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Growth performance in contrasting sets of mulberry (*Morus* Spp.) genotypes explained by logistic and linear regression models using morphological and gas exchange parameters

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ABSTRACT

Plant growth is key determinant for leaf yield and biomass accumulation in mulberry. Accurate prediction of yield ensures brushing of right quantum of silkworms for silk production. Repeated harvesting of leaves/shoots leads to stress with physiological and biochemical changes. This necessitates in-depth analysis of growth attributes leading to rejuvenation and biomass production. A total of 22 contrasting genotypes for growth (CSG) were shortlisted from a diverse set of germplasm based on Number of Days required for Bud Sprouting (NDBS), Shoot Elongation Rate (SER) and Number of Branches (NB) in two distinct seasons. The logistic regression analysis of CSG expressed in odds ratio showed positive regression coefficients for important traits *viz.*, inter-nodal distance (2.71), NB (1.31), Total Shoot Length (TSL, 1.38), Length of the Longest Shoot (LLS, 1.17) and Number of Leaves on the Longest Shoot (NLLS, 1.18) for high growth in August 2016. The growth curve estimates for SER was significantly ($P < 0.0001$) higher among High Growth Genotypes (HGG; 4–6.2 cm/day) compared to Low Growth Genotypes (LGG; 2.6–4.1 cm/day). Repeated measure ANOVA showed significance *A* ($P < 0.0001$), gs ($P < 0.01$), IWUE ($P < 0.01$) between three growth periods. *A*, *Tr*, and gs were higher among HGG compared to LGG and opposite was true in case of IWUE. Similarly, Amax and AQL were higher in HGG whereas Vcmax and Jmax were higher in LGG. Significant linear relationship of *Tr*, gs and IUWE was observed with NB, TSL and LLS. The study concludes that mulberry growth can be predicted using important morpho-metric traits and gas exchange parameters as supported by logistic and linear regression analysis.

1. Introduction

Mulberry (*Morus* spp.) is one among the fastest growing plants with high biomass turnout in comparatively short period of about 70 days. It is a perennial crop of high economic importance and the only source of food for the silkworm (*Bombyx mori* L.) for the production of natural silk. But, the growth potential of different cultivated varieties varies and not exploited fully due to lack of understanding on the morpho-physiological factors contributing to the trait. Plants varieties differ considerably in relative growth rate under identical or optimal condition. This has been observed in both cultivated crops and wild species (Poorter and Bongers, 2006; Van Kleunen et al., 2010). The typical characteristics that distinguish between the fast and slow growth in C_3 species are nutrient acquisition and allocation, morphology, physiology, biochemical composition *etc.,* (Comas and Eissenstat, 2004; Grotkopp and Rejmánek, 2007). Fast growing species have the advantage of rapid canopy development, which should improve the light interception and opportunity to acquire large share in nutrient or water than the slow growing plants (Zhao et al., 2006). In many crop plants such as rice, barley and wheat high growth depends on leaf appearance rate, tiller outgrowth, length of the coleoptile and photosynthetic rate (Spielmeyer et al., 2007; Rebolledo et al., 2012). Conversely, woody plants invest varying proportion of their photosynthate in perennial lignified structure such as shoot, stem, root which allow them to reach tall structure (Cornelissen et al., 1998). On the other hand associations of physiological aspects like photosynthesis and stomatal characteristics influencing the biomass production have been well studied (Kundu and Tigerstedt, 1999).

The mulberry crop is also gaining importance as a forage crop for animals in many Asian and European countries due to the rich nutritional components such as proteins, minerals and antioxidants (Guha et al., 2010). Mulberry foliage is repeatedly harvested by leaf plucking

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or shoots pruning (4–5 times in a year) resulting in stress and altering the physiology and biochemical composition of the plant. This necessitates an in-depth analysis of differential growth and developmental attributes leading to the rejuvenation and biomass production.

The present study was undertaken with the objectives of understanding the mechanism of morpho-physiological parameters in mulberry growth. Contrasting sets of genotypes (CSG) for growth were utilized for the assessment of the contribution of morphological and gas exchange parameters along with light and CO_2 curve analysis to predict the growth performance of mulberry through statistical models.

2. Materials and methods

2.1. Plant materials and experimental design

The experimental plot with red sandy loam soil was located at Central Sericultural Research and Training Institute (CSRTI), Mysuru, India (12°15′39.3″ N, 76°37′30.5″ E). A diverse set of 210 mulberry germplasm, each with 4 clonal ramets was established and maintained under Augmented Random Block Design (ARBD). The crown height was maintained at 0.9 m by pruning with spacing of 1.5 m between the plants. The plantation was maintained under standard package of practices recommended by the Institute (Sarkar et al., 2000).

The growth parameters *viz.*, Number of Days required for Bud Sprouting (NDBS), Shoot Elongation Rate (SER) and Number of Branches (NB) were recorded in three individual plants (clonal ramets) during the cultivation period (70 days) in July–September 2015 and October–December 2015. A substantial variability in growth traits *viz.*, NDBS, NB and SER among the 210 diverse mulberry germplasm was observed in two growth cycles (Supplementary Table 1). A set of CSG was selected (Table 1) based on the assessment of growth parameters showing consistently higher (High Growth Genotypes, HGG) and lower (Low Growth Genotypes, LGG) performance in two cycles (Supplementary Table 2) along with fresh leaf-shoot biomass accumulation (Thangavelu et al., 1997, 2000; Tikader et al., 2006). Mean temperatures of 25.3 °C and 24.4 °C and precipitations of 66.5 mm and 110.9 mm were recorded during the corresponding data measurement periods at the Meteorological Station, CSRTI, Mysuru located 0.6 km from the experimental site.

Table 1
Short-listed contrasting set of genotypes (CSG) based on growth performance in mulberry.

	Genotype	Species	Origin
High Growth Genotypes (HGG)	1. School Salem	*Morus indica*	India
	2. Sujanpur-5	*Morus indica*	India
	3. Jalalgarah-3	*Morus indica*	India
	4. Naudan-1	*Morus indica*	India
	5. *M. macroura*	*Morus* spp.	Japan
	6. *M. laevigata(H)*	*Morus* spp.	India
	7. Mother Graft	*Morus* spp.	India
	8. Georgia	*Morus* spp.	Unknown
	9. Thailand Local	*Morus* spp.	Thailand
	10. Xuan-5	*Morus* spp.	China
	11. S-1	*Morus alba*	Myanmar
Low Growth Genotypes (LGG)	1. Acc.8	*Morus* spp.	India
	2. Kajali	*Morus* spp.	India
	3. China Black-B	*Morus* spp.	China
	4. Meghamalai-1	*Morus indica*	India
	5. Valparai-05	*Morus* spp.	India
	6. Harmutty	*Morus indica*	India
	7. UP-9	*Morus* spp.	India
	8. Matigara White	*Morus* spp.	India
	9. M. indica Black	*Morus* spp.	India
	10.Vadagaraparai-2	*Morus indica*	India
	11. Meergund-6	*Morus* spp.	India

2.2. Plant growth and gas exchange measurements

Growth and photosynthetic traits were evaluated among the CSG in August 2016. All the observations were recorded in 3 individual plants (triplicates) of each genotype unless and otherwise specifically stated. The growth traits *viz.*, NDBS, Inter-nodal Distance (IND), NB, Length of the Longest Shoot (LLS), Total Shoot Length (TSL) and Number of Leaves on Longest Shoot (NLLS) were recorded in all genotypes during the cultivation period of 70 days. Length of the shoot (cm) was measured on 3 well grown branches in three different replicates in specific intervals from the 1st day pruning to 70th day (*i.e.*, on 15th, 30th, 45th, 60th and 70th day). Each time measurement was recorded from the shoot base to the apex and SER was calculated as described by Fukui (2000) using the following equation: SER = (L2–L1)/T. Where, L2 is the length of the shoot (cm) at the time of measurement, L1 is the initial shoot length (cm) and T represent the time-interval in days. Growth functions were used to extrapolate two reliable parameters: maximum growth rate (μ, maximum slope of growth curve) and asymptotic value for shoot growth (A, where the slope of the growth curve reaches zero) for all the genotypes (Ricklefs, 1968). The best fit was assessed based on Akaike Information Criteria (AIC) model selection procedure and the growth parameters were extracted from the best model. The logistic model provided a best fit for all the genotypes. This procedure has been automated in the grofit R library (Kahm et al., 2010).

Gas exchange parameters such as photosynthetic rate (*A*, μmol (CO_2) $m^{-2} s^{-1}$), stomatal conductance (gs, mol $m^{-2} s^{-1}$), transpiration rate (*Tr*, mmol (H_2O) $m^{-2} s^{-1}$) were measured on three leaves per plant in three replicates of each genotype during 45–50 days after pruning on fully expanded leaf using Portable Photosynthesis System LI-6400 (LiCor Inc., USA). The intrinsic water use efficiency (IWUE, μmol (CO_2) $mmol^{-1}$ (H_2O)) was calculated as the ratio between *A* and gs (*A*/gs; Cregg et al., 2000). The photosynthetic photon flux density (PPFD, 1250 μmol $m^{-2} s^{-1}$), leaf cuvette temperature (28 °C) was maintained constant throughout the experiment and fluctuation in ambient CO_2 in air was minimized by passing through a protective enclosure. Soil was moisture saturated during photosynthetic measurements. Before initiating the measurements, reference CO_2 concentration was stabilized for ~5 min and the chamber CO_2 was equilibrated to reference CO_2.

2.3. Physiological measurements

Photosynthetic light response curve was measured on fully elongated, sun-exposed leaf on 22 CSG in triplicates. Initial chamber condition was set at PPFD 2000 μmol $m^{-2} s^{-1}$, cuvette temperature 28 °C with ambient CO_2 concentration (~390 μmol mol^{-1} CO_2). Afterwards the light intensity was reduced stepwise from 2000 to 1500, 1250, 1000, 750, 500, 250 and 50 μmol $m^{-2} s^{-1}$. About 2–3 min were required to reach constant chamber condition in each step for data logging. Maximum photosynthetic rate at light saturation (Amax, μmol $m^{-2} s^{-1}$), apparent quantum yield (AQL) and light compensation point (LCP, μmol $m^{-2} s^{-1}$PPFD) was estimated by fitting the non rectangular hyperbola by non-linear least squares (Marshall and Biscoe, 1980). A total of 66 CO_2 response curve (A/Ci curve) were also developed among CSG. The CO_2 concentration was controlled with external CO_2 mixer. The CO_2 concentration was changed from 400 to 300, 200, 100, 50, 600, 800 and 1000 μmol mol^{-1} CO_2. The data was logged after 2–3 min when the chamber condition was equilibrated. The photosynthetic model (Farquhar et al., 1980) was used for estimating the parameters of A/Ci curve. The plantecophys R package by Duursma (2015) was used for simultaneous estimation of maximum rate of carboxylation (Vcmax, μmol $m^{-2} s^{-1}$), rate of electron transport (Jmax, μmol $m^{-2} s^{-1}$) and intercellular CO_2 concentration (C_{itr}, μmol mol^{-1}) at which the rate of CO_2 assimilation changed from rubisco carboxylated limited to ribulose bisposphate limited (RuBp). The model was fitted with the leaf temperature (28 °C), PPFD (1250), coefficients, Kc,