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JUTE (*CORCHORUS OLITORIUS* VAR. O-72) STEM LIGNIN: VARIATION IN CONTENT WITH AGE

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Abstract

Stem lignin content of a mature jute plant was found to be around 29% giving a deposition rate of 0.21% per day. The study was made on individual plants of different ages starting from five weeks when they were big enough to produce sufficient dry mass for lignin estimation to 16 weeks of age when a jute plant is ready for retting. An intense rate of increase (1.15%) was found during the 7th week of growth, probably due to a rise in temperature. Present data and the average rate of increase of lignin production can be considered as a reference value for projects working on producing low-lignin - jute varieties.

Introduction

The bast fiber of two plants, namely *Corchorus capsularis* and *C. olitorius* (Sparrmanniaceae) are known as jute (Heywood *et al.* 2007). The fiber is collected after retting from the bast or outer region of the stem. This serves as a good source of different grades of pulp, since it has about 60% of cellulose. Became of its non-abrasive, light weight and biodegradable properties, the industrial applications of jute fiber have been augmented (Kuroda *et al.* 2002).

In Bangladesh, jute could be a suitable alternative not only as the raw material for paper pulping, but also for other wood-based industries. Chemically, the extracted jute fiber has about 59 - 61% of cellulose while lignin content varies from 11.0 - 13.5% (Islam and Sarkanen 1993, Giwa and Akwu 2007, Del Río *et al.* 2009). However, till now, not much data is available about the lignin content of either the jute stem or the whole plant from where the jute fiber originates.

Since lignin is the most recalcitrant component of the plant cell wall (Angelidaki and Ahring 2000), the higher the proportion of lignin the lower the bioavailability of the substrate (Richard 1996). Therefore, the downstream processing of jute would benefit from a reduced amount of this polymer. Researchers working to lower the lignin content of jute fibers by gene silencing will be benefited by knowledge of the amount of lignin a normal jute plant produces during different phases of its growth cycle. This will also help them to make comparison with their jute variety under development. Thus, to facilitate such initiatives, the present research was undertaken to study the lignin content of jute stem, focusing different growth stages of the plant.

Materials and Methods

Corchorus olitorius var. O-72 was used for the present study. Seeds were sown at two days intervals in two different plots of the Bangladesh Jute Research Institute (BJRI), and are named sample sets 1 and 2, respectively. Lignin estimations were made at one week intervals, starting from 5 weeks of age until the plants were ready for retting (approximately 16 weeks). Height of each plant recorded during the present study has been presented in Table 1.

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Klason lignin estimation method (Moore and Johnson 1967, Ehrman and Himmel 1994, Templeton and Ehrman 1995) was used to estimate the acid insoluble lignin (AIL). Lignin content of the jute fiber samples were measured to ascertain that the method was suitable for determining the amount of stem lignin in jute. To get the average lignin value of a plant, only the middle 16 cm section of the stem was used in this study. Two plants were taken per sample and estimated separately. All the samples were estimated twice to get an average value.

According to the estimation procedure, plant stems were first collected and cut off according to length. Then, they were dried at 105°C to get the dry weight (Ehrman 1994) and ~ 0.5 g of sample was taken for estimation, denoted as W_1.

These dried samples were subjected to 72% H_2SO_4 hydrolysis at room temperature, followed by 4% H_2SO_4 hydrolysis at boiling temperature. The hydrolyzed solutions were then vacuum-filtered using filtering crucibles (glass crucibles with silica filter). After filtration, the crucibles with the residual content were heated at 105° C in a heating oven. Then, the weight of the crucibles with the dried residual content was taken, and denoted as W_2.

The next step was to heat the filtering crucibles with residual contents at 575 ± 25°C in a muffle furnace (carried out at the Bangladesh Council of Scientific and Industrial Research, BCSIR). The crucibles were next cooled in a desiccator, weighed and the weight designated as W_3.

With these three different weights, the percentage of acid-insoluble lignin was calculated using the following formula (Templeton and Ehrman 1995):

$$\text{Acid insoluble lignin (\%)} = \{(W_3 - W_2)/W_1\} \times 100$$

The data were analyzed individually for two different sample data sets using MS excel. The correlation coefficient between the heights and lignin contents were estimated separately for both the sample sets. The square of correlation coefficient and slope of regression line were calculated individually for both the sample data sets.

Results and Discussion

Lignin content of jute fiber was estimated to verify our method. The estimated value was found to be 12.83 - 13.32% (5 different samples with duplication). Since the value is within the previously reported range, (12.5 - 13.5%) (Rahman 2010, Del Río *et al.* 2009) the method was considered appropriate for estimating jute lignin.

The estimated stem lignin contents of 5 - 16-week old jute plants are given in the Table 1. Total increase in stem lignin content was found to be 16.11% (avg.) in 77 days. Highest increase, 8.04% was found during the 7th week (43rd to 52nd days) of growth. During this period, the daily increase was 1.15% whereas the average increase in lignin content was 0.21% per day during the whole study period.

The correlation coefficient between the height and lignin content was found to be 0.89 and 0.91, respectively for sample sets 1 and 2, indicating a strong correlation between these two variables (height and lignin content).

The square of correlation coefficient (correlation of determination, R^2) for both the sample sets were found to be 0.72 and 0.71, respectively suggesting that more than 70% variables in both data sets are in linear relationship. The slopes for linear correlations were found to be 0.17 and 0.19 implying a positive linear relationship. A t-test (two-tailed, unequal variance) was done to verify the similarity of two sample sets (Null hypothesis, H_0: means of both the sample set are equal at 5% confidence interval). A p value of 0.96 (accepts the H_0) implies that the two sample sets were similar.

As the second most abundant biopolymer on earth, lignin plays a critical role in the biogeochemical carbon cycle and affects the interaction between plants and other living organisms in a myriad of ways (Halpin *et al.* 2007). Huge variability is found in the lignin structure and content between different plant species (Halpin *et al.* 2007).

Table 1. Table for both the sample sets showing age, height, estimated and average lignin content of jute plant stem with the increment rate (increase per day).

Sample set 01			Sample set 02			Average	
Age (days)	Height (cm)	Lignin content (%)	Age (days)	Height (cm)	Lignin content (%)	Lignin content (%)	Increase per day (%)
37	48.6	13.42	35	64	13.61	13.515	-
45	81	15.74	43	97	15.98	15.86	0.2931
52	124	24.29	50	140	23.525	23.9075	1.1496
58	149	25.07	56	142	24.68	24.875	0.1613
65	180	26.325	63	190	26.895	26.61	0.2479
73	198	25.96	71	214	27.32	26.64	0.0038
79	205	26.61	77	221	27.865	27.2375	0.0996
86	197	26.41	84	223	27.615	27.0125	–0.032
93	277	27.43	91	260	28.015	27.7225	0.1014
100	305	28.66	98	242	28.34	28.5	0.1111
107	314	29.43	105	300	28.88	29.155	0.0936
114	279	29.62	*	*	*	29.62*	0.1329

*The plants for sample set 2 was unavailable, as they were subjected to retting on day 112.

In one study, lignin content of *C. capsularis* (JRC 212) fiber was found to be 21.36% (Sengupta and Palit 2004) whereas a fully grown jute plant stalk (or stick) is known to have between 24 - 25% of Klason lignin (Islam and Sarkanen 1993, Roy *et al.* 1991, Bhattacharyya 1996). Before retting, Haque *et al.* (2001) found the lignin content of the fiber to be about 15% which eventually came down to 12 - 13% on completion of retting. However, this bast fibrous part is only 4.5 - 7.5% of whole plant or stem (non-dried) (Islam and Sarkanen 1993). It may also be noted that about 10% of the fiber (of a 120 day old plant) is lost during retting (Ahmed and Akhter 2001). Taking this into consideration together with the different values obtained for fiber and stick or stalk lignin, whole plant or stem lignin content could certainly be more than 25%. Most measurements for lignin have been made for *C. capsularis* while little work has been done to know the content for either the stem or the stick of *C. olitorius*. Mosihuzzaman and co-workers found the stick lignin content of *C. olitorius* to be about 22% (Mosihuzzaman *et al.* 1989). However, the sample collection method and plant growth condition of that study was not clear. In another study, lignin content of *C. olitorius* was found much higher than *C. capsularis* (Sinha 2004). The average fiber lignin content of *C. olitorius* was 17.06% whereas for *C. capsularis* it was 12.71% (Sinha 2004). It is therefore not unexpected that the whole plant lignin content will be much higher for *C. olitorius* than *C. capsularis*.

Pappas *et al.* (1998) had studied the lignin content in the bark, wood, and pith of four kenaf varieties and found values from 10.4 - 10.8, 20.5 - 20.6 and 14.9 - 15.3% of plant material, respectively. For flax, the lignin content in the bast fibre was found to vary between 1.5 and 4.2% of the dry cell wall residues as compared to values varying from 23.7 and 31.4% in the flax xylem tissue (Day *et al.* 2005).

In our study, a mature jute (*C. olitorius*) plant stem (stick and bast fiber) was found to have on an average 28 - 29% of lignin (Table 1). For only the retted and dried bast fiber part, lignin was found to be ~13% which is consistent with previous studies carried out in standard conditions (Palit and Meshram 2004). This ensured the accuracy of our measurements.

Our results also suggest that the lignin production rate in jute is not uniform. A huge increase in percentage was found during the 7^{th} week of its growth. In this particular week, total increase was found to be about 8% giving a rate of 1.15% per day, almost six-fold greater than the average increase (0.21%) throughout the life cycle (Table 1). This sudden increase could possibly have occurred due to an increase in temperature during this period (usually between May and June) as a positive correlation between lignin deposition and temperature has been previously reported (Ford *et al.* 1979, Villavicencio *et al.* 2007).

From the correlation coefficient between height and lignin content, it is evident that lignin deposition increases with height. The R^2 value for both the sample sets (0.72 and 0.71) indicates a good linear correlation between lignin production rate and time. Moreover, the slopes of the line for linear correlations, 0.17 and 0.19 suggest similarity between the samples sets in the rate of linear increment (Motulsky and Christopoulos 2004).

With such a rate of increase in lignin production, it is difficult to use jute in pulp and paper industry, since the main challenge in during the process is to remove lignin in a selective manner while preserving to the greatest possible extent the cellulose and hemi-celluloses (Santos 2013). High percentage of lignin interferes with chemical processes during the industrial processing, as it is embedded within a complex matrix with cellulose in the cell-wall (Li *et al.* 2008) called ligno-cellulose. This polymer also hinders the degradation of cell wall polysaccharides to simple sugars destined for fermentation to ethanol in case of biofuel production (Keating *et al*. 2006).

However, advances in plant transformation technology have facilitated researchers in manipulating lignin content and composition in a variety of plant species (Humphreys and Chapple 2002, Boudet et al. 2003). These efforts have resulted in transgenic plants with improved pulping efficiency or digestibility (Baucher *et al.* 2003, Halpin 2004, Li *et al.* 2008). Hybrids of brown midrib sorghum and Sudangrass are already in use in agriculture (Li *et al.* 2008). Moreover, there are reported transgenic varieties of different crops to have reduced lignin content like in alfalfa (Guo *et al.* 2001), tobacco (Ni *et al.* 1994, Sewalt *et al.* 1997), and maize (Piquemal *et al.* 2002).

Unfortunately, no such study has been undertaken for jute, in spite of its enormous potentiality. The present study is expected to help researchers working to produce low-lignin containing hybrid or GM-variety of jute. In the absence of any reference data it will take at least five months to confirm (time for growth till maturity and retting) if the lignin content has been successfully reduced in such studies. Early morphological changes (unpublished data) that occur due to the reduction in lignin content could also be of some indication, but not enough to confirm. Researchers will be able to use our data as a reference at an early stage (5 weeks of growth) to determine the success of their work. More than six trials a year will be achievable using these data while conducting even 3 trials per year is difficult without such knowledge. Possibilities of more trials would facilitate a researcher to try more combinations of cross-hybridization or gene silencing at a time.

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