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Identifying Quantitative Trait Loci for Fiber Content and Fiber Components in Sugarcane Using Amplified Fragment Length Polymorphism Markers

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ABSTRACT

The cane yield, fiber content and fiber components (cellulose, hemicellulose and lignin) were determined from the segregation of 171 hybrid sugarcane clones derived from crossing K 84-200 and Kps 94-13 at 12 mth after planting. The results revealed that the average cane yield and fiber content in the hybrid sugarcane clones were 11.54 t.rai^{-1} (1 rai = 0.16 hectare) and 12.66%, respectively. The bagasse or fiber consisted of cellulose, hemicellulose and lignin with values of about 37.88–47.75, 30.15–40.73 and 4.81–10.23% dry weight, respectively. For amplified fragment length polymorphism identification, a total of 107 simplex markers were used to analyze the inheritance of quantitative trait loci (QTLs) for fiber, cellulose, hemicellulose and lignin contents. Eleven putative QTLs from the simplex markers were identified for these four traits. Each QTL explained from 2.5 to 4.3% of the variation in the fiber content. In parts of the fiber components, each QTL explained from 3.3 to 5.6% of the variation in the cellulose content and 2.3 to 3.0 % in the hemicellulose content, while the QTL explained 2.4 to 5.0 % of variation in the lignin content. Moreover, there were two putative QTLs related to more than one trait.
Keywords: sugarcane, fiber content, cellulose, hemicellulose, lignin

INTRODUCTION

Sugarcane (*Saccharum* species hybrids) is among the most genetically complex crop species. Commercial cultivars of sugarcane are highly polyploid, aneuploid and of multispecific origin, resulting in difficulties in genetic studies for more than two decades (Hogart, 1987). Cultivated sugarcane varieties have 80–140 chromosomes which are comprised of 8–18 copies of a basic X = 8 or X = 10 (D'Hont *et al*., 1998; Ha *et al*., 1999; Irvine, 1999). Understanding the genetic diversity could lead to successful genetic manipulation in a sugarcane breeding program, especially for the identification of quantitative trait loci (QTLs) for fiber content and fiber components.

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Generally, important agronomic traits are controlled by large numbers of QTLs (Hoarau *et al*., 2002). The identification of QTLs based only on conventional phenotypic evaluation is not possible. A number of molecular techniques, especially DNA-based markers, have been developed to provide information on the genetic diversity. Amplified fragment length polymorphism (AFLP) analysis is a technique through which selected fragments from the digestion of total plant DNA are amplified by a polymerase chain reaction. The resulting DNA fingerprinting provides a large number of information points per experiment. The higher the number of information points, the more types of markers; thus, the AFLP technique been used successively to assess the genetic diversity, for genome mapping and for sugarcane breeding (Ana *et al*., 2005).

The current study concentrated on the use of bagasse. Normally, the sugarcane stalk consists of two parts—the inner pith containing most of the sucrose and the outer part consisting of lignocellulosic fiber. During sugar processing, the sugarcane stalk is crushed to extract sucrose (Boopathy, 2004). This procedure produces a large volume of bagasse which contains both crushed rind and pith fibers (Dawson and Boopathy, 2008). Bagasse has a composition, properties and structure that make it suitable for uses such as compost, textiles, pulp and paper manufacture, and animal feed (Narendra and Yang, 2005). Cellulose (32–48%), hemicellulose (23–32%) and lignin (18–24%) are the main organic components of bagasse (Han and Rowell, 1997; Nordin, 1997; Dawson, 2005; Yong, 2005)

In addition, bagasse can also be used to produce fuel such as ethanol (Galvez, 1994; Lois, 2009). Ethanol conversion from bagasse should focus on the composition of insoluble fiber in the cell wall. A high ethanol yield can be achieved from fiber containing a high cellulose content. On the other hand, hemicellulose and lignin are constraints to ethanol production as they cause high production costs (Ho *et al*., 1998; Dien *et al*., 2003; Van Maris *et al*., 2006; Kim *et al*., 2007).

The purposes of the current research were: (1) to determine the cellulose, hemicellulose and lignin contents in hybrid sugarcane clones and parental clones, (2) to study the correlation among fiber content, cellulose content, hemicellulose content and lignin content and (3) to identify the AFLP markers linked to each trait.

MATERIALS AND METHODS

Agronomic trial and field data

A segregated population of 171 hybrid sugarcane clones derived from crossing the sugarcane varieties K 84-200 and Kps 94-13 were planted on March 2010 in a randomized complete block design with two replications at the Cane and Sugar Research and Development Center, Nakhon Pathom province, Thailand. Each clone was planted in a basic plot consisting of a 1.5 m row with three plants (stools) per row. Rows were spaced 1.5 m apart. The cane yield, fiber content and fiber components were evaluated at 12 mth after planting. The yield was estimated from the stalk weight and the number of stalks per stool. The fiber content was calculated using the formula given by Thangavelu and Rao (1982).

Fiber components analysis

Two samples of each hybrid sugarcane clone were analyzed for acid and neutral detergent fiber by the method of AOAC (1995) and European Group on Rabbit Nutrition (2001). This method determines three fiber residues after successive hydrolysis on the same sample using the following procedures: first, a neutral detergent (NDF), second, an acid detergent (ADF) and finally an acid hydrolysis (ADL). For the NDF analysis, approximately 1 g of each sample was placed in a clean and dried crucible then 100 mL of neutral detergent solution was added and heated to boiling for 60 min. Next, the solution was filtered and then washed twice with boiling distilled water. The crucibles were transferred to the cold extraction

unit and washed with acetone at least twice. Finally, the crucibles were oven dried at 103 °C for at least 6 h and weighed after cooling. For the ADF analysis, the crucibles after weighing were placed in the room with a temperature of 20–22 °C and three quarters filled with acid detergent solution and then stirred every hour and refilled as necessary. After 3 h, the solution was filtered and washed with hot distilled water at least six times until the pH was neutral, and then oven dried at 103 ° C overnight and weighed after cooling. In the third of the analyses, the ADL residue was burned at 550 °C for at least 3 h, and then transferred to an oven for 1 h at 250 °C. Finally, the ADL residues were weighed after cooling. The fiber components were calculated using the method described by Van Soest *et al.* (1991) and Eid *et al.* (2011).

Amplified fragment length polymorphism genotyping and quantitative trait loci detection

DNA was extracted from freshly-rolled leaves using a genomic DNA purification kit #5012 (Fermentas; Vilnius, Lithuania). The AFLP protocol (Vos *et al*., 1995) was followed with some modifications. Silver staining was applied for AFLP fingerprinting. A set of 316 polymorphic markers was produced using 20 AFLP primer combinations. Each AFLP marker was identified by a primer combination consisting of four letters plus a band number indicated as a suffix. The first two letters represented the *Eco*RI selective nucleotides and the last two letters represented the *Mse*I selective nucleotides. Clear and unambiguous bands were scored in a presence-versus-absence fashion. In part of the QTL detection, the simplex markers were identified based on the following criteria: (1) the fragment is absent in one of the parents; and (2) the fragment segregation at 1:1 (present:absent) ratio. Among the markers, 107 markers were found to be simplex (1:1) by using a chi squared test at the 95% confidence level. An analysis of variance was performed for each trait. Multiple linear regression was conducted to determine the proportion of phenotypic variance explained by the marker association between the AFLP markers and traits (R^2).

RESULTS AND DISCUSSION

Hybrid sugarcane clones were highly significantly different ($P < 0.01$) for all traits analyzed (Table 1). This indicated that there was more opportunity to select the desired varieties for both low fiber content and high cellulose content. The cane yield of hybrid sugarcane clones varied widely, ranging from 4.55 to 21.52 t.rai^{-1} (1 rai = 0.16 hectare) with an average of 11.54 t.rai^{-1}. The fiber content varied from 8.43 to 17.77% and the average was 12.66%. The cane yields of varieties K84-200 and Kps 94-13 (the parental varieties), were 19.22 and 23.82 t.rai^{-1}, respectively, while the fiber contents were 13.25 and 10.49%, respectively. A higher average content was observed in cellulose (43.19%) than in hemicellulose (35.91%) and lignin (7.51%). The cellulose content ranged from 37.88 to 47.75% while the hemicellulose and lignin contents ranged from 30.15 to 40.74% and from 4.81 to 10.23%, respectively (Table 2).

Table 1 Mean squares and significance levels of cane yield and fiber, cellulose, hemicellulose and lignin contents in parental sugarcane varieties and 171 hybrid sugarcane clones at 12 mth.

Source of variation	Mean square				
	Cane yield	Fiber	Cellulose	Hemicellulose	Lignin
Clones	22.08*	4.21**	5.34**	7.65**	1.80**
Blocks	732.37	203.74	0.79	0.00	0.23
Error	17.04	2.75	0.24	0.39	0.13
C.V.(%)	35.85	13.04	1.14	1.74	4.86

* = Significant at $P < 0.05$; ** = Significant at $P < 0.01$.

Table 2 Cane yield and fiber, cellulose, hemicellulose and lignin contents in parental varieties and 171 hybrid sugarcane clones.

Sugarcane groups	Agronomic traits				
	Cane yield ($t.rai^{-1}$)	Fiber (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Parental varieties					
K 84-200	19.22	13.25	41.48	32.87	7.61
Kps 94-13	23.82	10.49	41.94	33.85	7.09
Hybrid clones					
Maximum	21.52	17.77	47.75	40.73	10.23
Minimum	4.55	8.43	37.88	30.15	4.81
Average	11.54	12.66	43.19	35.91	7.51

Trait distributions are given in Figure 1. The distribution of cane yield was found to be skewed while a normal distribution was observed for the fiber content which was similar to the findings of Hoarau *et al.* (2002) and Anusonpornpurm *et al.* (2008). Cellulose, hemicellulose and lignin which are the fiber components were also found to be normally distributed.

The contents of cellulose, hemicellulose and lignin did not correlate with cane yield. On the contrary, they had significant correlation coefficients (0.9592, 0.8900 and 0.7433, respectively). This evidence indicated that the fiber component characters related to the fiber content more than to the cane yield which was consistent with the reports of Anusonpornpurm *et al.* (2008) and Zhang and Zhao (2007). They reported that the major composition of bagasse was fiber, cellulose, hemicellulose and lignin. Moreover, the correlation coefficients between cellulose and hemicellulose (0.7595) and between cellulose and lignin (0.7339) were higher than that between hemicellulose and lignin (0.4880) as shown in Table 3.

A total of 316 polymorphic bands were generated by the 20 primer combinations used on 168 of the 171 hybrid sugarcane clones and 2 parental varieties (K 84-200 and Kps 94-13). The average number of bands produced by each primer pair was 15.8 bands. This was consistent with Anusonpornpurm *et al.* (2008) who reported that the average number of bands produced by each primer pair from 26 primer pairs was 15.8 bands. The maximum number of bands (27) was produced by the primer AAC/CAG, while the minimum number (8) was produced by the primer AAC/CAC. All markers were used to identify the segregation by using a chi squared test at the 95% confidence level. Marker numbers of simplex, duplex, triplex and multiplex were 107, 77, 45 and

Table 3 Correlation coefficient among cane yield and fiber, cellulose, hemicellulose and lignin contents in 171 hybrid sugarcane clones at 12 mth after planting.

Traits	Fiber	Cellulose	Hemicellulose	Lignin
Cane yield	-0.1297^{ns}	-0.0993^{ns}	-0.1343^{ns}	0.1189^{ns}
Fiber		0.9592**	0.8900**	0.7433**
Cellulose			0.7595**	0.7339**
Hemicellulose				0.4880**

^{ns} = Not significant, ** = Significant at $P < 0.01$.

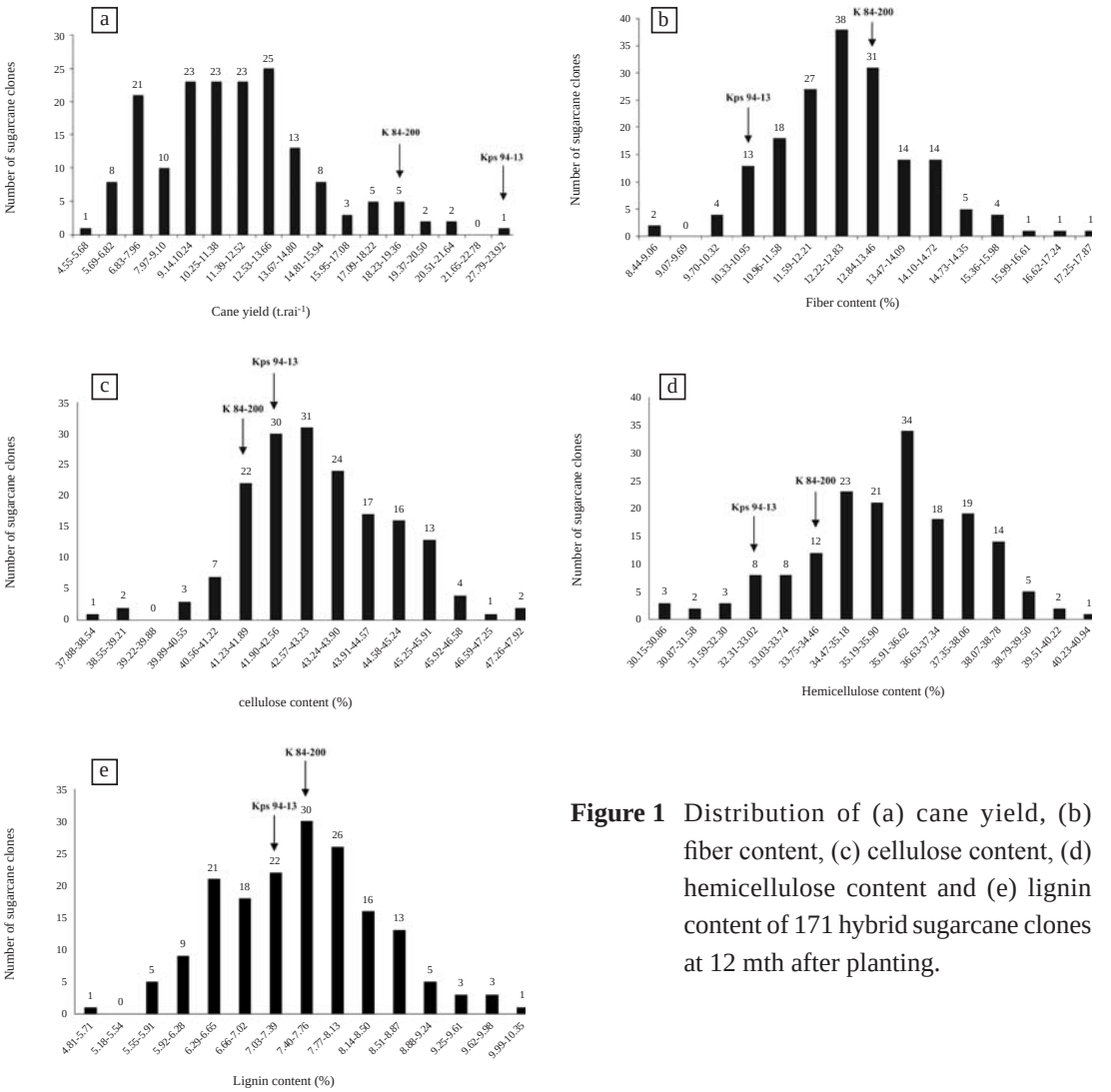

Figure 1 Distribution of (a) cane yield, (b) fiber content, (c) cellulose content, (d) hemicellulose content and (e) lignin content of 171 hybrid sugarcane clones at 12 mth after planting.

87, respectively. Only the 107 simplex markers were used to locate the QTLs (Table 4).

Marker-trait associations were identified for the fiber, cellulose, hemicellulose and lignin contents. There were 11 QTLs from the 107 simplex markers that were associated with traits. The numbers of QTLs associated with the fiber, cellulose, hemicellulose and lignin contents were 2, 2, 3 and 8, respectively, explaining phenotypic variance (R^2) of 2.5–4.3, 3.3–5.6, 2.3–3.0 and 2.4–5.0%, respectively. The two QTLs of fiber content were observed to be the same as those of the cellulose content. These two QTLs (cgag 7 and agtt 10) were identified to be associated with more than one trait. The cgag 7 was associated with the fiber, cellulose and lignin contents and explained 2.5, 3.3 and 4.6% of the phenotypic variation, respectively. The agtt 10 was associated with the fiber, cellulose and hemicellulose contents and explained 4.3, 5.6 and 3.0% of the phenotypic variation, respectively. All significant QTLs of the studied traits showed a negative effect. The QTL having the maximum effect on the fiber, cellulose and hemicellulose contents was agtt 10 and for the lignin content was tttt 15 (Table 5).

Table 4 Polymorphic band and segregation of 20 amplified fragment length polymorphism primer combination at $P \leq 0.05$

Primer combination	Number. of polymorphic bands	Segregation			
		Simplex 1:1	Duplex 11:3	Triplex 13:1	Multiplex >13:1
ACA/CAA	13	3	1	7	2
ACA/CAT	17	5	4	5	3
ACA/CAG	11	8	2	-	1
ACT/CAA	15	4	3	4	4
ACT/CAT	14	11	1	-	2
ACT/CTT	22	11	3	-	8
ACG/CAG	17	3	3	4	7
ACG/CTT	12	5	5	-	2
ACG/CTG	24	5	5	1	13
ACG/CAC	16	6	4	-	6
AAC/CAA	14	4	2	4	4
AAC/CAG	27	3	12	5	7
AAC/CTG	18	3	7	3	5
AAC/CAC	8	3	3	-	2
AAC/CTT	15	4	6	1	4
AAG/CAG	12	3	3	6	-
AAG/CTT	12	7	4	1	-
ATT/CAC	11	2	2	2	5
ATT/CTT	19	8	5	1	5
ATT/CGG	19	9	2	1	7
Average	15.8	5.35	3.85	2.25	4.35
Total	316	107	77	45	87
Percentage	100	33.86	24.37	14.24	27.53

A = Adenine, C = Cytosine, G = Guanine and T = Thymine.

Table 5 Marker effect and significant associations between simplex markers and traits at $P \leq 0.05$.

Markers	Parental variety	Fiber		Cellulose		Hemicellulose		Lignin	
		R^2	Effect	R^2	Effect	R^2	Effect	R^2	Effect
caag 3	*	-	-	-	-	-	-	5.0	-0.080
ctaa 1	Kps 94-13	-	-	-	-	-	-	2.7	-0.058
ctat 9	*	-	-	-	-	-	-	2.7	0.058
cgag 7	K 84-200	2.5	-0.458	3.3	-0.257			4.6	-0.076
acaa 1	*	-	-	-	-	2.3	-0.168	-	-
actt 7	Kps 94-13	-	-	-	-	-	-	3.5	-0.067
agag 8	*	-	-	-	-	-	-	4.6	-0.076
agtt 8	*	-	-	-	-	2.4	-0.170	-	-
agtt 10	*	4.3	-0.600	5.6	-0.336	3.0	-0.191	-	-
tttt 14	*	-	-	-	-	-	-	2.4	0.055
tttt 15	*	-	-	-	-	-	-	3.3	0.065

* = Present in K 84-200 and Kps 94-13.

a = Adenine, c = Cytosine, g = Guanine and t = Thymine used as marker codes.

CONCLUSION

Hybrid sugarcane clones from a cross between two commercial varieties having different fiber contents, produced fiber components (cellulose, hemicellulose and lignin) that were observed to have no relationship with the cane yield, but were observed to have a significantly positive correlation coefficient with the fiber content. Thus, selection for fiber content could be used to select for a high fiber component. A higher content was observed in cellulose than in hemicellulose, while the lignin content was less than 10%. As all the studied characters of the population had normal distributions, QTL identification using an AFLP technique was studied. A small effect (less than 6% of the phenotypic coefficient of determination) of markers in all studied characters was observed. Nevertheless, two QTLs were observed to have a significant effect on two traits of fiber components. These two QTLs could be potential markers for the selection of fiber components for use in the ethanol industry.

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