Influence of Hemp Plant Eccentric Growth on Physical Properties and Chemical Compounds of Hemp Hurd

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The directions of South, North, West, and East had no significant influence on hemp hurd thickness ($p > 0.05$) based on the data from 100 hemp plants measured and oriented in different directions. As the hemp hurd grew, the thickness of the plants at different orientations also changed. The pectin and ash content in the wide areas were higher than those in the narrow areas. The lignin, holocellulose, alpha cellulose, and hemicellulose content in the wide areas were lower than those in the narrow areas ($p = 0.05$) in different hemp plants of different genders and plant densities. Additionally, the cell numbers in the wide areas were higher than those in the narrow areas in different locations of hemp plants, of different genders, and varied plant densities ($p < 0.05$). Thus, the formation of the narrow areas and wide areas in the hemp hurd were caused by the division difference of cambium. The plant hormones were the main influence on the division of cambium. Three particular plant hormones cytokinin (CTK), abscisic acid (ABA), and auxin (IAA) in the wide areas of hemp plants were higher than those in the narrow areas of plants of different genders and plant densities ($p < 0.05$). The eccentricity had a great influence on the physical and chemical properties of hemp hurd.

Keywords: Hemp hurd thickness; Pectin content; Lignin content; Plant hormones; Cell numbers in cross section

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INTRODUCTION

Hemp (*Cannabis sativa* L.) has long been grown for the bast fibers that are used to produce rope, canvas, and a host of other materials (Broeck *et al.* 2008; Candy *et al.* 2017; Gallos *et al.* 2017; Liu *et al.* 2017). However, these fibers represent only a small percentage of the total biomass. Among the other hemp plant components is the xylem core, also called the hurd or shiv. Hurd has much weaker properties than those of the bast fibers, but can be used as an absorbent, as a filler for bricks, or for insulation.

Eccentric growth is manifested in almost all wood materials, such as trees (Shirai *et al.* 2015), crops of annual or perennial growth (Yadun 1999), and tension wood and opposite wood (Lautner *et al.* 2012). There are many reasons that cause the eccentricity of the wood, such as tree trunk leaning and the environment (Akachuku 1991; Leavitt 1993; Hellgren *et al.* 2004; Telewski 2006). Due to the eccentricity, the mechanical, physical, and chemical properties of the wood materials can be changed, including the chemical compounds content, density, and microfibril angle (MFA) (Stokes and Berthier

2000; Rodrigues *et al.* 2001; Andersson-Gunneras *et al.* 2006; Heinrich and Gärtner 2008; Sultana *et al.* 2010). A plant hormone has the potential to control the plant by inhibiting and stimulating growth, cell division, and cell expansion (Pierik *et al.* 2006; Love *et al.* 2009).

This study aims to focus on the influence of eccentric growth on the chemical and physical properties of the hemp plant. Moreover, this paper discusses the reasons behind the phenomenon. Based on the results, we can find some methods to control plant growth to get the ideal materials we need.

EXPERIMENTAL

Materials

Hemp hurd and hemp hurd thickness measuring

Cannabis sativa L. was planted by the Industrial Crops Institute of YAAS, Kunming, Yunnan, People's Republic China (PRC). The hemp plant and hemp hurd used in the experiments are shown in Fig. 1. One hundred hemp plants, randomly selected without considering gender and plant density, were used to measure the hemp hurd thickness at the directions of South, North, West, and East using a compass with a vernier caliper (Figs. 1B, 1C, and 1D, respectively), measuring point "a" (Fig. 1A) of the hemp plant was 5 cm above the base of the plant. The 10 locations between "b" (10 cm above the ground shown in Fig. 1A) and "c" (100 cm above the ground shown in Fig. 1A) of the three hemp plants were used to measure the hemp hurd thickness of different height at the directions of South, North, West, and East using a compass with a vernier caliper. The result is shown in Fig. 3.

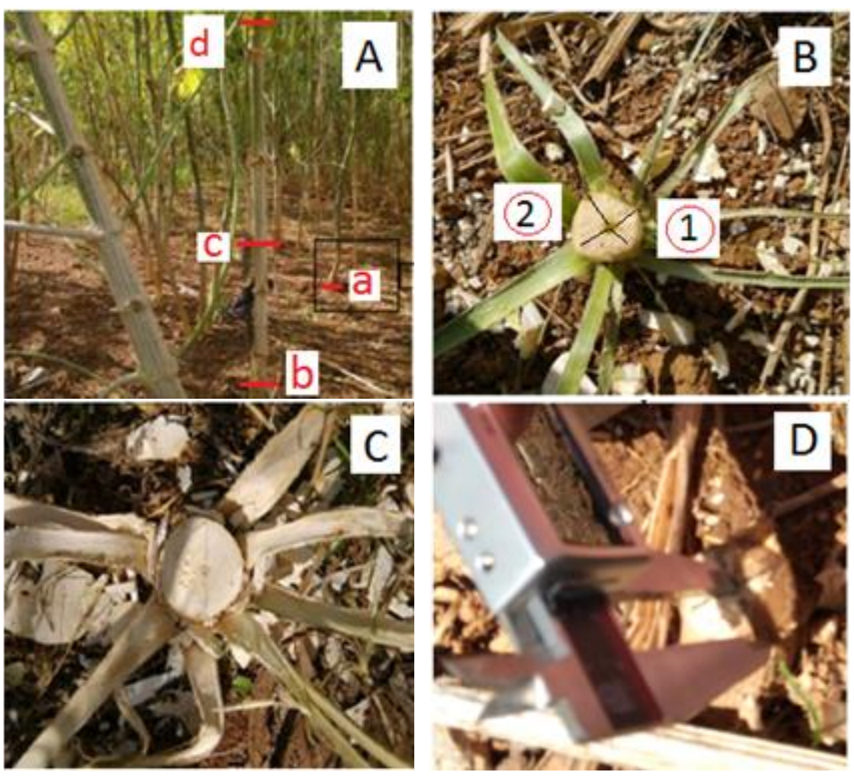

Fig. 1. Hemp plant and samples location for measuring

Chemical compounds measuring

To measure the effects of the eccentricity on the chemical compounds of the hemp hurd, the female and male hemp plant of two plant densities (9 plants per m^2 and 18 plants per m^2) were used. The whole hemp plants were cut into small samples of approximately 10 cm after the cortexes of the hemp plants were removed by hand. In each small sample, the wide and narrow sample areas were marked (1 for the narrow area, 2 for the wide area, as shown in Fig. 1B). Then, the wide and narrow samples were separated from the other samples and ground so they could pass through a 40-mesh screen but not a 60-mesh screen. The resulting powder was used to determine ash, klason lignin, holocellulose, alpha cellulose, and pectin contents using previously described procedures (ASTM 1985a; ASTM 1985b; ASTM 2001; ASTM 2015; Li *et al.* 2017) (Table 1). The hemicellulose content was estimated by calculating the difference between the holocellulose and alpha cellulose content.

Cell numbers measuring in cross-section

Locations “b” and “d” (Fig. 1A) were 10 cm and 250 cm above the ground, respectively. Two plant densities and two genders of hemp plant were used to measure the cell numbers in the narrow and wide areas of the hemp hurd (Fig. 1B) cross-section. Eight 1-cm samples were softened in boiling water for 48 h, then cut with a slicing machine, including the whole piece of the cross-section (from pith to cambium). Each slice was about 10 µm to 20 µm in thickness of hemp hurd, as shown in Fig. 2. The piece of cross-section hurd was stained with 1% safranine solution and placed on temporary slides; the coverslips were placed over the pieces and the pictures were captured with Motic Images Plus 2.0 image analysis system (Motic China Group Co., Ltd., Xiamen, P.R. China). Motic Images Plus 2.0 is a digital microscopy software suite provided free of charge with most Motic Digital Microscopy items. Images Plus is available in Windows versions as well as OSX and contains powerful tools necessary in a wide range of applications from Educational to Professional Digital Microscopy. Next, the pictures were printed and the cell numbers from pith to cambium were counted by hand.

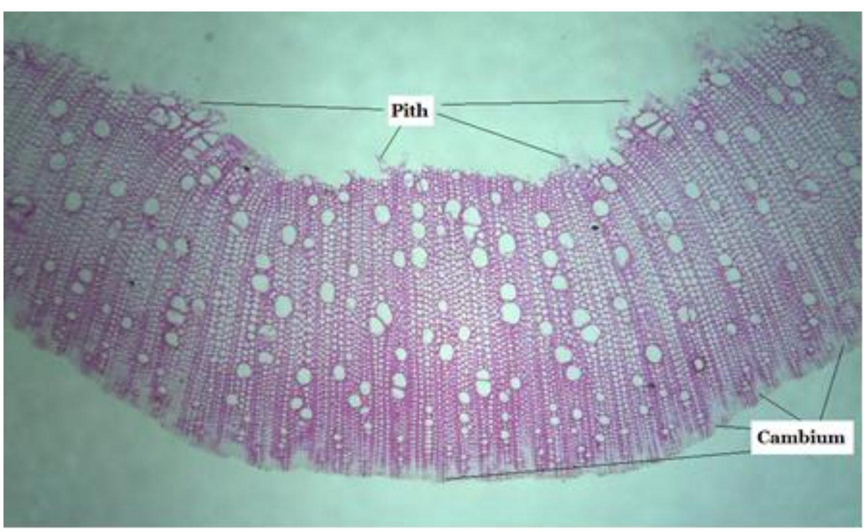

Fig. 2. The microstructure of hemp hurd cross-section

Plant hormones evaluation

Three kinds of plant hormones, cytokinin (CTK), abscisic acid (ABA), and auxin (IAA), respectively, were measured by the ELISA kit (Bio Take Corporation, Beijing, China). Eight samples of different gender and plant density were taken from the fresh hemp plant and dipped into liquid nitrogen for 15 min. Then the samples were stored in a refrigerator at -20 °C until they were tested and the results are presented in Fig. 4.

RESULTS AND DISCUSSION

The hemp hurd thickness of 100 hemp plants in the directions of south, north, west, and east were 8.08 mm (2.93), 8.37 mm (3.64), 8.20 mm (3.27), and 7.98 mm (3.14), respectively. Values in parentheses represent one standard deviation. The hemp hurd thickness of 52 hemp plants in the south direction was thicker than that of hemp hurd in the north direction, the hemp hurd thickness of 41 hemp plants in the south direction was thicker than that of hemp hurd in the west direction, the hemp hurd thickness of 57 hemp plants in the south direction was thicker than that of hemp hurd in the east direction, the hemp hurd thickness of 55 hemp plants in the north direction was thicker than that of hemp hurd in the west direction, the hemp hurd thickness of 51 hemp plants in the north direction was thicker than that of hemp hurd in the east direction, and the hemp hurd thickness of 52 hemp plants in the west direction was thicker than that of hemp hurd in the east direction. The thickness of the hemp hurd changed as the hemp plants grew in different directions (Fig. 3). This maybe because the hemp plants were affected by each other (allelopathy), or to keep the weight balance, or to get the enough sunlight in different directions. The reasons need to be confirmed in the future.

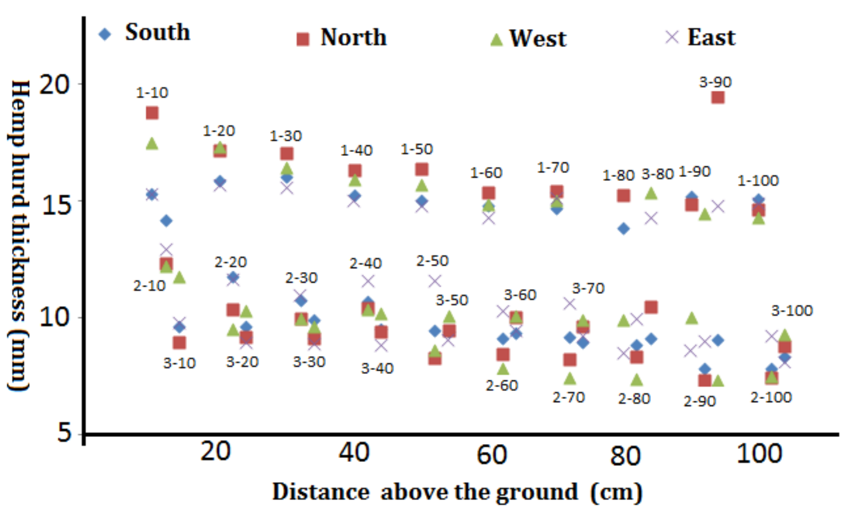

Fig. 3. The hemp hurd thickness of three hemp plants in different locations (1, 2, and 3 represent the hemp plant; 10 to 100 represent the height of hemp hurd above the ground)

Due to the changing hemp hurd thickness along the height of the hemp plant, the narrow areas and wide areas (Fig. 1B) were selected in every 10 cm length of hemp hurd. The influence of the wide and narrow areas on the chemical properties are shown in Table 1. The pectin and ash contents in the narrow areas were lower than those in the wide areas, and the lignin content, holocellulose, alpha cellulose, and hemicellulose content in the narrow areas were higher than those in the wide areas. The change in lignin content was in line with results from prior research (Rodrigues *et al.* 2001; Sultana *et al.* 2010). This might have been because the cell numbers in the narrow areas were less than those in the wide areas, the quality of the middle lamella was less in the narrow areas per unit volume than those in the wide part, and the pectin was rich in the middle lamella and primary layers of the cell (Palin and Geitmann 2012), yet the holocellulose, alpha cellulose, and hemicelluloses were rich in the cell wall. Thus, the pectin content was lower in the hemp hurd than the other chemical compounds that make up the cell walls. The pectin content and ash content were positively correlated, with a correlation coefficient of 0.13. The pectin content was higher in the wide parts, and the ash content in wide parts was higher too. This might be because pectin can bond with many heavy metal ions (Kartel *et al.* 1999; Rounds *et al.* 2011).

Table 1. Chemical Properties of Hemp Hurd

Gender	Plant Density (n/m^2)	Sample Part	Ash (%)	Pectin (%)	Lignin (%)	Holocellulose (%)	Alpha Cellulose (%)	Hemicellulose (%)
Male	9	Narrow	2.27 (0.01)	1.55 (0.01)	21.3 (0.01)	72.6 (0.01)	49.5 (0.01)	23.1 (0.01)
	9	Width	2.81 (0.01)	1.99 (0.01)	21.2 (0.01)	71.8 (0.01)	48.8 (0.01)	23.0 (0.01)
Female	9	Narrow	2.24 (0.01)	1.93 (0.01)	21.9 (0.01)	72.8 (0.01)	49.8 (0.01)	23.0 (0.01)
	9	Width	2.52 (0.01)	2.86 (0.01)	21.4 (0.01)	71.9 (0.01)	49.1 (0.01)	22.8 (0.01)
Male	18	Narrow	1.92 (0.01)	1.87 (0.01)	21.8 (0.01)	73.0 (0.01)	49.8 (0.01)	23.2 (0.01)
	18	Width	2.46 (0.01)	2.81 (0.01)	21.4 (0.01)	72.0 (0.01)	49.7 (0.01)	22.3 (0.01)
Female	18	Narrow	1.91 (0.01)	2.04 (0.01)	22.0 (0.01)	72.8 (0.01)	50.4 (0.01)	22.4 (0.01)
	18	Width	2.13 (0.01)	2.19 (0.01)	21.5 (0.01)	72.3 (0.01)	50.1 (0.01)	22.2 (0.01)

Note: Figures in parentheses represent one standard deviation

The cell numbers in the whole area of both the wide and narrow areas were counted by hand (Table 2). The cell number in the wide areas was higher than the cell number of the narrow areas in hemp plants of different genders and plant densities. The density of cells in the wide areas was lower than that of the narrow areas in hemp plants mentioned above. The difference was attributed to the cambium activity during the annual cycle (Villanueva *et al.* 2015), which was controlled by plant hormones (Pierik *et al.* 2006; Love *et al.* 2009). The plant hormones CTK, ABA, and IAA that correlated to the growth of the plant were higher in the wide areas than that in narrow areas (Fig. 4). The results differed from prior research (Hellgren *et al.* 2004; Jonathan *et al.* 2009). This might have been because hemp is different from the poplar plant and has different plant hormones. In the future, there can be further research about the influence of exogenous hormones on the cambium activity. Thus, one day it may be possible to control the plant growing speed to obtain the ideal materials by control the hormones rather than genetic technology.

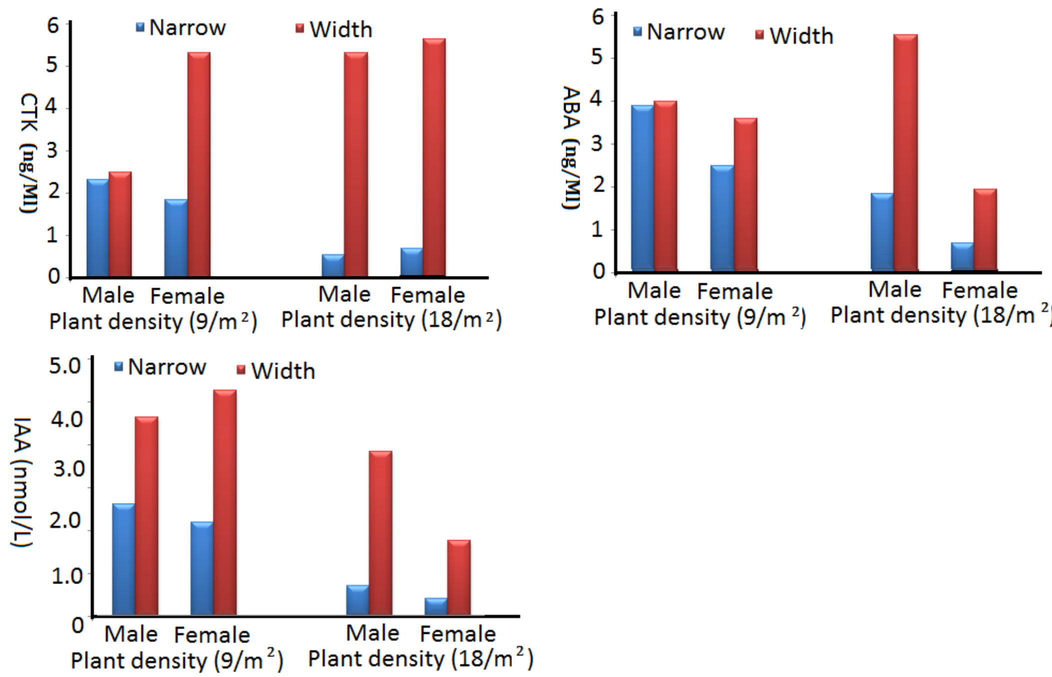

Fig. 4. Three kinds of plant hormones in hemp plants of different genders and plant densities

Table 2. Cell Numbers of the Hemp Hurd

Gender		Male		Female		Male		Female	
Plant Density (n/m^2)		9		9		18		18	
Sample Area		Narrow	Width	Narrow	Width	Narrow	Width	Narrow	Width
10 cm	Mean	275.3 (3.95)	283.2 (4.94)	141.6 (3.63)	176.4 (3.72)	197 (3.89)	242.6 (9.17)	137.9 (6.05)	157.3 (8.06)
	Range	269 to 281	276 to 290	136 to 149	169 to 181	193 to 205	226 to 257	128 to 147	146 to 169
250 cm	Mean	46.5 (4.53)	56.6 (2.99)	52.1 (1.73)	60.9 (2.38)	54.4 (3.57)	80.2 (1.81)	47.7 (0.95)	58.2 (3.71)
	Range	41 to 54	53 to 60	50 to 56	56 to 64	48 to 59	77 to 82	46 to 49	52 to 64

Note: Figures in parentheses represent one standard deviation.

CONCLUSIONS

1. The difference of hemp hurd thickness in different directions of East, South, West, and North was not significant ($p > 0.05$) based on the data from 100 hemp plants. As the hemp hurd grew, the thickness of the plants at four different directions also changed.
2. The pectin and ash content in the wide areas were higher than those in the narrow areas while the other chemical compounds were the opposite. The lignin,

holocellulose, alpha cellulose, and hemicelluloses content in the narrow areas were higher than those in the wide areas ($p = 0.05$) in two genders and plant densities.

3. The cell numbers in the wide areas were higher than those in the narrow areas in two locations of hemp plants (10 cm and 250 cm), of different genders, and varied plant densities ($p < 0.05$) due to the division difference of cambium caused by the plant hormones. Three particular plant hormones, CTK, ABA, and IAA, in the wide areas were higher than those in the narrow areas of hemp hurd plants of different genders and plant densities ($p < 0.05$). The cambium division speed should be controlled by the exogenous plant hormones to control the plant growing speed and obtain ideal materials based on what it is to be used for.

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