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RESEARCH ARTICLE

IDENTIFICATION OF MICROSATELLITE MARKERS FOR HIGH FIBRE CONTENT IN SUGARCANE

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Abstract

The high productivity and tolerance to various biotic and abiotic stresses in modern sugarcane varieties have been derived from the wild species *Saccharumspontaneum*, which is also a source for high fibre. In view of the importance of fibrein bioenergy and related sectors,an attempt was made to identify markers associated with fibrecontent in a hybrid population of sugarcane derived from an interspecific cross involving *S. officinarum* x *S. spontaneum*. Initially the DNA of the two parents and the DNA bulks representing high and low fibre progenies were screened with 124 sugarcane SSR primers, of which 102 primers generated polymorphic profiles between the two bulks. Ten of the primers generated specific markers consistently associated with high fibre content in the progenies. These markers will be useful in selecting high fibre clones during the various stages ofselection in sugarcane breeding.

Keywords : *Saccharum officinarum,Saccharum spontaneum,*interspecific hybrids, SSR markers, fibre%

Introduction

Sugarcane is one of the highest biomass producers among crop plants and the biomass produced by the crop is more than double that of any cereal crop. The sugarcane bagasse which is the fibrous material obtained after the extraction of sugar is used as a feed stock in the generation of electricity by the sugar mills. Bagasse is also used as a feed stock for paper and board Industries. Considerable research is in progress for the economically viable production of cellulosic ethanol from sugarcane bagasse. Consequently sugarcane fibre is viewed as an important source for producing fuel (ethanol) and energy (electricity). The present day commercial varieties have an optimum fibre content of 12-14% to suit the existing milling conditions. The fibre content in modern sugarcane varieties has been largely contributed by *S.spontaneum,* the wild relative of sugarcane.In the present context there is growing interest in developing energy canes with high fibre content which can be used for cogeneration as well as production of cellulosic ethanol. Thus, apart from sucrose, the fibre content of sugarcane is also considered an important trait for selection in sugarcane in the changing scenario. Estimating fibre% at early selection stages is cumbersome requiring considerable time and resources. In this context, identifying selectable markers for this trait will help in identification of genotypes with the required fibrecontent efficiently. In the present study an attempt was made to identify markers specific to fibre content in a hybrid population developed from a cross involving *S. officinarum* and *S. spontaneum*.

Materials and methods

A hybrid population of 92 progenies derived from a cross PIO-00-513 (*S. officinarum*) x IND99-904

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(*S. spontaneum)* formed the material for this study. The 92 progenies along with the parents were evaluated in a RBD with two replications of plot size 3m. The fibre content of the parents and progenies was estimated as per Thangavelu and Rao (1982) and the progenies were classified as high fibre (>20%) and low fibre (<20%).

SSR screening

DNA was extracted from the young leaves of the plant material used for the study as per Walbot (1988), quantified by UV spectrophotometry and further checked on 0.8% agarose gels. Based on the total DNA concentration, 10 ng dilutions of the DNA were made for PCR amplification. PCR reaction mix was prepared to a final volume of 15 µl, containing 1.5µl of 10X PCR buffer (Merck, India), 2.4µl of 200µM dNTPs (Merck, Germany), 1µl each of 30ng forward and reverse primers, 1unit of *Taq* polymerase enzyme (Merck, Germany) and 3µl of 10ng genomic DNA. The PCR reactions were performed on a PTC- 200™ Programmable Thermal Controller(Biorad, USA). The PCR cycles consisted of denaturing at 94°C for 5 min, followed by 30 cycles of 94°C for 1 min, 50°C to 55°C for 1 min and 72°C for 1 min followed by a final extension step of 72^0 C for 10 min. The amplification products were analysed on 4% non-denaturing polyacrylamide gel and visualized by silver staining.

Bulked segregant analysis

Tenµl DNA each from 10 high and 10 low fibre progenies were pooled separately to constitute the high and low fibre DNA bulks. The two bulks along with the high and low fibre parents were screened for polymorphism using 124 microsatellite primers. Seventeen primers which generated amplifications specific to the high fibre parent and the high fibre bulk were used to screen the mapping population of 92 progenies. The per cent correspondence between the trait and the markers identified based on the bulk segregant analysis was calculated as follows:

$$\% \text{ correspondence} = \frac{\text{No. of progenies with markers that match the corresponding phenotype}}{\text{Total progenies}} \times 100$$

Results and discussion

The fibre content in the parents and 92 hybrid progenies was estimated (Table 1). The female parent PIO-00-513 was found to have low fibre content (14.14%) while the male parent IND99-904 recorded a high fibre content of 28.64%. The fibre content in the progenies ranged from 15.19% to 27.40% with a mean of 20.75%, well above the fibre content observed in *S.officinarum* or other cultivated hybrids and significantly lower than that in *S. spontaneum* parent. The progenies were classified as high fibre (>20%) and low fibre (<20%) progenies. There were 49high fibre (HF) and 43 low fibre (LF) progenies in the population segregating in a 1:1 ratio.The fibre % in crosses involving *S. officinarum* and *S. spontaneum* is expected to be around 20%, closer to the mid parent value. High fibre in interspecific hybrids involving *S. officinarum* and *S. spontaneum* had been universally reported. Ramdoyal and Domaingue

Table 1. Fibre % in parents and progenies

Parameter	Progenies (N=92)	PIO 00-513	IND99-904	Mid parent value
Mean Fibre%	20.75	14.14	28.64	21.39
% Improvement in the progeny over PIO 00-513		46.53		
% Improvement in the progeny over IND99-904			-27.54	
% Improvement in the progeny over mid parent value				-2.99

(1994) reported that the hybrid population involving *S. officinarum* and *S. spontaneum* showed higher means for fibre% compared to the *S. officinarum* parent. Roach (1968) and Shang et al. (1968) reported fibre % of ~19 in hybrid progenies involving *S. spontaneum* while Walker (1971) reported fibre % ranging from 14.5 to 21.9 in the progenies depending on the *S. spontaneum* parents used. Our results are in agreement with these reports. When the midparent value was considered, there was a marginal reduction in the mean fibre % of the progenies. However, the mean fibre % of the progenies was too high (20.75%) for acceptance as commercial varieties and further back crosses will be required for reducing the fibre% to the desirable levels.

The high biomass potential of sugarcane makes it an ideal candidate for biofuel production. The total dry biomass derived from the crop depends on the fibre% and thus high fibre content is viewed as an important trait for fuel or energy canes. The energy cane breeding programs world over depends on the use of the wild relative of *Saccharum*, particularly *S. spontaneum*for developing sugarcane varieties with high energy potential. In the present study nearly 50% of the population recorded more than 20% fibre while 20 clones recorded more than 23% fibre. The results further endorse the potential of *S. spontaneum*in imparting high fibre characteristics to its progeny.

Bulked Segregant analysis

The hybrid population was classified into high (>20%) and low fibre groups (<20%). The DNA selected from high and lowfibre progenies were pooled to form the high and lowfibre DNA bulks. The DNA bulks along with the two parental DNAs were subjected to bulkedsegregant analysis using 124 SSR primer pairs. Of the 1670 bands amplified by these primers, 383 bands were polymorphic between high fibre and low fibre bulks/parents and 1287 bands were monomorphic. The mean number of bands amplified by each primer pair was 13.46 with a range from 2-32. The size of the bands ranged from 41bp- 2040bp. Primer Soms49 amplified maximum number of polymorphic bands (14), followed by Soms155 (12), Sogl35(11), Sogl2(10) and Soms60(10). The percent polymorphisms obtained with individual primer pairs ranged from 5% (Soms 64) to 80% (Soms88) with an average of 21.8%.

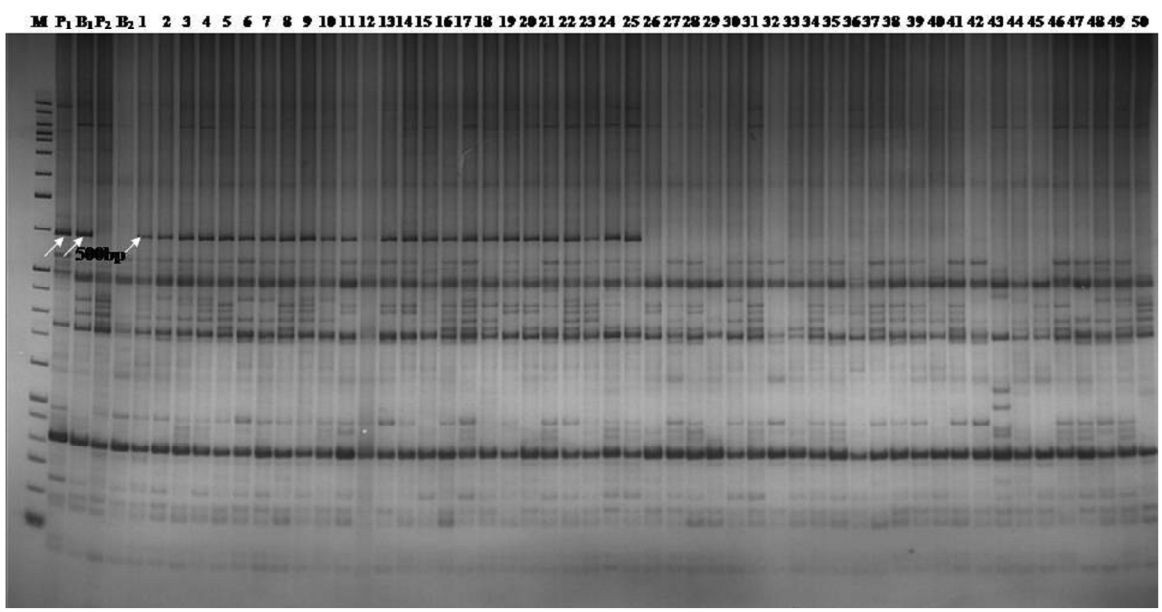
Seventeen primers which generated polymorphism between high and low fibre bulks and parents were used for screening the hybrid population. Of the 17 primers used, 10 primers, viz. Soms 88, Soms109, Soms154, Soms156, Soms167, Soms106, Sogl34, Sogl36, Sogl41 and Sogl50 (Table 2) produced specific amplification in high-fibre progenies (Fig.1). These markers were designated as Soms-$88_{(300bp)}$, Soms-$109_{(400bp)}$, Soms-$154_{(380bp)}$, Soms-$156_{(330bp)}$, Soms-$167_{(500bp)}$, Soms-$106_{(200bp)}$, Sogl-$34_{(400bp)}$, Sogl-$36_{(290bp)}$, Sogl-$41_{(410bp)}$ andSogl-$50_{(100bp).}$ The markers showed 61.95 to 90.2% correspondence with the high and low fibre progenies (Table 3). The markers Soms88-300 and Soms106-200 recorded very high correspondence with the trait viz. 90.20% and 81.52% respectively. These markers were present in most of the high fibre progenies and absent in most of the low fibre progenies and could serve as effective markers for selecting high fibre progenies. The markers $Soms88_{(300bp)}$, $Soms109_{(400bp)}$, $Soms106_{(200bp)}$, and $Sogl36_{(290)}$werefound to segregate in 1:1 ratio.

DNA markers have been developed for various agronomic traits in sugarcane for their use in breeding (Honeycutt et al. 1995; Sills et al. 1995, Daugrois etal. 1996; Guimares et al. 1997; Ming et al. 2001;Hoarau et al. 2002). Msomiand Botha (1995) reported RAPD markers linked to fibre content in *Saccharum*species through bulked segregant analysis. From a segregating population, two bulks representing low and high fibre extremes were screened for RAPD variation. Of the 749 fragments amplified, eight were polymorphic and six of the fragments were shown to be segregating in a 1:1 ratio, indicating single dosage. In a simulated marker based selection, use of one or more of the

Table 2. Primers which amplified markers specific to high fibre content

S.No.	Primer	Forward sequence	Reverse sequence
1	SOGL50	GCTACTATGGACAACAGGG	ATGAAGAGACGAGACGAAGA
2	SOMS106	TCTCAAGGATACACCATCAAG	ATCATCAGCACGACAGACA
3	SOMS109	ATCCTTTGTCGTCTCCGT	AGTTGGGTGTGTATTTGGTG
4	SOMS154	CTCGTTTCATAGCAGACCTT	GCAACTGGAGGAACTGATG
5	SOMS156	ATCGTCTCTGGTTGTTGGT	ATCCTCCATTTCCACCTC
6	SOMS167	AGCAGAGACACACGCACA	ACAAGAGGAGGTTCAGGG
7	SOMS 88	AGATGGATGAGGGTTTCTTT	CCTACGAGTTTATTCTTCAGT
8	SOGL 34	CATTGTTCTGTGCCTGCT	CCGTTTCCCTTCCTTCCC
9	SOGL 36	TCCTCATTACCATTTGTTCC	CCTCCTCTTGCTGGACTT
10	SOGL 41	TGAGGACGGGATGAAGAC	CGGTTACTGTTTGAGGGAG

fragments as marker for fibre led to a significant increase in the number of high fibre clones in the selected progeny. However, reliability of the RAPD markers is far less compared to the SSR markers and hence the SSR markers identified from the present study could be of considerable value in identifying high fibre clones in the early selection stages. The marker Soms88-300 which segregated in 1:1 ratio was found to be associated with over 90% of the high fibre progenies indicating its potential as an effective marker in selecting high fibre clones during different stages of selection. There is considerable interest in sugarcane varieties with high fibre at present in view of the growing



P1 : high fibre parent (IND 99-904), B1 high fibre bulk
P2 : low fibre parent (PIO 00-513), B2 low fibre bulk

Fig. 1. Marker specific to high fibre amplified by primer Soms167

Table 3. Correspondence between the markers and fibre% in the progenies

S.No	Marker	Presence of the markers in high and low fibre progenies				% correspondence between marker and the trait
		HF (49 progenies)		LF (43 progenies)		
		P	A	P	A	
1	Soms-88$_{(300bp)}$	42	4	5	41	90.20
2	Soms-109$_{(400bp)}$	31	15	14	32	68.47
3	Soms-154$_{(380bp)}$	28	18	17	29	61.95
4	Soms156$_{(330bp)}$	28	18	15	31	64.13
5	Soms167$_{(500bp)}$	28	18	6	40	73.90
6	Soms106$_{(200bp)}$	35	11	6	40	81.52
7	Sogl34$_{(400bp)}$	31	15	19	27	63.04
8	Sogl36$_{(290)}$	30	16	10	36	71.73
9	Sogl41$_{(510)}$	30	16	10	36	71.73
10	Sogl50$_{(100bp)}$	31	15	10	36	72.82

HF -High fibre progenies, LF- Low fibre progenies
P –number of progenies in which the marker is present
A –number of progenies in which the marker is absent.

demand for feed stock for cogeneration, cellulosic ethanol production and paper industries. The markers identified in this study hold promise in developing high fibre varieties of sugarcane which will strengthen the bio-energy sector.

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