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BRIEF COMMUNICATION

Variation in photosynthetic rates and biomass productivity among four mulberry cultivars

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Abstract

Among four mulberry (*Morus alba* L.) cultivars (K-2, MR-2, BC2-59, and S-13), highest net photosynthetic rate (P_N) was observed in BC2-59 while the lowest rates were recorded with K-2. Significant differences among the four cultivars were found in leaf area, biomass production, activities of ribulose-1,5-bisphosphate carboxylase and sucrose phosphate synthase, and glucose and sucrose contents. The P_N and the activities of photosynthetic enzymes in the four cultivars were significantly correlated with the growth and biomass production measured as leaf yield, total shoot mass, and aerial plant biomass.

Additional key words: branches; chlorophyll; DCPIP photoreduction; glucose; *Morus*; ribulose-1,5-bisphosphate carboxylase; shoot; starch; sucrose; sucrose phosphate synthase.

Mulberry is one of the most important cash crops in India, which is the only source food material for developing silkworm. Mulberry is capable of thriving under a variety of agro-climatic conditions. Recommendations for cultivar selection based on photosynthetic productivity in mulberry have seldom been done. Mulberry is sensitive (Agastian *et al.* 2000), responding extremely well to the optimum agricultural inputs but showing practically no growth when plant nutrients and moisture operate as limiting factors (Susheelamma *et al.* 1990, Dorcus and Vivekanandan 1997).

The photosynthetic performance of plants could be used for the prognosis of plant productivity. Induction of photosynthesis in nature usually takes place by orderly light mediated activation of the enzymes of PCR cycle, thus establishing a system for its regulation (Leegood 1985, Dietz 1986). The ecological behaviour of the plants is determined by photosynthetic productivity, coupled to the use of photosynthetic products in growth and development. Manipulation of the photosynthetic system by genetic engineering using molecular biological modification of source and sinks will result in the production of the plants with resistance to different agro-climate. We report the use of morphological, physiological, and biochemical analyses to understand the potential variations among four different mulberry cultivars. Attempts were also made to examine the morpho-physiological characters with a view to build up the preliminary selection criteria for best performance of mulberry cultivars.

Mulberry plants (*Morus alba* L. cvs. K-2, MR-2, BC2-59, and S-13), obtained from the regional sericultural research station, Coonoor, Tamilnadu, India, were propagated in 30 cm pots under 12 h natural photoperiod [irradiance (400-700 nm) of 1 600-1 800 μmol m^{-2} s^{-1}] with day and night temperatures of 30/23 °C and approximate air humidity of 60 %. The plants were well watered and periodically fertilised with nutrient solution. Third or fourth leaf from top of one-year-old plant was collected for all the physiological and biochemical measurements. Plant height, number of branches, total length of branches, leaf yield, shoot mass, and aerial biomass were determined.

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Abbreviations: Chl, chlorophyll; DCPIP, 2,6-dichlorophenol indophenol; DTT, dithiothreitol; FeCN, ferricyanide; PVP, polyvinylpyrrolidone; PS2, photosystem 2; RuBPC, ribulose-1,5-bisphosphate carboxylase; SPS, sucrose phosphate synthase; UDP, uridine-diphosphate.
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P_N was measured according to Ramachandra Reddy and Nandan Kumar (1996), being monitored after pre-irradiation of the leaves for 40 min. Irradiation was by halide flood lamps behind a water screen. P_N measurement in individual leaves from different shoots was repeated three to four times. Chloroplasts were isolated according to Leegood and Walker (1993). Freshly harvested leaves were irradiated for 15 min to degrade leaf starch. 5 g of leaves was cut into strips and homogenised in a semifrozen grinding medium which consisted of 0.33 M sorbitol, 10 mM $Na_4P_2O_7$, 5 mM $MgCl_2$, 1 % *PVPP-40*, 0.5 mM DTT, and 2 mM sodium ascorbate at pH 6.5. The crude extract was squeezed through two layers of cheese-cloth and the filtrate centrifuged at 250×*g* for 5 min to remove cell debris. The supernatant was then centrifuged at 2 500×*g* for 10 min. The pellet was suspended in a cold medium consisting of 0.33 M sorbitol, 2 mM EDTA, 1 mM $MnCl_2$, and 50 mM HEPES (pH 7.6). A portion of this chloroplast preparation was layered on a sucrose gradient comprising 1.50, 1.00, and 0.75 M sucrose in 10 mM tricine-KOH (pH 7.6) and centrifuged at 2 500×*g* for 15 min. The chloroplasts at the interface between 1.00 and 1.50 M sucrose were diluted with a suspension medium consisting of 0.33 M sorbitol, 50 mM HEPES (pH 7.6), 2 mM EDTA, 1 mM $MgCl_2$, and 1 mM $MnCl_2$. This suspension was centrifuged at 5 000×*g* for 5 min to yield a pellet of intact purified chloroplasts. The intactness of the purified chloroplasts used in the present study was 80-85 % according to Lilley *et al.* (1975). The photochemical activities in the isolated chloroplasts were determined spectrophotometrically as described by Raghavendra and Das (1976).

Enzyme extractions were performed at 4 °C. The leaf blades (10 g) were homogenised with a grinding medium which consists of 100 mM Tris-HCl (pH 7.8) containing 5 mM DTT, 10 mM $MgCl_2$, 1 mM EDTA, 5 mM magnesium acetate, and 1.5 % *PVP-40*. The homogenate was squeezed through four layers of cheesecloth and then centrifuged at 10 000×g for 10 min. The protein was precipitated with 75 % (m/v) ammonium sulphate and spun at 30 000×*g* for 30 min. The precipitate was dissolved in 50 mM Tris-HCl buffer (pH 8.0) which contained 1 mM DTT and 0.2 mM NADPH. Ribulose-1,5-bisphosphate carboxylase (RuBPC) activity was assayed at 30 °C by the incorporation of $^{14}CO_2$ into acid stable products (Lorimer *et al.* 1977) and the radioactivity was measured in the liquid scintillation counter. Sucrose phosphate synthase (SPS) was assayed at 30 °C by measuring the production of UDP (Huber 1981). Chl content was determined spectrophotometrically in 80 % acetone extract (Arnon 1949).

The contents of starch and sucrose in the leaf tissues were estimated enzymatically according to Ramachandra Reddy *et al.* (1996). The total sugar content in the 80 % ethanolic extract was determined using the anthrone method (Dubios *et al.* 1956).

Cultivars BC2-59 and S-13 possessed significantly higher biomass yields than other cultivars (Table 1). Plants were significantly higher in the BC2-59 cultivar (176.3 cm) while cultivar S-13 contained more branches (13.3) with a maximum branch length of 1 418 cm (Table 1). The biomass production was high in cultvars BC2-59 and S-13 (1 249.1 and 1 139.0 g, respectively) compared to that of K-2 or MR-2 (404.0 and 785.1 g, respectively).

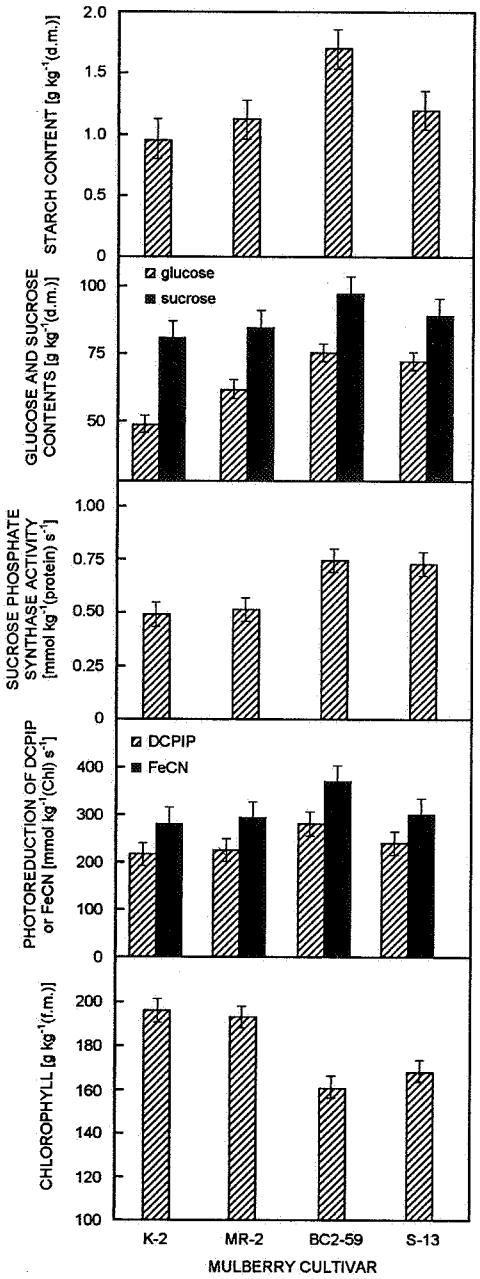

Fig. 1. Total chlorophyll content in the leaves, photosystem 2 activities measured as photoreduction of DCPIP or FeCN in isolated chloroplasts, sucrose phosphate synthase activity, and contents of glucose, sucrose, and starch of four mulberry cultivars (K-2, MR-2, BC2-59, and S-13). Means of at least four independent determinations ± one SE.

Table 1. Morphological characteristics, photosynthetic rates, and RuBPC activity of four mulberry cultivars under preliminary yield trial. Average values for three seasons: spring (February to April 1999), summer (May to July 1999), and rainy (August to October 1999).

Parameter	Mulberry cultivar			
	K-2	MR-2	BC2-59	S-13
Plant height [cm]	115.0	145.0	176.0	160.0
Number of branches per plant	8.1	12.7	9.3	13.3
Total length of branches [cm]	576.0	1053.0	1002.0	1418.0
Leaf yield per plant [g]	252.0	465.7	726.3	638.0
Mass of shoot per plant [g]	152.0	319.4	522.8	501.0
Aerial biomass per plant [g]	404.0	785.1	1249.1	1139.0
Photosynthetic rate [mg(CO_2) m^{-2} s^{-1}]	0.91	0.99	1.28	1.12
RuBPC activity [mol kg^{-1}(Chl) s^{-1}]	76.40	77.45	91.70	88.50

Chloroplasts from cvs. S-13 and BC2-59 possessed high activity of DCPIP and FeCN reduction associated with relatively high Chl content and this indicates a high PS2 capacity (Fig. 1). P_N depends on the development of the photosynthetic system including the energy-transducing components and on enzymes of the carbon reduction cycle (Evans and Terashima 1988, Geiger and Servaites 1994). A significant variation in P_N among the mulberry cultivars was presumed to result from the rate-limiting enzyme activity of RuBPC (Table 1), and this might strongly influence the productivity of mulberry plants. Hence the cvs. S-13 and BC2-59 possessed relatively high activity of the carboxylating system thus assuring high P_N. We presume that the carbon assimilating capacity and the biomass productivity in mulberry might be limited by the activity of RuBPC. Our results also show that the cultivars with high carboxylating activities possess relatively large biomass productivity.

Sucrose phosphate synthase, which is important in sucrose partitioning, regulates sucrose synthesis. Source-sink studies of whole plants showed that the photosynthetic carbon can be differentially partitioned into sucrose and starch available for export during periods of high sink demand or retained as starch when the sink demand is low (Rama Das and Ramachandra Reddy 1988). When sucrose synthesis is insufficient to use all of the triose phosphate made available by photosynthesis, photosynthesis becomes feedback limited and this is characterised by loss of sensitivity of photosynthesis to CO_2 and O_2 partial pressures (Vassey and Sharkey 1989). The determined activities of sucrose phosphate synthase, along with the glucose, sucrose, and starch contents, demonstrate that starch and sucrose syntheses and their accumulation indirectly play a crucial role in partitioning the photosynthetically fixed carbon (Fig. 1).

Although activities of the photosynthetic enzymes and photoreduction in the chloroplasts varied among all the four mulberry cultivars, the cvs. S-13 and BC2-59, with their high photosynthetic activity and high biomass yields, are useful for the sericulture industry. These results can be used in mulberry breeding programs or transgenic mulberry research to generate plants with higher photosynthetic activity to obtain superior biomass yields.

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