QUANTIFICATION OF CELLULOSE CONTENTS BY TRANSMISSION SPECTRA OF PLANT TISSUES

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Determination of cellulose is a part of numerous scientific studies and provides an interlinked base for many developmental and physiological variations among living entities. So, quick and accurate quantification of cellulose can never be over-emphasized. The present study leads towards the development of a method for quantification of cellulose contents under microscope. It is basically dependent upon transmitted color spectrum of plant tissues and assesses accurate cellulose quantity with an error of ±0.46%. By adopting this method, inter- and intracellular variations in cellulose concentrations can efficiently be determined by measuring variations in color intensity. Moreover, it introduces a technique of physiological studies and also provides a base for development of similar formulas for other biochemicals of living tissues.

Keywords: transmission spectrum, cellulose staining, colorimetric analysis, equation of line, area under line, percentage error

INTRODUCTION

Measuring biochemicals in plant tissues, especially cellulose, is a frequently required research assignment.[1] This has been done for different purposes by using different cellulose measuring methods,[2,3] but all those methods can only estimate cellulose in unit mass of material. It is very difficult to precisely measure cellulose concentrations at microscopic points of an object. So, it is a hard job to determine the strength of cellulose and cellulose-related phenomena at micro-points of tissues. Presently, a method has been developed to estimate cellulose contents at very precise points of cell walls by using the transmission spectrum of light. The subject plant selected in this study is *Sorghum bicolor*, which is a commonly cultivated fodder and food crop globally.[4] This method will not only be helpful in finding out cellulose concentrations, but will also facilitate the developmental research of plants.

EXPERIMENTAL

Acquisition of germplasm

Genomically pure sorghum seeds were obtained from Fungal Biotechnology Laboratory, Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan, and were grown in plastic pots of 5" diameter, under controlled conditions (25±2 °C). The plants were watered for one month according to the water requirements and then used for laboratory assays.

Section cutting and cellulose color scale

Morphologically different stem tissues of sorghum were selected at different heights from soil surface and cellulose contents of fresh tissues were determined by adopting the method of Updegraff.[5] Moreover, plant tissue was processed by the method of Himmel *et al*.,[6] for the quantification of cellulose in pure and dried plant cell walls only. Surface area, crystallinity index and water retention value of cellulose in both fresh and processed plant samples were determined according to Chandra *et al*.,[7] Jeihanipour *et al*.[8] and Teghammar *et al*.,[9] respectively. The values of physical properties of cellulose were analyzed through Duncan's Multiple Range Test (DMRT) in order to determine the change in properties of processed tissue.

Then, transverse sections of fresh and processed tissues of 0.1 mm thickness were cut with the help of microtome and stained for 15 minutes with Updegraff solution.

These sections were microscopically examined and images were captured at ISO=100 and EV=0. Whereas the microscope of LABOMED (CXR2) was adjusted at maximum light pass through shutter. Meanwhile, a microscopic Halogen lamp of 12 V and 20 Watt was set to the highest level of illumination to obtain crisp quality images. Then color proportions (red, green and blue (R:G:B)) of images were determined through COLORS (Shizuoka Red Cross Hospital, Shizuoka, Japan) and different combinations of R, G and B were plotted in a graph against respective cellulose concentrations. Plotted graph defined a color-based scale of cellulose contents (Figure 1), which provided complete information about the behavior of different combinations of R, G and B with respect to smooth change in cellulose contents of the tissue.

Colorimetric analysis of cellulose contents

From Figure 1, the color slope showing the smoothest and the most regular trend towards cellulose contents was selected in order to develop mathematical formula for cellulose quantification by using "Equation of line". The values under that particular line, calculated through the mathematical method of integration, represent the cellulose quantity with the respective color combination. Moreover, an ideal slope was also drawn by keeping in mind most of the points of the actual color line in order to determine the efficacy of this method. The differences between the ideal cellulose line and the actual cellulose line generated a space for the percentage error of the formula, which was calculated to get more precise cellulose quantification from tissue photographic images.

Table 1
Physical properties of cellulose samples investigated

	Cellulose surface Dye ratio (Orange/Blue)	Crystallinity	Water retention
Fresh tissue	3.03±0.019a*	0.29±0.007a	1.48±0.07a
Processed tissue	3.01±0.011a	0.28±0.01a	1.51±0.02a

*Letters beside each value describe the level of significance, calculated through DMRT

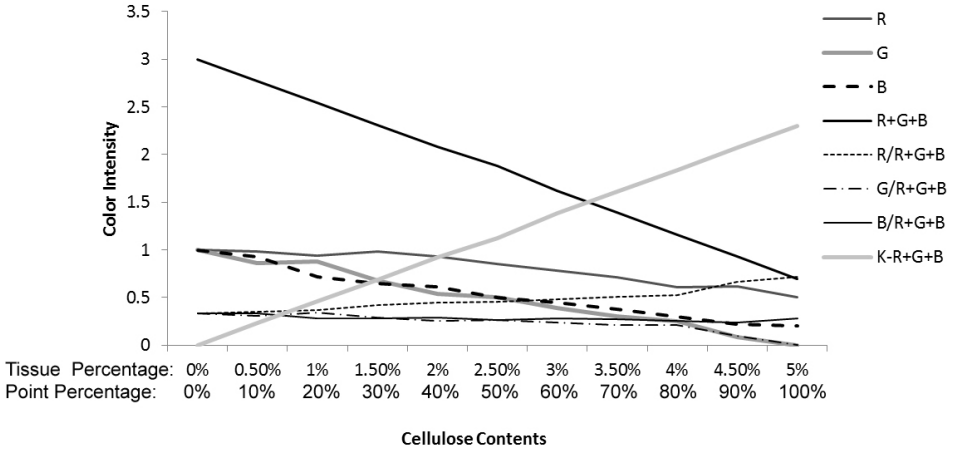

Figure 1: Cellulose concentrations showing intensities of different colors. The lines of colors show each specific behavior against varying cellulosic amounts (K= sum of the maximum intensities of all three basic colors, i.e. R, G and B; tissue percentage denotes percentage cellulose contents in sorghum tissue; while point percentage stands for percentage of cellulose at a particular point of pure and dried plant cell wall)

RESULTS AND DISCUSSION

The physical properties studied did not produce any significant difference before and after the treatment (Table 1), which revealed that both tissue samples (fresh and processed) were comparable to each other, exhibited the same characteristics (as differences among them were insignificant) and could be analyzed by intrinsically similar methods.

The intensities of different color combinations plotted against cellulose contents generated different slopes, but it was noted that the slope of R+G+B exhibited a smooth trend against constantly varying quantities of cellulose (Figure 1). The line drawn by R+G+B (y-axis) with cellulose contents (x-axis) had a negative slope, while K-R+G+B was a line showing exactly an opposite trend to R+G+B and had a positive slope.

The line created by R+G+B showed the smoothest and the nearest to cellulose quantity dependent behavior among all color lines. So, it was selected as the representative line of cellulose quantity and denoted as "cellulose line" in downstream analysis.

By applying the equation of line on the "cellulose line", we obtain:

$$\frac{y-y_1}{y_2-y_1}=\frac{x-x_1}{x_2-x_1}$$

$$\Rightarrow \frac{y-2.08}{1.85-2.08}=\frac{x-2}{2.5-2}$$

$$\Rightarrow \frac{y-2.08}{-0.23}=\frac{x-2}{0.5}$$

$$\Rightarrow y-2.08=\frac{-0.23}{0.5}(x-2)$$

$\Rightarrow$ *y = -0.46x + 0.92 + 2.08*

y = -0.46x + 3 (the required equation of the obtained line)

It was compared with the standard equation of line (*y = mx + c)*

m = -0.46 = Slope of line

C = 3 =y interception of line

and $m = \tan\theta$

where Θ is the angle between line and x-axis (cellulose axis)

$\tan\theta = -0.46$

$\theta = \tan^{-1}(-0.46)$

$= 155.297^{o}$

The area under this line:

$$A = \int_{x1}^{x2} y\,dx$$

$$A = \int_0^5 (-0.46x + 3)dx$$

$$A = \int_0^5 -0.46x\,dx + \int_0^5 3\,dx$$

$$A = -0.46\int_0^5 x\,dx + 3\int_0^5 dx$$

$$A = -0.46\left|\frac{x^2}{2}\right|_0^5 + 3|x|_0^5$$

A = -0.23 (25-0) + 3 (5-0)

A = 9.25

The graph can be shown as a function, which is the combination of three straight lines viz.:

$$Y=\begin{cases} 3-0.46x & 0\le x\le 2 \text{ and } 3\le x\le 5 \\ 2.88-0.4x & 2<x<2.5 \\ 3.18-0.52x & 2.5<x<3 \end{cases}$$

Where y-intercepts are 3, 2.88 and 3.18 (Figure 2); slopes are -0.46, -0.4 and -0.52; and angles with x-axis are 155.3°, 158.2° and 152.52°, respectively (Figure 2).

Now the area under this graph is A':

$$A' = \int_0^5 y\,dx$$

$$A' = \int_0^2 (3-0.46x)\,dx + \int_2^{2.5} (2.88-0.4x)\,dx + \int_{2.5}^3 (3.18-0.52x)\,dx + \int_3^5 (3-0.46)\,dx$$

$$A' = 3\int_0^2 dx - 0.46\int_0^2 x\,dx + 2.88\int_2^{2.5} dx - 0.4\int_2^{2.5} x\,dx + 3.18\int_{2.5}^3 dx - 0.5\int_{2.5}^3 x\,dx + 3\int_3^5 dx - 0.46\int_2^5 x\,dx$$

$$A' = 3|x|_0^2 - 0.46\left|\frac{x^2}{2}\right|_0^2 + 2.88|x|_2^{2.5} - 0.4\left|\frac{x^2}{2}\right|_2^{2.5} + 3.18|x|_{2.5}^3 - 0.5\left|\frac{x^2}{2}\right|_{2.5}^3 + 3|x|_3^5 - 0.46\left|\frac{x^2}{2}\right|_3^5$$

A' = 3(2 – 0) – 0.23 (4 – 0) + 2.88 (2.5 – 2) – 0.2 (6.25 – 4) + 3.18 (3 – 2.5) – 0.25 (9 – 6.25) + 3 (5 – 3) – 0.23 (25 – 9)

A' = 6 – 0.92 + 1.44 – 0.45 + 1.59 – 0.6875 + 6 – 3.68

A' = 9.2925

A = Area beneath ideal line

A' = Area beneath actual line

The deviation between two areas has been denoted as A":

A" = A' - A

A" = 9.2925 – 9.25 = 0.0425

Percentage error = 100x (Deviated area/actual area)= 100x (0.0425/9.2925) = 0.46%

Hence the equation of the graph line, which is plotted by ideal values, is y = -0.46x + 3

$\Rightarrow$ X = -2.17y + 6.52

Biochemical concentrations in plant tissues affect their staining processes and cause differential staining, indicating concentration variations of a particular biochemical.[10,11] By observing the color pattern after staining, a lot of desired information about plant metabolism and physiological processes can be gathered easily. This color change strictly depends upon the accumulation of a particular biochemical for which the specific targeting stain is being used.[12] So, the biochemical contents in plant tissues can be quantified based on the intensities of staining colors – a technique that has been used in the present study. In this research, cellulose quantities have been correlated with the stained color intensities and then this correlation has been used to derive a mathematical formula for color-based quantification of cellulose. So, this investigation positively follows all the above-mentioned studies and proves that specific staining of cellulose was successfully carried out in those investigations.

The area under the cellulose line represents cellulose amounts and the best method to deal with such lines is by using integration.[13,14] So, the rate of change of the area under micro-points of the cellulose line (with the width $\rightarrow$ 0), was analyzed as the rate of change of cellulose contents with respect to change in R+G+B. On the other hand, experimental biology is a cascade of unwanted errors and it is very difficult to completely eliminate the origin of these errors.[15, 16] But these errors can only be determined either by repeated experimentations or through appropriate physical science methods.[17-19] Therefore, these methods were used to calculate the percentage error in the present formula and in this way the efficiency of the present methods was determined.

Because colorimetric value of cellulose measurement has 0.46% more amount of cellulose. So, it should be subtracted from the obtained cellulose amount for getting more precise value.

Cellulose= 6.52-2.17 (R+G+B) $-$ Percentage Error

Cellulose= 6.52-2.17 (R+G+B) $-$ 0.46% = α

α = Percentage cellulose in fresh plant tissue

Point percentage of α x (Maximum cellulose content of processed tissue sample/ Maximum cellulose content in fresh tissue sample)

$$\text{Point percentage of cellulose} = \frac{100}{5} \times \alpha$$

Point percentage of cellulose =20 × α

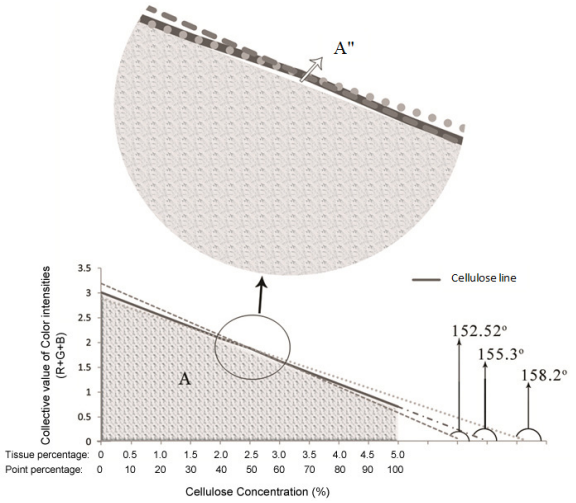

Figure 2: Resolving single cellulose line into three straight lines (slopes), completely covering the actual cellulosic area (A′) ((A) is the ideal cellulosic area covered by the ideal cellulose line and (A") is the difference between ideal and actual cellulosic areas (area of error); collective value of color intensities, i.e. red (R), green (G) and blue (B), have been mentioned on y-axis)

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CONCLUSION

The intensity of light transmission spectrum has an inverse relation with cellulose contents of plant tissue. This intensity is defined by the additive interconnections of red, green and blue components of the transmitted light. All these three components individually possess a negative relation to cellulose concentration with variable slope values. The collective value of red, green and blue color intensities give the exact quantity of cellulose present in the transmission medium (Cellulose = 6.52-2.17 (R+G+B)). Moreover, the value of cellulose contents obtained through this method has an error value of 0.46%, which is subtracted from the initially calculated value.

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