A new technology for predicting the fiber content in hemp bast

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Hemp (*Cannabis sativa* L.) is a traditional fiber crop, which is becoming one of the most important industrial fibers, with a promising future in many fields. To accelerate the breeding of hemp cultivars with increased fiber content, it is important to establish a pre-flowering method that can be used to predict the potential fiber content in hemp bast. This study investigated the correlation between fiber content in the stem bast portion and in the entire stem. In addition, the variation in the bast fiber content during the sampling period was studied in three hemp cultivars. It was clear that the bast fiber content in hemp stems was determined up to 40 d before emergence of the staminate buds. The fiber content of the bast (in a sample piece 30cm long and covering one-quarter of the stem girth, sampled at two-fifths of the plant height from the soil, 20 d before the staminate buds emerged) was shown to be representative of the fiber content of the entire stem. In conclusion, this new method would allow breeders to select the hemp plants for high bast fiber content during the early to middle growth periods, before the male buds emerged, potentially accelerating the genetic improvement of fiber content in industrial hemp.

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ABSTRACT

Hemp (*Cannabis sativa* L.) is a traditional fiber crop, which is becoming one of the most important industrial fibers, with a promising future in many fields. To accelerate the breeding of hemp cultivars with increased fiber content, it is important to establish a pre-flowering method that can be used to predict the potential fiber content in hemp bast. This study investigated the correlation between fiber content in the stem bast portion and in the entire stem. In addition, the variation in the bast fiber content during the sampling period was studied in three hemp cultivars. It was clear that the bast fiber content in hemp stems was determined up to 40 d before emergence of the staminate buds. The fiber content of the bast (in a sample piece 30cm long and

covering one-quarter of the stem girth, sampled at two-fifths of the plant height from the soil, 20 d before the staminate buds emerged)was shown to be representative of the fiber content of the entire stem. In conclusion, this new method would allow breeders to select the hemp plants for high bast fiber content during the early to middle growth periods, before the male buds emerged, potentially accelerating the genetic improvement of fiber content in industrial hemp.

Keywords: hemp (*Cannabis sativa* L.), fiber content, prediction, fiber yield potential, hemp breeding

INTRODUCTION

Hemp (*Cannabis sativa* L.) is an ancient and eco-friendly cultivated crop that was first cultured in China, and is used for the manufacture of clothes, household supplies, paper pulp, drugs, food, recyclable composite materials and so on (*Blade, Gaudiel & Kerr, 1999; Dalotto, 1999; Zhou, Zhang & Zhang,2009*).Hemp has been used to make more than 2,500 known products (*Johnson, 2014*). Hemp textile industries first began in Europe and Asia around8000BC (*Hemphouse, 2017*). In the middle of the 20thcentury, hemp was banned from cultivation by governments as an illegal drug crop. However, in recent years, governments and researchers became more interested in the cultivation of hemp, as one of the most important green fibers and drugs (*Decorte, 2010*), and the cultivation of a number of hemp cultivars with low THC (tetrahydrocannabinol,<0.3%)

content has been allowed, to the point where some European government seven provide agricultural subsidies for hemp cultivation (*Forapani et al., 2001*). Hemp can grow with little or no chemical fertilizers, herbicides or pesticides and the cropis now cultivated all around the world (*Hemphouse, 2017*).

Asa dioecious (separate male and female individual plants) and hence obligately out breeding species, hemp has a very high level of genetic variation with respect to biological traits, even within a cultivar, or between parents and their progeny (*Faeti et al., 1996; Yang et al.,2003b; Deng et al., 2010*). Bredemann was the first author to select high-yield in hemp cultivars on the basis of the fiber contents of the male and female parents, and then crossing those high-fiber parents (*Ranalli, 2004*). For efficient hemp cultivar production, it is necessary to select parent plants with high fiber contents before flowering, in order to achieve seed production in the current year, and then to select within the offspring to identify individuals with high fiber content and fiber yield potential.

The bast fiber content differs among various parts of the hemp stem. *18. Mediavilla, Leupin & Keller (2001)*observed that, if the hemp stem was separated into three equal portions, approximately 54% of the fibers were in the bottom third, 34% in the middle third and only 12%in the top third. *de Meijer & van der Werf (1994)* reported that 70.4%of the stem dry matter

53 and 71.6% of the hemp fiber product were found in the first meter of stem from the soil surface
54 upwards. According to another report, the amount of pure fiber per unit length of stem (g cm^{-1})
55 decreased linearly from the bottom to the top (*Amaducci, 2003*). However, *Westerhuis et al.*
56 *(2009)* reported that the ratio of the total fiber content to wood content was highest in the middle
57 part of the hemp stem, and decreased towards both the bottom and the top of the stem.
58 Generally, it is commercially important to maximize hemp fiber quality and yield. Many
59 scientists have engaged in the breeding of hemp cultivars with high yield and good quality, and
60 many new cultivars have been released (*van der Werf, Wijlhuizen & de Schutter, 1995; Yang et*
61 *al., 2003b;Westerhuis et al., 2009a, b; Guo et al., 2017; Kang et al., 2017*). However, a method
62 for selecting hemp plants with high fiber contents before flowering is not currently available
63 (*Ranalli, 2004*), resulting in a decrease in selection efficiency, and an increase in the duration
64 (and cost) of hemp breeding programs.
65 In this study, we investigated the correlation between fiber content in the stem bast portion
66 and the whole stem, as well as how the bast fiber content varied during the pre-flowering
67 sampling period in three hemp cultivars. The goal of this research was to try to develop a non-
68 destructive methodology to evaluate the potential fiber content in the hemp bast, by which to
69 select elite high-fiber in dividuals before flowering as the parents for hybridization, for the

multiplication of seed of commercial cultivars, and for the identification of elite cultivars.

MATERIALS AND METHODS

Cultivation of the hemp plants

The hemp plants were cultivated in a field of the experimental station of Yunnan University at Kunming, Yunnan Province in south-western China, in the years 2014and 2015. Kunming lies at 25°01′E, 102°41′N, at an altitude of 1896 m, and it has a dry season from November to April, with a monthly average temperature over the year of 8.1–19.9°C, a monthly mean relative humidity of 58–83%, and an annual rainfall of 1011 mm. The soil nutrients were determined in the trial site before sowing to be 145.3–148.5 mg kg^{-1}alkali hydrolysable nitrogen, 23.5–25.4 mg kg^{-1} available phosphorus, and 249.3–255.8 mg kg^{-1} available potassium in 2014 and 2015.

The plant material was represented by three recently bred cultivars in Yunnan Province, namely Ym 1, Ym4 and Ym5, which exhibited different time periods from seedling emergence to male bud emergence, approximately120, 110 and 130 days, respectively. All cultivars were sown on 29 and 28 April in 2014 and 2015, respectively. The distance between plant rows was 40 cm, the sowing depth was 8cm and final density was 300,000 plants ha^{-1}. The experimental set up of the cultivars Ym 1, Ym 4 and Ym 5. The area of the plots of each cultivar was $20m^2$, and the width of the walking area between each plot was 80cm, and two lines of the Ym5 cultivar (80cm

wide) were planted in each guard row. The fields were cultivated by following local hemp cultivation practice, without spraying herbicides or pesticides

The experiment carried out in 2015 was conducted to verify the results in 2014

Sample collection and treatment

For the experiment in 2014, the first sample collection was carried out about 70 d after seedling emergence, by which time the plants had grown to 1.5min height. Another five to seven sample collections (depending on the reported time between sowing and the emergence of the male buds occurred for each cultivar) were done at 10-d intervals. However, for the experiment in 2015, only three sample collections were carried out, at the times corresponding to the last three sample collections for the three cultivars in 2014.

At each sample collection, five plants of similar growth status for each cultivar were selected at each time and a mark was made on the east side of each plant. For convenience, the stem was cut off at ground level and plant height was measured, then a piece of bast (representing one-quarter of the stem girth) was stripped off from the marked side, from the bottom to the top of the stem. The sampled bast was separated equally into five portions by length and designated as portion 1 (P1) at the bottom of the stem through to portion 5 (P5)at the top. The remainder of the bast was collected to test the fiber content of the whole stem (the fiber in the sampled bast was also added). The bast was dried in an oven set at 38°C (to keep microbes alive) until constant

105 weight was achieved.

106 The bast strips were degummed by incubation in a water bath at 36°C for 5 d.When the
107 incubation was completed, the bast strips were washed, then the fiber was collected and dried at
108 60°C to constant weight. The fiber content in each portion of the sampled bast and in the bast of
109 the whole stem was calculated from the ratio of fiber dry weight to bast dry weight

110 **Data analysis**

111 The correlations between the fiber content in the bast of each stem portion and the whole stem for
112 each cultivar, and for the three cultivars together, were analyzed using the statistical software
113 SPSS 16.0.Based on the data of the whole stem bast from each sample collection, the variation of
114 the fiber content in each of the three cultivars throughout the sampling period was presented,
115 using Excel 2007. Data points were presented as the mean value, while error bars represented
116 represent ± standard error from mean.

117 # RESULTS

118 **Variation in bast fiber content in the hemp cultivars during the sampling period**

119 The bast fiber contents in the hemp cultivars in 2014 varied clearly, but the plot lines of the three
120 cultivars did not intersect (Fig. 1).Cultivar Ym4 showed higher bast fiber content than did Ym1
121 and Ym5, and it was observed that Ym 5 had the lowest fiber content during the sampling period.

Moreover, although the variation of fiber content in the three cultivars in 2015 differed somewhat from that in 2014, the overall trends within the two years were similar to one other (Figs. 1, 2).The results showed that the difference between the bast fiber content of the three cultivars (and maybe the potential fiber yield) was determined early in plant growth (40 d before emergence of the staminate flower buds), and that this difference was quite stable throughout the sampling period. Therefore, the fiber content of the different cultivars in the growth period before flowering could be a reflection of variation in the potential fiber yield at harvest among the hemp cultivars.

Correlations between fiber content in the bast of the different stem portions and in the whole stem

In general, moderate to high positive correlations were observed between the fiber content in the bast of the different stem portions and in the whole stem, with the exception of the top portion, P5 (Tables 1, 2). Generally, there was a highly significant positive correlation between the fiber content in the bast from the first (P1) to the third portions (P3) and that in the whole stem, with the exception of the third portion of Ym 1 in 2015 (Tables 3, 4).

The fiber content in the bast of portion 2 (P2) was highly correlated with that of the whole stem in cultivars Ym 1, Ym 4 and Ym 5 in the samples collected 20 days before the male buds emerged (Table 3). The correlation coefficients were 0.940, 0.970 and 0.903, respectively (Table

3, values underlined), with the corresponding coefficients of determination being 0.884, 0.941 and 0.815, respectively. This indicated that the reliability of predicting the fiber content of the whole stem via testing the fiber content in the bast of P2 20 d before flowering could be more than 80%.

The above results were verified by the data from the2015 trial. The correlation coefficients between the fiber content in the bast of stem portion 2 (P2) and that of the whole stem in the three cultivars from the samples collected 20 days before the male buds emerged were 0.971, 0.964 and 0.970, respectively (Table 4, values underlined), with the coefficients of determination being 0.942, 0.929 and 0.941, respectively. This means that the reliability of predicting the fiber content in the whole stem via testing the fiber contents in the bast of P2 20 d before flowering was more than 90% for the three cultivars.

Putting the data from the three cultivars together, the correlation between the fiber contents in the bast of stem portion 2 (P2) and the whole stem from the samples collected 20 days before the male buds emerged was very high in both 2014 and 2015 (Tables 1, 2). The correlation coefficients were 0.949 and 0.960, in 2014 and 2015, respectively. Implying reliabilities of more than 90%for this method of predicting the fiber content of the whole stem 20 days before decisions had to be made, regarding which parents to hybridize (Tables 1, 2, values underlined).

DISCUSSION

The high degree of genetic variation in hemp is an advantage with respect to breeding cultivars with high fiber yield (*Faeti et al., 1996; Yang et al., 2003b*). Hemp is an anemophilous obligately cross-pollinated crop, and its pollen can travel over 100km (*Cabezudo et al., 1997; Cariñanos et al., 2004*), which causes difficulty for hemp breeders, with respect to preventing uncontrolled pollination. Therefore, the selection of elite plants early in growth would be very important for increasing breeding efficiency. However, due to the absence of an applicable method for predicting the bast fiber content at an early growth stage before flowering, the evaluation of the fiber yield of prospective parent plants could not be quantified or assessed on an objective basis. As a consequence, evaluation of the hemp cultivar or germplasm as a prospective parent is either delayed to post-harvest or could only be carried out on a subjective basis by an experienced breeder (*Ranalli, 2004*).This is one of the main impediments to enhancing the efficiency of hemp breeding.

In this paper, a new method for predicting the hemp bast fiber content was developed, which entailed stripping off a small piece of the stem bast (one-quarter of the stem girth and 30cm long) from the lower part (P2) of the stem 20 d before flower bud emergence, and measuring the fiber content. Inter-cultivar differences with respect to bast fiber content, and maybe potential fiber

yield, were determined at an early stage, and the differences were quite stable throughout the sampling period. Moreover, using this technology and sampling a small piece of stem bast did not stunt the growth of the hemp plants (data not shown).The treatment could be used to estimate the bast fiber content of the whole stem and the potential fiber yield of the hemp cultivars before flowering (permitting the use of the selected high-fiber individual plants as parents in hybridization in the current year), and showed reliability values(in terms of estimating whole stem fiber content) as high as greater than 80%. Using this method, breeders can select plants with high fiber content for hybridization, to accelerate the production of high-fiber hybrid progeny, and to enhance breeding efficiency in industrial hemp. This method could also be used to select individual plants with high fiber content for seed multiplication from the highly heterogeneous populations which make up a commercial hemp cultivar.

Conclusion

The difference between the bast fiber content of the three hemp cultivars (and maybe the potential fiber yield) was determined early in plant growth (40 d before emergence of the staminate flower buds), and that this difference was quite stable throughout the sampling period. The fiber content of the bast (in a sample piece 30cm long and covering one-quarter of the stem girth, sampled at two-fifths of the plant height from the soil, 20 d before the staminate buds

191 emerged) was shown to be representative of the fiber content of the entire stem, and showed
192 reliability value as high as greater than 80%. Using this technology and sampling a small piece of
193 stem bast did not stunt the growth of the hemp plants. The technology studied in this paper can
194 accelerate the production of high-fiber hybrid progeny, and to enhance breeding efficiency in
195 industrial hemp.

196 ## REFERENCE

197 **Amaducci S. 2003.** HEMP-SYS: design, development and up-scaling of a sustainable production system for
198 hemp textiles—an integrated quality systems approach. *Journal of Industrial Hemp* **8(2):** 79-83.
199 **Decorte T. 2010.** The case for small-scale domestic cannabis cultivation. *International Journal of Drug Policy*
200 **21(4):** 271-275.
201 **Blade SF, Gaudiel RG, Kerr N. 1999.** Low-THC hemp research in the black and brown soil zones of Alberta,
202 Canada. Perspectives on new crops and new uses. *ASHS Press, Alexandria* **VA:** 306-310.
203 **Bocsa I, Karus M. 1998.** The Cultivation of Hemp: Botany,Varieties, Cultivation and Harvesting. Sebastopol
204 CA: Hemp Tech.
205 **Cabezudo B, Recio M, Sánchez-Laulhé J, Trigo MDM, Toro FJ, Polvorinos F. 1997.** Atmospheric
206 transportation of marihuana pollen from North Africa to the southwest of Europe. *Atmospheric*
207 *Environment* **31(20):** 3323-3328.
208 **Cariñanos P, Galán C, Alcázar P, Domínguez E. 2004.** Analysis of the particles transported with dust-clouds

reaching Cordoba, southwestern Spain. *Archives of environmental contamination and toxicology* **46(2):** 141-146.

de Meijer EPM, van der Werf HMG.1994. Evaluation of current methods to estimate pulp yield of hemp. *Industrial Crops and Products* **2:** 111–120.

Deng G, Guo HY, Dun HY, Li F, Yang M, Liu FH. 2010. A review on the impacts of environmental and physiological factors on fiber yield and quality of industrial hemp. *Plant fiber sciences in China* **32:** 176-182.

Faeti V, Mandolino G, Ranalli P. 1996. Genetic diversity of Cannabis sativa germplasm based on RAPD markers. *Plant Breeding* **115:** 367-370.

Forapani S, Carboni A, Paoletti C, Moliterni VMC, Ranalli P, Mandolino G. 2001. Comparison of hemp varieties using random amplified polymorphic DNA markers. *Crop Science* **41:** 1682–1689.

Guo HY, Hu XL, Chen Y, Xu YP, Guo MB, Wu JX, Zeng M, Deng WP, Yang M. 2009. Research on breeding of new early-maturing seed-using hemp variety 'Yunma 2'. *Plant fiber sciences in China* **31:** 285-287.

Guo HY, Yang M, Xiu XH, Gao YH, Yuan H. 2002. Discussion on industrial hemp production and industrialization in Yunnan Province. *Plant fiber sciences in China* **24:** 24-27.

Guo L, Wang ZM, Che Ye, Wang DK, Li ZY. 2017. The breeding reportof new industrial hemp variety Qingdama No.1. *Plant fiber sciences in China* **39(2):** 61-63.

Hemphouse. 2017. Hemp history. *http://www.hemphousemaui.com/hemp-history/* (accessed 22 November 2017).

Johnson R. 2014. *Hemp as an agricultural commodity*. Library of Congress Washington DC Congressional Research Service.

Kang HM, Zhao MS, Kong JQ, Gao JH, Meng XK, Feng XP. 2017. Breeding of a new hemp variety Fenma No. 3. *Seed* **36(6):**114-116.

MediavillaV, Leupin M, Keller A. 2001. Influence of growth stage of industrial hemp on the yield formation in relation to certain fibre quality traits. *Industrial Crops and Products* **13:** 49-56.

Ranalli P. 2004. Current status and future scenarios of hemp breeding. *Euphytica* **140:** 121-131.

van der Werf HMG, Wijlhuizen M, de Schutter JAA. 1995. Plant density and self-thinning affect yield and quality of fibre hemp (*Cannabis sativa* L.). *Field Crop Research* **40:**153-164.

Westerhuis W, Amaducci S, Struik P C, Zatta A, van Dam JEG, Stomph TJ. 2009a. Sowing density and harvest time affect fibre content in hemp (*Cannabis sativa*) through their effects on stem weight. *Annals of Applied Biology* **155:** 333- 348.

Westerhuis W, Struik P C, van Dam JEG, Stomph TJ. 2009b. Postponed sowing does not alter the fibre/wood ratio or fibre extractability of fibre hemp (*Cannabis sativa*). *Annals of Applied Biology* **155:** 225-244.

Yang M, Guo HY, Gao YH, Hu XL, Chen Y, Yin PX, Liu ZB. 2003a. Character and cultivation of a new

industrial hemp variety ‘Yunma 1’. *Plant fiber sciences in China* **6:** 21-23.

Yang M, Guo HY, Wen GS, Wu W, Qian JL. 2003b. Breeding of a new hemp variety ‘Yunma 1’ and its cultivation. *Plant fiber sciences in China* **25:** 1-3.

Zhou YK, Zhang J, Zhang JC. 2008. Structure andpropertiesof hemp xyloidstem based viscose fiber. *Journal of Textile Research* **29(4):** 22-26.

Table 1(on next page)

Table 1

Table 1 Pearson’s correlation coefficients between fiber content in the bast of stem portions and the whole stem of three hemp cultivars during the sampling period of 2014

1

Time before male	Different portion of stem bast				
bud emergence (d)	P1(bottom)	P2	P3	P4	P5(top)
40	0.890**	0.357	0.719**	0.708**	0.482
30	0.955**	0.901**	0.932**	0.790**	0.565
20	0.900**	0.949**	0.674*	0.571	0.047
10	0.871**	0.783**	0.864**	0.711**	0.009
0	0.774**	0.969**	0.948**	0.881**	0.724**

2 * $p<0.05$, ** $p<0.01$. The underlined value represents that for P2 sampled 20 d before male bud emergence, as described in

3 the text.

4

Table 2(on next page)

Table 2

Table 2. Pearson’s correlation coefficients betweenfiber content in the bast of stem portions and the whole stem of three hemp cultivars during the sampling period of 2015

Time before male bud emergence (d)	Different portion of stem bast				
	P1(bottom)	P2	P3	P4	P5(top)
40	0.767**	0.892**	0.588*	0.785**	0.531
30	0.762**	0.805**	0.850**	0.552	-0.012
20	0.900**	0.960**	0.935**	0.848**	0.631*

Notes.

* $p<0.05$, ** $p<0.01$. The underlined value represents that for P2 sampled 20 d before male bud emergence, as described in the text.

Table 3(on next page)

Table 3

Table 3. Pearson’s correlation coefficients of fiber contents in the bast of the stem portions and the whole stem in different hemp cultivars at different collection dates during the sampling period of 2014

1

Cultivar	Time before male bud emergence (d)	Different portion of stem bast				
		P1(bottom)	P2	P3	P4	P5(top)
Ym 1	40	0.715	0.304	0.739	0.323	0.135
	30	0.940*	0.975*	0.924*	0.880	0.508
	20	0.949*	0.940*	0.827	-0.054	-0.287
	10	0.966*	0.986**	0.668	0.988**	-0.726
	0	0.911*	0.910*	0.882	0.980**	0.647
Ym 4	40	0.980**	0.668	0.723	0.790	0.809
	30	0.700	0.861	0.979*	0.878	0.359
	20	0.798	0.970*	0.722	0.908*	0.956*
	10	0.719	0.510	0.985**	0.808	0.693
	0	0.937*	0.991**	0.962*	0.845	0.359
Ym 5	40	0.837	-0.139	0.607	0.325	0.663
	30	0.965*	0.707	0.988**	0.466	0.497
	20	0.964*	0.903*	0.220	0.283	-0.992**
	10	0.895	0.647	0.985**	0.992**	-0.715
	0	0.438	0.949*	0.915*	0.652	0.182

2 **Notes.**

3 * $p<0.05$, ** $p<0.01$. The underlined value represents that for P2 sampled 20 d before male bud emergence, as described in

4 the text.

5

Table 4(on next page)

Table 4

Table 4. Pearson’s correlation coefficients betweenfiber content in the bast of the stem portions and the whole stem in different hemp cultivars during the sampling period of 2015

1

Cultivar	Time before male bud emergence (d)	Different portion of stem bast				
		P1(bottom)	P2	P3	P4	P5(top)
Ym 1	40	0.975*	0.766	-0.613	0.901*	0.677
	30	-0.010	0.982**	0.850	0.182	0.519
	20	0.923*	0.964*	0.939*	0.935*	0.891
Ym 4	40	0.969*	0.963*	0.859	0.969*	0.666
	30	0.980**	0.761	0.943*	0.993**	0.897
	20	0.981**	0.971*	0.988**	0.891	0.945*
Ym 5	40	0.504	0.951*	0.786	0.895	-0.510
	30	0.624	0.446	0.411	-0.319	0.207
	20	0.833	0.970*	0.853	0.928*	0.754

2 **Notes.**

3 * $p<0.05$, ** $p<0.01$. The underlined value represents that for P2 sampled 20 d before male bud emergence, as described in

4 the text.

5

Figure 1

Figure 1

Figure 1 Fiber contents in the stem bast of the hemp cultivars Ym 1, Ym 4 and Ym 5 in the sampling period of 2014. Error bars represent ± standard error from mean (n=5).

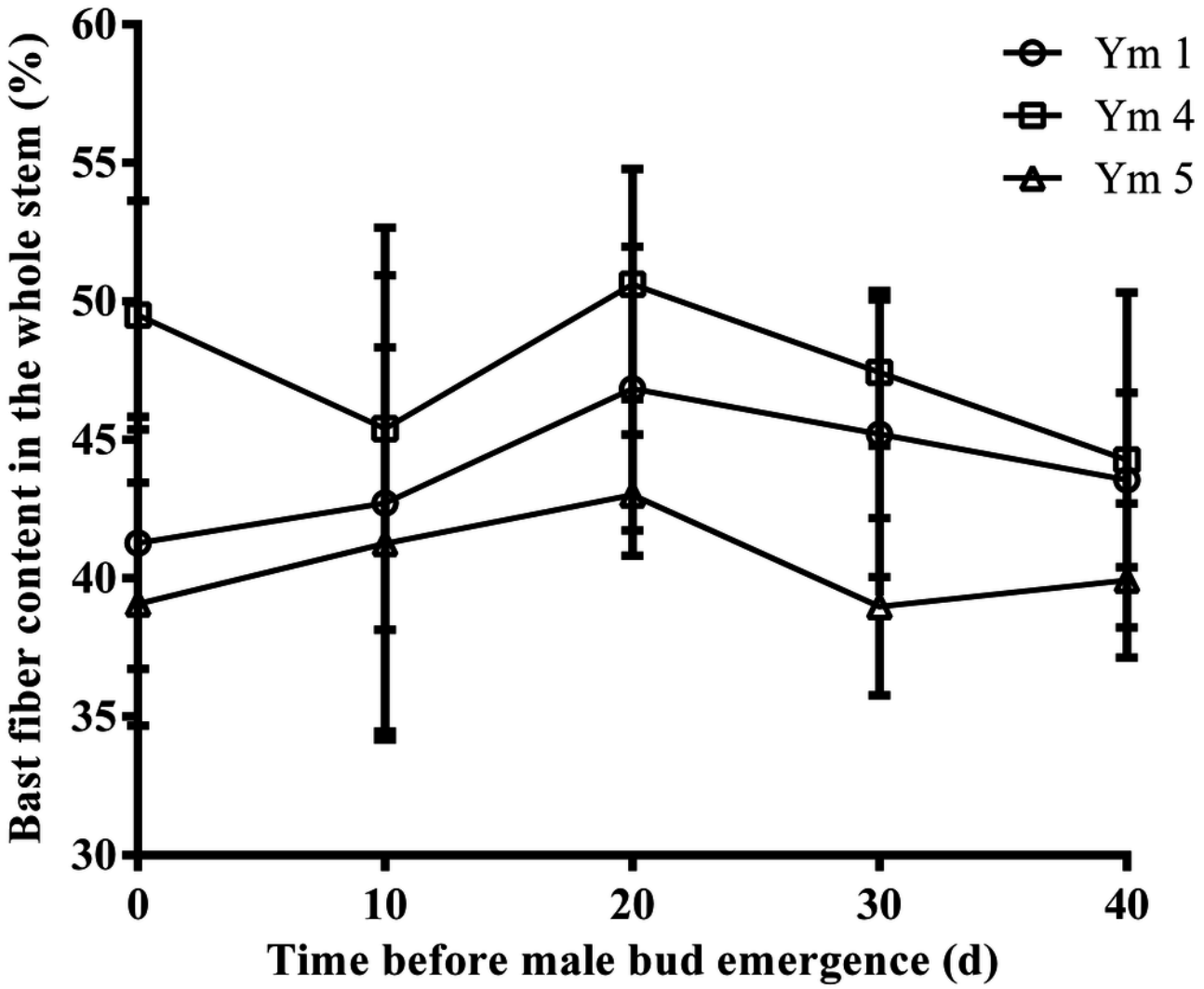

Figure 2

Figure 2

Figure 2 Fiber content in the stem bast of the hemp cultivars Ym 1, Ym 4 and Ym 5 in the sampling period of 2015. Error bars represent ± standard error from mean (n=5).