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An Easy and Simple Method for Estimating Total Shoot Length During Screening and Evaluation of Mulberry (*Morus* spp.) Genotypes

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Abstract

In mulberry (*Morus* sp.), grown for its foliage, which is the sole food for the silkworm (*Bombyx mori* L.), evolving high yielding varieties is a long drawn and laborious process. One of the important selection parameter that has significant positive correlation with leaf yield is Total Shoot Length [TSL] of the mulberry plant. Measuring the length of all the shoots of the test genotypes to get the total shoot length during several stages of screening and evaluation requires enormous skilled manpower and time. The enormity of the task itself most often than not leads to inaccuracies. Due to multi-collinearity among the characters such as number of shoots, length of the longest shoot and total shoot length, the expression of these characters as a single entity could be more accurate and time saving if appropriate statistical relationships are established. In view of this, a regression relationship was derived and a model developed for estimating total shoot length by measuring only the length of longest shoot and number of shoots per plant. The model was tested with four mulberry varieties that are often used as checks in evaluation experiments and significantly high coefficient of determination [R2] ranging from 0.81 to 0.91 were recorded. Further, evaluation of the models with two mulberry genotypes grown under two distinctly different growing environments also showed no significant difference between the estimated and actual total shoot length. These tests confirmed the efficacy of the models across varieties and growing environments, thus paving way for reduction in drudgery, savings in time and resources in mulberry breeding programmes.

Keywords: Evaluation, Models, Mulberry, Multi-collinearity, Total shoot length.

INTRODUCTION

Mulberry (*Morus* spp.) is an economically important crop plant grown for its foliage, which is the sole food of the silkworm (*Bombyx mori* L.). Mulberry, which is a tree in nature, is trained as low bushes and pruned about 5 times a year to harvest nutritious leaves for silkworm rearing. Generally, the number of shoots per plant varies between 5 and 20. Conventional plant breeding programme in mulberry involves evaluation of thousands of individual hybrids in the first phase of hybrid screening. The subsequent two stages are the Primary Yield Evaluation [PYE] and Final Yield Evaluation [FYE] of the short-listed hybrids in specific growing environments. The three-stage evaluation requires about eleven years and involves eight years of data recording. Leaf yield and growth data are recorded five times a year in all the three stages, rendering the process immensely laborious and time consuming.

Leaf yield in mulberry is determined by many growth parameters. It is reported by many workers such as Das and Krishnaswami (1969); Sarkar *et al.,* (1987); Susheelamma *et al.,* (1988); Bari *et al.,* (1989); Bindroo *et al.,* (1990) and Singhvi *et al.,* (2001) that the number of shoots per plant, length of the longest shoot and total shoot length are positively correlated with leaf yield. Hence, these characters are considered important determinants of leaf yield and are measured in screening and evaluation of large heterogeneous populations and shortlisted promising hybrids for final yield evaluation as reported by Sarkar *et al.,* (1987). However, measuring all the shoots of each of the thousands of hybrids during hybrid screening and short-listed ones during replicated trials at PYE and FYE stages, requires enormous skilled manpower and is a time consuming process. This is even more cumbersome in case of experiments conducted in rainfed conditions, where the measurements have to be recorded in a non-destructive method from standing crops. The enormity of the task and the drudgery involved, often leads to inaccuracies. Further, it appears that, while studying the association of morphological characters with leaf yield, collinearity among number of shoots, length of the longest branch and total shoot length was not exploited for establishing a relation to reduce time, manpower and avoid human errors.

Keeping the above in view, the present study was undertaken to establish correlation among number of shoots, length of longest shoot and total shoot length and to find out a way to estimate total shoot length based on number of shoots and length of longest shoot without actually measuring all the shoots of a plant.

MATERIALS AND METHODS

Data sets from primary yield evaluation trial (PYE) and final yield evaluation trial (FYE) under advanced generation breeding programme were considered for the study. Data on four mulberry genotypes, V1, G4, RC1, and RC2, which were used as checks in primary yield evaluation trial, and evaluated under two distinct environments of optimum and sub-optimum irrigation were considered in the first part of the study for developing and validating the models. In the later part of the study aimed at confirming the efficacy of the model across different growing environments, data on varieties V1 and RC1 in the final yield evaluation stage, under optimum and sub-optimum levels of irrigation, were considered. The methodology consisted of model development and model validation and confirmation.

Development of Models

For developing the models, leaf yield and growth data of varieties V1, G4, RC1 and RC2 grown under two environments of optimal and sub-optimal irrigated conditions, for primary yield evaluation [PYE], were considered. The entire package of practices recommended for mulberry was followed and leaf yield and growth data were recorded five times a year for two consecutive years. On 70th day after each pruning, data on number of shoots (NS), length of longest shoot (LLS) and total shoot length (TSL) were recorded from ten plants from each variety. A new variable NS × LLS (the product of NS and LLS) was obtained. Correlation of total shoot length (TSL) with number of shoots, length of the longest shoot and total shoot length (NS × LLS) were calculated. Linear regression relationships of total shoot length (TSL) to NS × LLS were developed for all the varieties under the two growing environments, to develop two models specific to the environment. These models served as original models for further validation. Further, the hypothesis (H0: $b1 = b2$) on the slopes of the two regression models under two conditions for each variety was tested using Z test.

Validation of Models

To confirm validity of the models across growing environments, an independent validation method was adopted, as they are statistically independent. The data from sub-optimum conditions of irrigation was considered as validation set. The regression constants developed (original model) for the four varieties under optimum conditions of irrigation and the data on NS × LLS from the validation set were used to estimate total shoot length (TSL). The estimated total shoot length and the actual total shoot length measured in the validation set were compared using paired t-test following the method of Snedecor and Cochran (1976).

Confirmation

To confirm validity of the developed models across growing environments, data on three variables viz., NS, LLS and actual TSL from two varieties V1 and RC1 grown under optimal and sub-optimal conditions, respectively, were collected for ten harvests over a period of two years. The new variable NS × LLS was computed and used in the regression models obtained earlier from the data of PYE, to get the estimated total shoot length. These estimated total shoot length data were compared to the actual total shoot length using one-way ANOVA with Tukey's multiple comparisons post-test.

RESULTS AND DISCUSSION

Leaf yield in Mulberry is a complex character that is influenced by total shoot length and various workers such as Fotadar (2000); Masilamani *et al.,* (1996) and Susheelamma (1988) have reported the direct and indirect effect of number of shoots, length of the longest shoot and total shoot length on leaf yield. It was also observed that number of shoots and length of the longest shoot in all the varieties tested are negatively correlated. This type of correlation between the component characters may lead to inappropriate evaluation. The current practice of measuring the length of all shoots [5-20/plant] of each plant several times during the three-stage screening and evaluation spread over eleven years, consumes enormous resources. The enormity of the task itself more often than not results in inaccuracies.

In an effort to develop a method that involves measurement of two variables per plant (number of shoots and length of the longest shoot) and then find a new variable, which is the product of these two, to accurately estimate the total shoot length of a plant, consistent and positive correlations were observed between total shoot length (TSL) and number of shoots ($p<0.01$) and also between TSL and NS × LLS ($p<0.0001$). However, no significant correlation was observed between TSL and length of the longest shoot (Table 1). The positive correlation of total shoot length [TSL] to number of shoots [NS] was relatively stronger in all genotypes in comparison to its correlation with length of the longest shoot. Scatter plots and linear regression relation between total shoot length and NS × LLS for the varieties V1, G4, RC1 and RC2 under optimal and sub optimal conditions of irrigation are shown in Fig.1. A linear function always best described the relationships. The regression model, coefficient of determination and the Z statistic for the comparison of the slopes of the models were also estimated (Table 2). High coefficient of determination (R2 = 0.81 to 0.91) was observed in all the genotypes, thus explaining the fitness of the model. The Z statistics for the comparison of the slopes of the models for two growing conditions in all the genotypes were found to be not significant at $p<0.01$. The model developed for optimal conditions was validated using the data on NS × LLS with sub optimum conditions, which revealed the consistency of the newly defined variable, NS × LLS in explaining the total shoot length across varieties and growing environments. The estimated total shoot length thus obtained was compared with the actual total shoot length measured using paired t-test (Table 3). The t-statistics were found to be not significant in all the genotypes confirming the validity of the developed model. Confirmatory tests conducted with the relevant data sets of two varieties, V1 and RC1, from the final yield evaluation (FYE) under two growing conditions to predict total shoot length showed that there is no statistical difference between the models' mean estimates, or estimated and actual mean total shoot length (Table 4).

The new method developed, is less laborious, simple, faster and avoids inaccuracies, since it involves measuring two variables, which are relatively easier when compared to the enormity of measuring all the shoots of all genotypes over several crops. Also the method is robust and consistent across different varieties and growth environments. Adopting the suggested method in mulberry screening and evaluation would lead to reduction in drudgery and effective utilization of resources without compromising on the accuracy of data.

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Tables

Table 1. Correlation among various characters under optimal and sub optimal conditions of irrigation

Character	Genotypes	Optimum Irrigation	Sub-optimum Irrigation
Number of shoots [NS]	V1	0.687**	0.675**
	G4	0.674**	0.613**
	RC1	0.585*	0.680**
	RC2	0.676**	0.621*
Length of the longest shoot (LLS)	V1	-0.035	0.105
	G4	0.153	0.136
	RC1	0.105	-0.107
	RC2	-0.004	-0.074
NS × LLS	V1	0.911***	0.953***
	G4	0.953***	0.943***
	RC1	0.902***	0.933***
	RC2	0.936***	0.922***

** p<0.001 and *** p<0.0001

Table 2. Original models for four varieties grown under optimal and sub optimal conditions of irrigation

Mulberry Variety	Original regression model				Z stat	p<0.01
	Optimum irrigation	R^2	Sub optimum irrigation	R^2		
Estimated	Y=2.162+0.647x	0.829	Y=1.110+0.729x	0.909	1.22	NS
	Y=1.687+0.666x	0.908	Y=1.538+0.669x	0.889	0.06	NS
	Y=2.235+0.604x	0.814	Y=1.095+0.715x	0.869	1.53	NS
	Y=1.378+0.701x	0.876	Y=2.232+0.637x	0.849	0.94	NS

NS= Not significant

Table 3. Estimation of total shoot length and comparison with actual total shoot length as measured

Mulberry variety	Original regression model (Optimum irrigation)	R2	Total shoot length (m)		t stat p<0.01
			Actual	Estimated	
V1	Y=2.162+0.647x	0.829	12.10	11.92	NS
G4	Y=1.687+0.666x	0.908	9.79	9.90	NS
RC1	Y=2.235+0.604x	0.814	8.98	8.90	NS
RC2	Y=1.378+0.701x	0.876	11.71	11.81	NS

NS = Not significant

Table 4. Comparison of Total shoot length estimated using the two models with the actual values as measured in the FYE trial

Mulberry variety	Estimated total shoot length (m)		Actual total shoot length (m)	p_a (Tukeys)
	Regression model -1 (Optimum irrigation)	Regression model -2 (Sub-optimum irrigation)		
V1	11.21	11.30	10.90	0.859
RC1	9.37	9.54	8.99	0.681

Figures

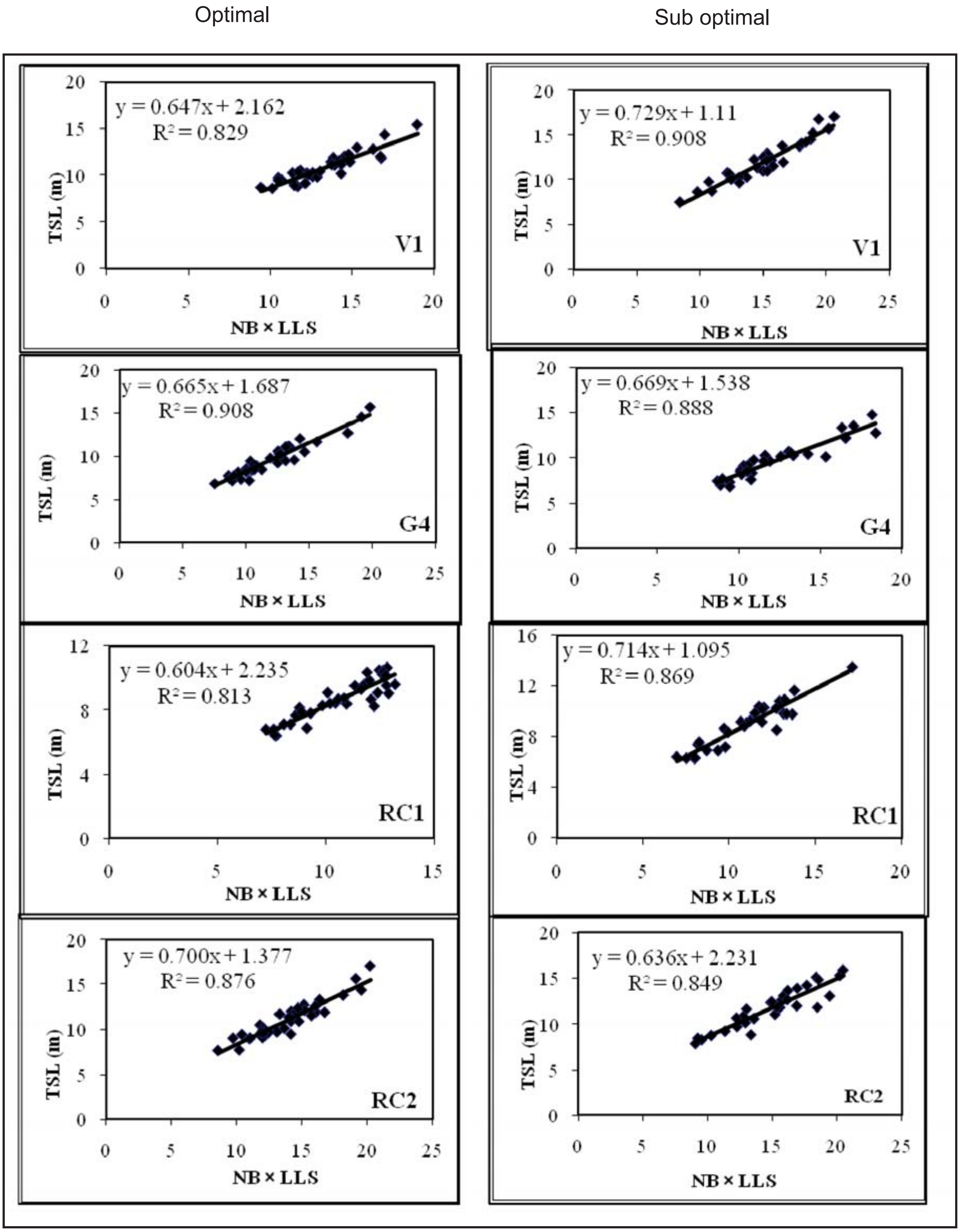

Fig.1. Relation between TSL and NS × LLS for four mulberry genotypes under optimal and sub-optimal conditions of irrigation.