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Identifying QTLs for Fiber Content and Agronomic Characters in Sugarcane Using AFLP Markers

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ABSTRACT

Sugarcane is a highly complex poly-aneuploid, heterozygous, interspecific polyploid and cultivars commonly have over 100 chromosomes. The population of 168 progenies from a cross between a sugarcane commercial variety (K 93-207) and a *S. spontaneum* clone (MPT 97-1) were evaluated in a replicated field trial. Stalk diameter and tillering were recorded at harvesting and subsequently plant material was evaluated for fiber content. Clones were significantly different (P<0.01) for all traits analyzed. A total of 180 AFLP simplex markers were used to analyze the inheritance of quantitative trait loci (QTLs) for stalk diameter, tillering and fiber content. Thirty putative QTLs from the simplex markers were identified for these three traits. Each QTL explained from 3.7 to 10.7% of the variation for stalk diameter and 3.7 to 9.9% for fiber content. For tillering each QTL explained from 4-8% of the variation. K 93-207 contributed a positive effect to the QTLs for stalk diameter and a negative effect for the QTLs for fiber content and tillering. MPT 97-1 contributed a negative effect to the QTLs for stalk diameter and a positive effect for fiber content and tillering. Ten of the QTLs from both parents were identified as contributing to more than one trait. However, these markers were not available for all parts of this very highly complex genome. Further work will be needed to identify more markers in this population.
Key words: AFLP, sugarcane, QTL, fiber content, simplex marker

INTRODUCTION

Sugarcane is a classic example of a complex autopolyploid. Cultivated sugarcane varieties have ~80–140 chromosomes, comprising 8–18 copies of basic x = 8 or x = 10 (D'Hont *et al.*, 1996, 1998; Ha *et al.*, 1999; Irvine, 1999; Grivet and Arrunda, 2001). Most chromosomes of cultivated sugarcane appear to be largely derived from *Saccharum officinarum* (Irvine, 1999). However, *in situ* hybridization data suggested that about 10% may be derived from *S. spontaneum* (D'Hont *et al.*, 1996). The commercial varieties are polyploid and aneuploid hybrid derivatives from two highly polyploid species, i.e. the domesticated sugar-producing species *Saccharum officinarum* (x=10, 2n=8x=80) and the wild species *Saccharum spontaneum* (x =

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8, 2n = 8x = 40–128). The first interspecific hybrids produced in the early twentieth century were backcrossed with *S. officinarum* (Bhat and Gill, 1985). *S. officinarum* commonly has high sucrose content, low fiber content, thick stalks, little pubescence, rare flowering and limited tillering. *S. spontaneum* does not accumulate sucrose and is fibrous, thin-stalked and pubescent, having profuse flowering and abundant tillering. In Brazil more than 90% of all electrical energy comes from hydroelectric sources. But at the end of 1992, the State of Sao Paulo passed a law permitting the sugar mills and/or distilleries to sell the excess electrical energy they produced back to the State. This electricity comes from burning the sugar cane trash and bagasse (sugar cane fiber stalk after crushing) (Ripoli *et al*., 2000)

Sugarcane is a complex polyploid derived from interspecific hybridization. Most agronomic traits of importance are controlled by large numbers of quantitative trait loci (QTLs). Genetic analysis of agronomic traits using molecular markers has been limited so far in sugarcane. One difficulty when addressing QTL detection in this high polyploid is the construction of a saturated map effectively covering all homo(eo)logous chromosomes. A second difficulty is the possibility that any quantitative trait allele (QTA) effects may be small, because of the many segregating alleles present at key loci for the investigated traits (Haorau *et al*., 2002). The bi-parent population seems more suitable for QTL detection especially if the two parents have highly-contrasted phenotypes for the trait of interest. Then, in bi-parent progeny: (1) the two populations to be compared are of much more balanced size (1:1) than the selfed progeny (3:1); (2) the buffering effect of the background of alternative alleles should be less strong, since half of this background is inherited from the "contrasted parent" (Raboin *et al*., 2006).

Genetic tools for sugarcane have only recently become adequate to quantify the effect of many genomic regions on a trait. Two prior studies reported the association of DNA markers with disease resistance and flowering time in sugarcane. Daugrois *et al*. (1996) identified a putative major gene for rust resistance linked at 10 cM with an RFLP marker CDSR0029 in sugarcane cultivar 'R570'. Guimares *et al*. (1997) found an RFLP marker associated with short-day flowering. However, the mapping populations used in these two studies were too small (83 and 100 individuals, respectively) for comprehensive QTL analysis. Jordan *et al*. (2004) detected numerous small QTLs for stalk number in sugarcane and found sorghum QTLs for tillering in synthetic positions. Similarly, the yield components (plant height, stalk diameter, stalk number and brix) in the selfed progenies of the modern cultivar R570 were controlled by numerous QTLs with smaller individual effects (Hourau *et al*., 2002). But the recent study by Aljanabi *et al*. (2007) detected a major QTL which was found to be linked at 14 cM to an AFLP marker and explained 23.8% of the phenotypic variation of yellow spot resistance.

In this study the primary objective was to identify the quantitative trait loci (QTLs) of sugarcane progeny from K 93-207 x MPT 97-1 by using AFLP simplex markers for three agronomic traits.

MATERIALS AND METHODS

Agronomic trial and field data

A segregated population of 168 clones derived from a cross between the sugarcane commercial variety (K 93-207) and a *S. spontaneum* clone (MPT 97-1) was planted on March 2004 in a randomized complete block design with 3 replicates at Mitr Phol Sugarcane Research Center, Phukieo, Chiyaphum. Each clone was planted in a basic plot consisting of a 1.5 m row with five plants (stools) per row. Rows were spaced 1.3 m apart. Standard commercial

cultivation practices were used. Stalk diameter and tillering were recorded on each individual plot a few days prior to the harvest of plant canes (February 2005). Ten stalks were randomly chosen to estimate fiber content at the sugar laboratory in the Mitr Phol Sugarcane Research Center. The number of stalks was counted for the whole row-plot and calculated for tillering. Ten stalks per plot were randomly chosen to evaluate stalk diameter. Stalk diameter was recorded at the mid length of the stalk.

AFLP genotyping

DNA was extracted from freshly-rolled leaves using a genomic DNA purification kit #5012 (Fermentas). The AFLP protocol (Vos *et al*., 1995) was followed with some modifications. Silver staining was applied for AFLP fingerprinting. A set of 474 polymorphic markers was produced using 19 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. The experiment was conducted at the DNA laboratory of the Cane and Sugar Research and Development Center, Kasetsart University Kamphaeng Saen Campus.

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, 180 were found to be simplex (1:1) by using a Chi squared test at the 95% confidence level. The remaining duplex, triplex and higher multiplex markers were not used for QTL detection. An analysis of variance was performed for each trait. Broad-sense heritability at the experimental design level (h^2_{BS}) was determined from the ratio between the genetic (σ_g^2) and phenotypic (σ_p^2) variance, with $\sigma_p^2 = \sigma_g^2 + \sigma_e^2/3$, where σ_e^2 is the error variance. Simple linear regression was conducted to determine the proportion of phenotypic variance explained by marker association between the AFLP markers and traits (R^2).

RESULTS AND DISCUSSION

Clones were significantly different (P<0.01) for all traits analyzed and broad-sense heritability in this population was 0.80 (80%) for fiber content, 0.83 (83%) for tillering and 0.84 (84%) for stalk diameter (Table 1). Phenotypic correlations among traits are shown in Table 2. Stalk diameter had a negative correlation with both fiber content and tillering. Fiber content had a positive correlation with tillering. The results were

Table 1 Analysis of variance and broad-sense heritabilities for stalk diameter, tillering and fiber content.

Source	df	Mean Square		
		Stalk diameter	Tillering	Fiber content
Clones	167	0.219**	18.90**	20.48**
Blocks	2	0.508**	70.29**	30.30**
Error	334	0.030	3.19	4.08
Total	503			
CV(%)		12.30	18.60	8.10
h^2_{BS}		0.84	0.83	0.80

*,** Significant at P<0.05 and P<0.01 respectively, h^2_{BS} = broad-sense heritability

the same as those reported by Badaloo and Ramdoyal (2003) and Hoarau *et al*. (2002). Broad-sense heritabilities were generally high, indicating good control of the environmental error in the field experiment. These heritabilities are similar to the findings of Hoarau *et al*. (2002) that the heritability of brix, stalk diameter, tillering and stalk length were higher than 80%. Raboin *et al*. (2006) reported the heritability of rust resistance scores at the experimental design level was very high (96%) and Milligan *et al*. (1990) also reported the heritability of fiber content from two plots was higher than 90%. Trait distributions are given in Figures 1 to 3. The distribution could be considered

Table 2 Correlation coefficients for fiber content, stalk diameter and tillering.

Traits	Fiber content	Stalk diameter	Tillering
Fiber content	1	-0.609**	0.417*
Stalk diameter		1	-0.559**
Tiller number			1

*,** Significant at $P<0.05$ and $P<0.01$ respectively

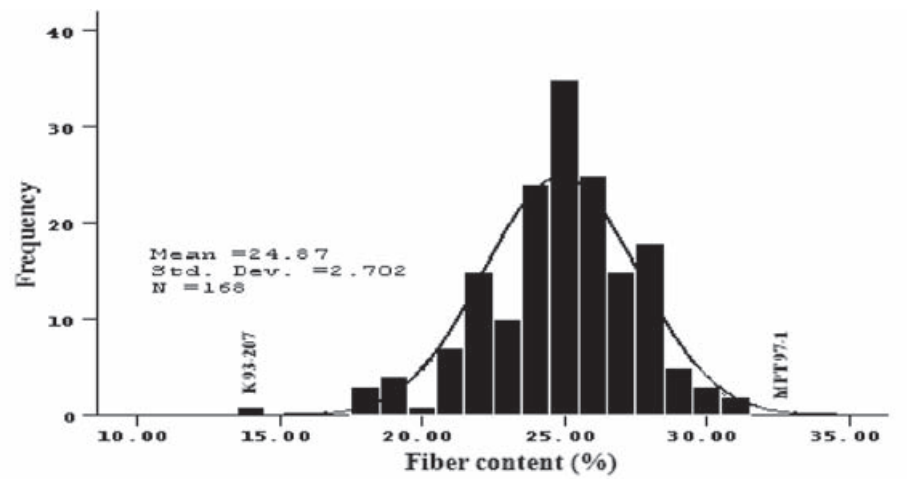

Figure 1 The distribution of fiber content (%) from 168 sugarcane progenies.

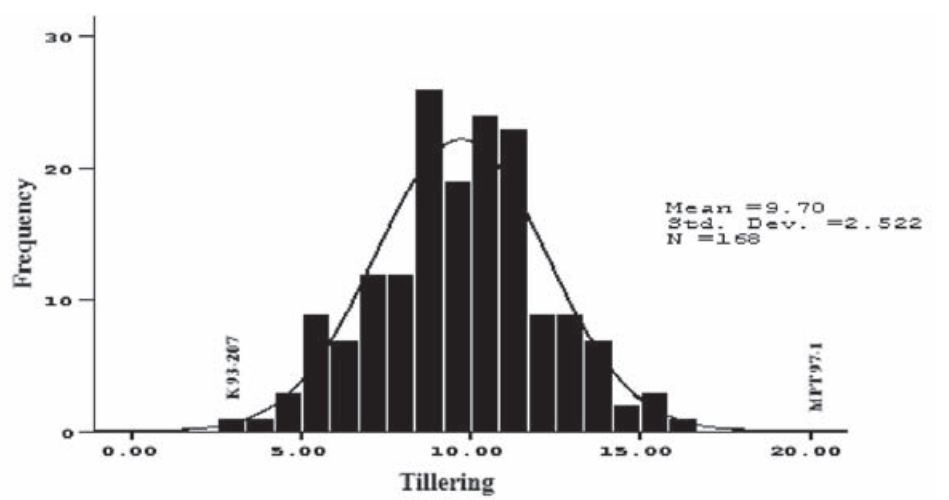

Figure 2 The distribution of tillering from 168 sugarcane progenies.

normal for fiber content and tillering (Figures 1 and 2), but was found to be a little skewed for stalk diameter (Figure 3). The fiber content and tillering varied widely, ranging from 15-33% for fiber content and 2-25 tillers for tillering. The stalk diameter varied from 1-3 cm. The female parent (K 93-207) was a clone with thick stalk, low fiber content and tillering. The male parent (MPT 97-1) was a clone with thin stalk, very high fiber content and tillering.

Marker-trait associations were identified for all traits tested. For all traits, numerous simplex marker QTLs were identified which explained a small proportion of the phenotypic variance (R^2), consistent with other studies in sugarcane for agronomic traits such as sugar content (Ming *et al*., 2001) and brix, stalk diameter, tillering and stalk length (Hoarau *et al*., 2002). The QTL effect to explain traits such as yellow spot resistance (Aljanabi *et al*., 2007) or stalk color (Raboin *et al*., 2006) differed in that they reported R^2 values as high as 20%. From the 19 QTLs from the male parent (the marker band in MPT 97-1), 17 QTLs were identified for stalk diameter, 6 for tillering and 9 for fiber content, explaining from 3.7 to 10.7%, 4.1 to 7.9% and 4.2 to 8.5% of the variation, respectively (Table 3). Of these, nine QTLs were identified as associated with more than one trait. The maximum QTL effect was ccaa18 for stalk diameter, agtt10 for tillering and catc5 for fiber content. All QTLs from the male parent showed a negative effect for stalk diameter and a positive effect for fiber content and tillering. Among the 11 QTLs from the female parent (K 93-207), 10 QTLs were identified for stalk diameter and 2 for fiber content, which explained from 3.9 to 6.2% and 3.7 to 9.9% of the variation, respectively (Table 3). Among these, only one QTL was identified as associated with more than one trait. The maximum QTL effect was ctat5 for stalk diameter and fiber content. The QTLs from the female parent showed a negative effect for fiber content and a positive effect for stalk diameter, except for ctat12 which showed a negative effect for stalk diameter.

The low R^2 values in this study suggested that these traits have a large number of loci each with a low effects control of the phenotypic variation. Fewer single-dose markers were identified for the K 93-207 parent than for the *S. spontaneum* parent. This could be have been due to higher numbers of multi-copy alleles present in this commercial species compared to the *S. spontaneum* parent and *S. spontaneum* is an important species for introgression with respect to important traits such as fiber content, tillering, high biomass, ratooning ability and disease resistance (Ming *et al*., 2002). Most of the QTLs

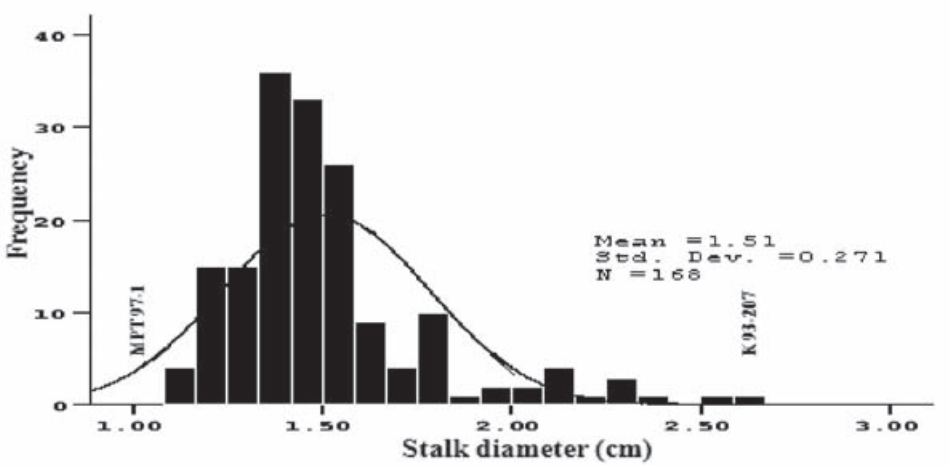

Figure 3 The distribution of stalk diameter (cm) from 168 sugarcane progenies.

for stalk diameter had a negative effect, but for tillering and fiber content, the QTLs had a positive effect. These effects were the same as reported in the phenotypic correlation.

CONCLUSION

Using a cross between a commercial variety and a *S. spontaneum* clone, AFLP markers were identified for stalk diameter, stalk number and fiber content with the phenotypic coefficient of determination at different levels for each trait. The small effect markers explained lower than 10% of the variation. These results showed that, *S. spontanuem* is an important species that can be used for introgression to commercial clones with respect to important traits such as fiber content and tillering. Further work is required to identify

Table 3 Marker effect and significant associations between simplex markers and traits at $P \leq 0.005$.

Marker	Parent	Stalk diameter		Tillering		Fiber content	
		R^2 (%)	Effect	R^2 (%)	Effect	R^2 (%)	Effect
agtt10		7.6	-0.168	7.9	1.611		
agtt14		4.1	-0.128				
catc4		5.2	-0.142	6.7	1.496	4.7	1.290
catc5		4.4	-0.133	4.2	1.211	8.5	1.697
catc30		8.5	-0.177	6.1	1.436	4.4	1.253
ccta5		7.6	-0.169				
ccaa18		10.7	-0.198				
ccaa22		8.0	-0.173	4.4	1.238	4.2	1.233
ccta8	MPT 97-1					6.9	1.534
cgtg19		4.4	-0.134				
cgtg20	(male)	5.9	-0.153				
ctag10		10.2	-0.194			6.7	1.514
cttt3		4.3	-0.131			5.1	1.338
cttt11				4.1	1.199		
cttt13		3.7	-0.135	5.1	1.329	4.3	1.239
cttt30		4.3	-0.130				
gctt3		4.2	-0.129			5.3	1.372
gctt19		7.1	-0.164				
ggat3		4.0	-0.127				
cata15		4.4	0.133				
catc3		4.4	0.133				
cgtg7		4.6	0.134				
cgtg9		5.0	0.140				
ctat5	K 93-207	6.2	0.153			9.9	-1.813
ctat11		4.3	0.131				
ctat12	(female)	4.4	-0.131				
ctat32		3.9	0.125				
gcta1		5.3	0.131				
gctt12		4.9	0.139				
ggtg2						3.7	-1.196

more markers for this population. This in turn will help with the genetic map construction, including an analysis of the linkage configuration in the coupling phase that will give us more information on this large genome of a complex polyploidy crop.

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