



Quality assessment of planting stock using chlorophyll content analysis

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Abstract

The present work envisages chlorophyll pigment analysis to assess the quality of planting material for the agroforestry programs using tree species. Data on Chlorophyll-a, chlorophyll-b, total chlorophyll, and chlorophyll a/b ratio was obtained from the samples namely TRMC, TRMV, TRMP, TRMG, and a control TM of *Terminalia bellerica* (Gaertn) Roxb. The seedling chlorophyll content to assess the impact of rooting media and the management practices on the increase in chlorophyll content was a focal area in the investigation series. Fresh seeds of *Terminalia bellerica* gathered from the tropical natural stands of southwest peninsular India were used for raising the seedlings. The seeds were sown after processing in the experimental field. The fresh leaves of seedlings were used for the analysis. The result showed that the mean values of chl a ranges from 0.01167 ± 0.000577^c (TRMC) - 0.05800 ± 0.001000^a (TM), chl b in the range of 0.02433 ± 0.000577^c (TRMG) - 0.04200 ± 0.001000^a (TRMP), Total chl in the range of 0.03267 ± 0.000577^c (TRMC) - 0.09000 ± 0.001000^a (TM) and chl a/b ratio in the range of 0.55700 ± 0.051449^d (TRMC) - 1.81333 ± 0.075976^a (TM). The values of these parameters significantly differed between seedlings of different mediums. Data and the findings of the present study show that there is a positive influence for rooting media together with management practices in the production of chlorophyll in *Terminalia bellerica* seedlings. Since chlorophyll content is a measure of photosynthesis capability, it can be further predicted that a higher amount of chlorophyll from the least unit area of leaves is an indication of plants with higher vigor.

Keywords: chlorophyll a/b ratio, rooting media, terminalia, root-trainers, abiotic stress in seedlings

Introduction

The characteristic green color of the green plants is imparted by their chlorophyll content. The quantity of chlorophyll in a plant is the indicator of its capacity to carry out the process of photosynthesis [1]. Thus leaf chlorophyll content act as an indicator of the photosynthetic mechanism of the plants. The amount of chlorophyll in a plant-primarily depends upon the amount of sunlight it receives, however the soil quality and nutrient uptake also play an important role in providing the molecule synthesis of chlorophyll pigment [2]. The essential components of plant photosystems are chlorophyll-a and chlorophyll-b, in which chlorophyll-a act as an important factor of the energy production in plants [3]. The concept of photosynthetic capacity and its role in plant growth is not verified in forest tree plantation programs. The variation and role of chlorophyll pigments and their role in seedling growth remain unclear [4]. The total amount of leaf chlorophyll and chlorophyll ratio (Chl a/b) directly impacts the photosynthetic capacity of plants.

The chlorophyll content is reported to have medicinal properties such as blood sugar control, detoxification, digestive metabolism, and allergy control [2, 5]. The variation in the chlorophyll content will give evidence regarding the physiological fitness of the plant/leaf [6]. Thus it is important to study the influence of different rooting mediums in determining the quality and quantity of chlorophyll content in the leaf/plant, and will help to find or improve the efficiency of rooting media, to enable maximum production of chlorophyll, which will be helpful in plantation programs.

Terminalia bellerica is a medicinally as well as economically valuable forest tree species that are under high threat of overexploitation. Proper plantation programs have to be developed for the successful regeneration of this plant before it goes to be extinct. The quality and vigor of the plant seedlings will depend upon their health status and physiological metabolism such as photosynthesis. Thus in this study, the effect of different rooting media in maintaining the healthy condition of the seedlings of *Terminalia* sp. for the production of quality seedlings has been identified by the spectrophotometry method.

Materials and Methods

All the leaf samples for the study were collected from the raised seedlings of *Terminalia bellerica* in the nursery of Prof. T. C. Joseph memorial Garden, Department of Botany, Union Christian College, Aluva, (+10°7'30'65''

+76°20'33.2''), Kerala, India during March 2018. Terminalia seedlings were raised in four different rooting media as coir pith and soil combination (TRMC), vermiculite and soil combination (TRMV), perlite and soil combination (TRMP), raised standard nursery bed-garden soil (TRMG), and a control (TM). The chlorophyll content data was developed according to Arnon's (1949) method. Fresh leaves grounded in acetone using a mortar and pestle. Chlorophyll extracted into acetone solution was collected by filtering the homogenate using Whatman filter paper No.1. The homogenate was washed 2-3 times with 5 ml of 80% acetone each time and the final volume was made up to 25 mL. The color absorbance of the solution was estimated by a spectrophotometer.

Statistical analysis

To calculate the means of mediums, three replicates (independent samples) were taken for each variable (growth medium). Homogeneity of the variance was tested by the Levene test and found that the group variances are homogenous. The mean values, Standard deviation (SD), standard error (SE), coefficient of variation (CV) of the pigments in all the mediums were calculated. The results were subjected to analysis of variance to assess the role of growing medium on the plant pigment and the means were compared by the Tuckey test at the significance level of 0.05 and 0.01. The statistical analysis was performed using the IBM SPSS software package.

Result and Discussion

1. Seedling production of Terminalia bellerica seeds

The fresh seeds were sowed in four different media as raised standard nursery bed (TRMG), coir pith and soil combination (TRMC), vermiculite and soil combination (TRMV), perlite, and soil combination (TRMP). Seeds were planted in each medium and maintained for the collection of sample leaf analysis. The germination and growth of the seedlings were noted periodically.

2. Chlorophyll estimation of Terminalia bellerica seedlings in different rooting media

Leaf samples were taken from the seedlings raised in four different rooting mediums and the number of chlorophyll pigments was quantified by spectrophotometric method. It was revealed that the content of chlorophyll-a, b, and total chlorophyll content of the leaf showed variation among the four mediums (Table 1), these values were compared with the chlorophyll content of leaves from a Terminalia tree maintained in the natural environment. The result showed that the mean values of chl a ranges from .01167±.000577^e (TRMC) - .05800±.001000^a (TM), chl b in the range of .02433±.000577^c (TRMG) - .04200±.001000^a (TRMP), Total chl in the range of .03267±.000577^e (TRMC) - .09000±.001000^a (TM) and chl a/ b ratio in the range of .55700±.051449^d (TRMC) 1.81333±.075976^a (TM). The values of these parameters significantly differed between seedlings of different mediums. From the data, it could be inferred that the difference in the media composition could contribute to the difference in the amount of chlorophyll content. It may be due to the difference in the nutrient supply by the medium. The supporting explanation could be that the ratio of K/Na plays an important role in chlorophyll content [8]. The chlorophyll pigment composition is of great importance since it plays a crucial role in the physiological functions of a plant. In which the amount of chlorophyll-a is of much importance since it is the primary pigment [2]. There was a significant difference among the five samples in chlorophyll content. Post-hoc testing revealed a significant difference between the mediums. The average value of chlorophyll-a ranged from 0.01167±.000577^emg/gm to .05800±.001000^amg/gm. Chlorophyll b ranged from 0.02100±.001000^dmg/gm to .04200±.001000^a mg/gm. Total chlorophyll ranged from .05100±.001732^d to .09000±.001000^a and Chlorophyll a/b ratio ranged from .55700±.051449^d to 1.81333±.075976^a. Content of chlorophyll b was close to similar in TRMV and TRMG (0.02600±.001000^cmg/gm & 0.02433±.000577^cmg/gm) and both the mediums were treated as similar subset in Tukey test.

Table 1: Statistics of leaf chlorophyll content (mg/ g) for different Rooting medium Mean values (n=3) ± SD are shown. Within each variable, mean values followed by different letters (a-e) in columns are significantly different ($P<0.05$); Tuckey test.

Pigments	Medium	Minimum	Maximum	Mean	CV (%)
Chl a	TRMC	.011	.012	.01167±.000577 ^e	4.944
	TRMV	.024	.026	.02500±.001000 ^d	4
	TRMP	.027	.029	.02800±.001000 ^c	3.571
	TRMG	.031	.033	.03200±.001000 ^b	3.125
	TM	.057	.059	.05800±.001000 ^a	1.724
Chl b	TRMC	.020	.022	.02100±.001000 ^d	4.762
	TRMV	.025	.027	.02600±.001000 ^c	3.846
	TRMP	.041	.043	.04200±.001000 ^a	2.381
	TRMG	.024	.025	.02433±.000577 ^c	2.372
	TM	.031	.033	.03200±.001000 ^b	3.125
Total chlorophyll	TRMC	.032	.033	.03267±.000577 ^e	1.766
	TRMV	.049	.052	.05100±.001732 ^d	3.396
	TRMP	.069	.071	.07000±.001000 ^b	1.429

	TRMG	.055	.058	.05633±.001528 ^c	2.713
	TM	.089	.091	.09000±.001000 ^a	1.111
Chl a/ Chl b ratio	TRMC	.500	.600	.55700±.051449 ^d	9.237
	TRMV	.925	1.000	.96167±.037528 ^c	3.902
	TRMP	.627	.690	.66633±.034298 ^d	5.147
	TRMG	1.291	1.333	1.31467±.021502 ^b	1.636
	TM	1.727	1.870	1.81333±.075976 ^a	4.190

The content of chl a was 0.5800 ± 0.001000^a mg/gm with a fluctuation from 0.057-0.059 and was found to be the highest among the other four mediums. The lowest chl a with an average of 0.01167 ± 0.000577^e with a fluctuation from 0.011-0.012 was found in TRMC. Chl b ranged from 0.04200 ± 0.001000^a mg/gm with a fluctuation of 0.041-0.043 mg/gm in TRMP and the lowest 0.02100 ± 0.001000^d mg/gm was found in TRMC with a fluctuation of 0.020-0.022. Total chl was highest in TM (0.09000 ± 0.001000^a mg/gm) with a fluctuation of 0.089-0.090.

Chl a/b ratio was 1.81333 ± 0.075976^a mg/gm with a fluctuation of 1.72-1.87 and was the highest in TM.

Variations were observed in the amount of chl a and chl b between the medium. Kamble *et al* (2015) studied the difference in chlorophyll content in young and adult leaves of some selected plants and found that amount of chl a was higher than that of chl b in most of the plants. Contradiction to that in the present study expects for TM and TRMG medium (both contain natural soil without soil additives) all the other three medium amount of chl b was highest than that of chl a. The possible justification could be the variation of micronutrient availability in each medium.

This can be explained by the fact that the ratio of chlorophyll-a and chlorophyll-b changes with the degree of abiotic stress and nutrient ^[10]. The changes in temperature during growth affect leaf pigment composition and photosynthesis in *Zea mays* genotypes differing in sensitivity to low temperature have been recorded by Haldimann ^[11]. The change in total chlorophyll content of the seedlings raised in different mediums indicates the importance of the rooting medium for the healthy production of seedlings ^[12]. The accurate nutritional status of *salvia splendens* chlorophyll contents has been traced in plants grown in nutrient solutions ^[9]. Total chlorophyll content varies from species to species and is influenced by biotic components signifies the growth vigor and biomass development of a plant ^[13]. It was highest (0.09000 ± 0.001000^a) in TM followed by TRMP (0.07000 ± 0.001000^b) indicate the growth vigor and health status of the plants in TM medium.

Conclusion

This study demonstrates that chlorophyll content varies among plants raised in different rooting media. The impact of rooting media and the management practices on the increase in chlorophyll content has been objectively explained through the findings. Terminalia bellerica is a well-known medicinal tree of peninsular India, which is suitable for reforestation activities and planting as an avenue tree. Data and the findings of the present study show that there is a positive influence for rooting media and the management practices in the production of chlorophyll in Terminalia bellerica seedlings. Since chlorophyll content is a measure of photosynthesis capability, it can be further predicted that a higher amount of chlorophyll from the least unit area of leaves is an indication of plants with higher vigor. The decline of growth patterns due to pests and diseases can be also correlated through this method, which is yet to be covered.

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