins	Free amino acids	Soluble sugars	Sucrose	Starch	Phenols	Proline	Glycine betaine
M-5	0	97.74 \pm 1.95	16.55 \pm 0.33	51.01 \pm 1.13	31.33 \pm 0.63	17.75 \pm 0.35	8.38 \pm 0.17	1.30 \pm 0.02	2.11 \pm 0.04
	1	115.12 \pm 2.30	18.52 \pm 0.37	42.43 \pm 0.84	36.34 \pm 0.72	19.87 \pm 0.39	8.18 \pm 0.16	1.32 \pm 0.03	4.70 \pm 0.09
	2	120.63 \pm 2.41	19.32 \pm 0.48	46.87 \pm 0.93	30.56 \pm 0.61	23.75 \pm 0.47	8.86 \pm 0.81	1.83 \pm 0.03	5.27 \pm 0.10
	4	126.55 \pm 2.53	22.73 \pm 0.50	32.47 \pm 0.64	28.18 \pm 0.56	27.26 \pm 0.54	6.48 \pm 0.12	1.44 \pm 0.03	5.34 \pm 0.12
	8	105.65 \pm 2.11	19.03 \pm 0.42	32.88 \pm 0.65	27.82 \pm 0.55	21.60 \pm 0.43	6.28 \pm 0.12	1.13 \pm 0.02	3.13 \pm 0.06
	12	84.54 \pm 1.69	12.53 \pm 0.27	31.44 \pm 0.78	17.37 \pm 0.34	18.13 \pm 0.36	5.70 \pm 0.11	0.90 \pm 0.01	2.73 \pm 0.05
BC2-59	0	118.72 \pm 2.37	23.53 \pm 0.47	52.84 \pm 1.06	31.93 \pm 0.63	21.66 \pm 0.43	8.30 \pm 0.16	1.40 \pm 0.02	3.07 \pm 0.06
	1	146.32 \pm 2.92	23.63 \pm 0.47	50.44 \pm 1.01	39.87 \pm 0.79	22.13 \pm 0.44	11.12 \pm 0.22	1.73 \pm 0.03	4.67 \pm 0.09
	2	146.21 \pm 2.92	23.72 \pm 0.47	54.67 \pm 1.09	34.13 \pm 0.68	29.82 \pm 0.59	10.37 \pm 0.21	3.28 \pm 0.06	6.10 \pm 0.12
	4	127.63 \pm 2.55	29.07 \pm 0.58	56.46 \pm 1.12	33.67 \pm 0.67	28.42 \pm 0.56	9.33 \pm 0.18	2.40 \pm 0.04	7.03 \pm 0.14
	8	125.21 \pm 2.50	23.03 \pm 0.46	45.87 \pm 0.91	30.53 \pm 0.61	23.52 \pm 0.47	8.97 \pm 0.17	1.36 \pm 0.02	5.70 \pm 0.11
	12	106.71 \pm 2.13	20.73 \pm 0.41	45.65 \pm 0.91	27.45 \pm 0.55	19.90 \pm 0.39	8.63 \pm 0.17	1.26 \pm 0.02	4.07 \pm 0.08
S-30	0	100.31 \pm 2.01	26.22 \pm 0.52	50.73 \pm 1.01	32.04 \pm 0.64	24.77 \pm 0.49	12.73 \pm 0.25	1.17 \pm 0.02	2.86 \pm 0.05
	1	159.02 \pm 3.18	33.05 \pm 0.66	51.88 \pm 1.03	30.29 \pm 0.60	27.83 \pm 0.55	13.20 \pm 0.26	2.10 \pm 0.04	5.10 \pm 0.10
	2	145.01 \pm 2.90	29.53 \pm 0.59	55.44 \pm 1.10	38.05 \pm 0.76	24.88 \pm 0.49	10.86 \pm 0.21	3.62 \pm 0.07	5.98 \pm 0.12
	4	143.74 \pm 2.87	24.53 \pm 0.49	53.67 \pm 1.07	34.33 \pm 0.68	20.23 \pm 0.40	10.43 \pm 0.20	2.63 \pm 0.05	6.73 \pm 0.13
	8	131.13 \pm 2.62	20.52 \pm 0.41	51.47 \pm 1.02	32.08 \pm 0.64	19.63 \pm 0.39	8.94 \pm 0.17	1.84 \pm 0.03	4.37 \pm 0.08
	12	126.84 \pm 2.53	20.07 \pm 0.40	40.83 \pm 0.81	22.62 \pm 0.45	19.43 \pm 0.38	8.73 \pm 0.17	1.71 \pm 0.03	3.86 \pm 0.07

References

- Agastian, P.: Identification of Mulberry Genotypes for Coastal Salinity through Chemo- and Bio-Assay Tests. - Ph.D. Thesis. Bharathidasan University, Tiruchirappalli 1997.
- Anas, S.S.M., Vivekanandan, M.: Salinity responses of the triploid mulberry varieties. - Indian J. Seric. **33**: 92-94, 1994.
- Arnon, D.I.: Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. - Plant Physiol. **24**: 1-15, 1949.
- Bates, L.S., Waldron, R.P., Teare, I.D.: Rapid determination of free proline for water stress studies. - Plant Soil **39**: 205-207, 1973.
- Dubois, M., Gilles, K.N., Hamilton, J.K., Revers, P.A., Smith, F.: Colourimetric method for determination of sugars and related substances. - Anal. Chem. **28**: 300-356, 1956.
- Flowers, T.J., Troke, P.F., Yeo, A.R.: The mechanism of salt tolerance in halophytes. - Annu. Rev. Plant Physiol. **28**: 89-121, 1977.
- Goodwin, T.W.: Carotenoids. - In: Peach, K., Tracey, M.V. (ed.): Handbook of Plant Analysis. Pp. 272-311. Springer-Verlag, Berlin 1954.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with the Folin phenol reagent. - J. biol. Chem. **193**: 265-275, 1951.
- Mc Cready, R.M., Guggloz, J., Silvier, V., Owens, H.S.: Determination of starch and amylose in vegetables. - Anal. Chem. **22**: 1156, 1950.
- Peiris, B.D., Ranasinghe, A.: Effect of sodium chloride salinity on chlorophyll content in rice (*Oryza sativa*) leaves. - Indian J. Plant Physiol. **36**: 257-258, 1993.
- Ramanjulu, S., Sreenivasulu, N., Giridhara Kumar, S., Sudhakar, C.: Photosynthetic characteristics in mulberry during water stress and rewetting. - Photosynthetica **35**: 259-263, 1998a.
- Ramanjulu, S., Sreenivasulu, N., Sudhakar, C.: Effect of water stress on photosynthesis in two mulberry genotypes with different drought tolerance. - Photosynthetica **35**: 279-283, 1998b.
- Ramanjulu, S., Veeranjaneyulu, K., Sudhakar, C.: Physiological changes induced by NaCl in mulberry var. Mysore local. - Indian J. Plant Physiol. **36**: 273-275, 1993.
- Stewart, C.R., Hanson, A.D.: Proline accumulation as a metabolic response to water stress. - In: Turner, N.C., Kramer, P.J. (ed.): Adaptation of Plants to Water and High Temperature Stress. Pp. 173-189. Wiley and Sons, New York 1980.
- Stiborová, M., Kšínská, S., Březinová, A.: Effect of NaCl on the growth and biochemical characteristics of photosynthesis of barley and maize. - Photosynthetica **21**: 320-328, 1987.
- Storey, R., Wyn Jones, R.G.: Quaternary ammonium compounds in plants in relation to salt resistance. - Phytochemistry **16**: 447-453, 1977.
- Swain, T., Hillis, W.O.: The phenolic constituents of *Prunus domestica*. The quantitative estimation of phenolic constituents. - J. Sci. Food Agric. **10**: 63-68, 1959.
- Troll, W., Canan, K.: A modified photometric ninhydrin method for the analysis of amino-imino acids. - J. biol. Chem. **200**: 803-811, 1953.
- Van Handel, E.: Dried microdetermination of sucrose. - Ann. Biochem. **22**: 280-283, 1968.