IDENTIFICATION OF MICROSATELLITE MARKERS ASSOCIATED WITH YIELD COMPONENTS AND QUALITY PARAMETERS IN SUGARCANE

By

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Abstract

MARKER assisted selection depends on the identification of tightly linked association between marker and the trait of interest. In the present work, functional (EST-SSRs) and genomic (gSSRs) microsatellite markers were used to detect putative QTLs for sugarcane yield components (stalk number, diameter and height) and as well as for quality parameters (Brix, Pol and fibre) in plant cane. The mapping population (200 individuals) was derived from a bi-parental cross (IACSP95-3018 x IACSP93-3046) from the IAC Sugarcane Breeding Program. As the map is under construction, single marker trait association analysis based on the likelihood ratio test was undertaken to detect the QTLs. Of the 215 single dose markers evaluated (1:1 and 3:1), 90 (42%) were associated with putative QTLs involving 43 microsatellite primers (18 gSSRs and 25 EST-SSRs). For the yield components, 41 marker/trait associations were found: 20 for height, 6 for diameter and 15 for stalk number. An EST-SSRs marker with homology to non-phototropic hypocotyls 4 (NPH4) protein was associated with a putative QTL with positive effect for diameter as also with a negative effect for stalk number. In relation to the quality parameters, 18 marker trait associations were found for Brix, 19 for Pol, and 12 for fibre. For fibre, 58% of the QTLs detected showed a negative effect on this trait. Some makers associated with QTLs with a negative effect for fibre showed a positive effect for Pol, reflecting the negative correlation generally observed between these traits.

Introduction

The identification of molecular markers tightly linked to agronomic traits of breeders' interest is an important step to make possible the implementation of marker assisted selection in a breeding program (Morgante and Salamini, 2003; Charcosset and Moreau, 2004).

Besides this practical application, molecular markers are also suitable to study the genetic architecture of agronomic traits, especially the complex ones (quantitative traits) that can be resolved into single Mendelian components.

Sugarcane is an important cash crop with a complex genome that, certainly, will benefit from molecular marker technology, as most of its agronomic traits have a multigenic and/or multi-allelic nature. Several QTLs have been detected for basic yield components such as plant weight,

stalk number, stalk diameter and also for Brix and suckering through molecular mapping involving sugarcane bi-parental crosses (Hoarau *et al*. 2002; Jordan *et al*. 2004; Reffay *et al*., 2005; Aitken *et al.,* 2008). The identification of genomic regions responsible for a phenotype of a trait probably will be accelerated by the utilisation of molecular markers derived from expressed sequence tags (ESTs). This type of marker can provide the direct mapping of genes while, at the same time, they themselves may be responsible for the trait of interest (Cato *et al.,* 2001).

EST databases, such as the Sugarcane EST (SUCEST), have being used in the development of molecular markers for mapping (Oliveira *et al.,* 2007) and QTL detection (Pinto *et al.,* 2009) in sugarcane. Microsatellites, as co-dominant markers, have a great potential to allow the comparison among sugarcane maps from different mapping populations. Nowadays, several microsatellites derived from ESTs (EST-SSRs) are available for sugarcane (Oliveira *et al.,* 2009) and together with the genomic ones (Cordeiro *et al.,* 2000) will extend the access to the sugarcane genome, especially to the expressed ones.

In the present work, functional (EST-SSRs) and genomic (gSSRs) microsatellite markers were used to detect putative QTLs for sugarcane yield components (stalk number, diameter and height) as also for quality parameters (Brix, Pol and fibre) in plant cane. As the map is under construction, single marker trait association analysis based on the likelihood ratio test was undertaken to detect putative QTLs at $P < 0.05$.

Material and methods

Mapping population and field data

The mapping population was composed of 200 individuals derived from a bi-parental cross between the elite clone IACSP95-3018 (female parent) and the variety IACSP93-3046 (male parent) from the IAC Sugarcane Breeding Program. The progeny were planted in July 2005 at IAC Sugarcane Station in Ribeirão Preto in an augmented randomised block design, with five replications and including varieties SP81-3250 and RB835486 as controls.

Yield components (stalk number, stalk diameter and stalk height) as well as Brix and Pol (Pol value; gram of sucrose /kg/100g of fresh cane) and fibre percent were collected for each genotype at harvest in plant cane in August 2006. Stalk number was counted in one metre plot. Stalk diameter was measured in a sample of 5 stalks in the middle of the internode (one third of the stalk height from the base to the top).

The same sample was also used for the stalk height measurement conducted with a tape measure. The phenotypic data of each clone was adjusted for differences among blocks by the SAS statistical package (SAS Institute Inc., NC, USA).

Microsatellite analysis

Total genomic DNA was extracted from 300 mg of powdered lyophilised young leaf tissues using a CTAB-method (Hoisington *et al*., 1994) modified for sugarcane. PCR reactions were performed in a 20 µL final volume containing 40 ng of template DNA, 0.2 µM of each forward and reverse primer, 100 µM of each dNTP, 2.0 mM $MgCl_2$, 10mM Tris-HCl, 50 mM KCl, and 0.5 Unit Taq DNA polymerase. Reactions were amplified as follows: 94^0C for 3 min; followed by 30 cycles of 94^0C for 1 min; annealing temperature specific for each primer for 1 min; extension of 72^0C for 1 min and a final elongation step at 72^0C for 2 min. Amplification products were separated by electrophoresis on 6% denatured polyacrylamide gels and using a 25-bp ladder as size standard and silver-staining according to Creste *et al.* (2002).

Single-marker trait association analyses

Marker segregation types were identified in a chi-square test for deviations from the expected segregation ratios of 1:1 and 3:1 (markers in single-dose in only one of the parents and markers in single-dose in both parents, respectively) with Bonferoni correction to control type I error for multiple tests. Markers were identified by the name of the SSRs plus a number according

to the fragment size (molecular weight) followed by a letter to denote parent polymorphism origin: D_1 for marker present on ISCSP95-3018, D_2 marker present on IACSP93-3046 and C for marker present in both parents.

Associations between the 215 single dose markers evaluated and QTL for yield components and quality parameters were done by comparing, through a likelihood ratio, the full regression model $(Y_j = \mu + bx_j + \varepsilon_j)$ assuming a lack of association between marker and QTL $(b = 0)$ (QTL search). The criteria adopted to declare putative marker-trait associations were p-values smaller than 0.05 and 0.01 (not correcting for multiple tests). The 215 single dose markers were derived from 43 microsatellite primers, of which, 18 genomic and 25 functional were chosen at random.

Results and discussion

The genetic map of the population used in the present work is under construction and therefore the number of single dose markers available until now is insufficient for a QTL mapping analysis. Thus, a single marker trait association analysis based on the likelihood ratio test (Liu, 1998) was undertaken to give previous information of putative QTL associations without the need of a genetic map. Moreover, this approach allowed maker trait association for all single dose markers obtained to be investigated.

Single-marker trait associations

Ninety marker trait associations ($P < 0.05$) were found for the phenotypic measures obtained on plant cane of which 14 (15%) were found at 1% ($P < 0.01$) significance level and 11 (121%) at 0.5% ($P < 0.005$) significance level. These 90 putative marker trait associations represented 42% of the total single dose markers evaluated in the single-marker analysis.

The number of associations detected (90) exceeded the average number of statistical false positive association that might exist due to statistical chance. A statistical average of 64 potential false positive associations due to statistical chance is assumed at $P<0.05$ (0.05 x 215 markers x 6 traits).

Putative QTLs for yield components

The range of the progeny phenotypic values ranged from 5 to 30 for stalk number (average of 14.14 ± 4.04), from 1.77 to 3.43 cm for stalk diameter (average of 2.71 ± 0.32 cm) and 0.93 to 3.29 m for stalk height (average of 2.36 ± 0.39 m).

For the phenotypic evaluations related to the yield components (height, diameter and stalk number), 41 marker associations were found: 20 (17.8%) for height, 6 (5%) for diameter and 15 (13%) for stalk number. Of the total number of markers associated with height, 6 (30%) showed a negative effect on this trait. SCA17.1C marker was associated either for height or diameter, with negative effect on both traits.

For stalk number, 8 (53%) markers showed negative effect contributing to reduction in tillering. Cir32.5D2 marker derived from IACSP93-3046 (D2) parent had a high positive effect on stalk number (7.48 stalks). SCB130.1D1 marker was associated with a putative QTL with positive effect (0.13 cm) for diameter and negative (–1.40 stalks) for stalk number (Table 1) reflecting the negative correlation usually observed between diameter and stalk number. Moreover, SCB130.1D1 marker is derived from an EST with homology to a hypocotyl protein.

Putative QTLs for cane quality parameters

For the quality parameters 18 (20%) single dose markers were found associated with putative QTLs for Brix, 19 (21%) for Pol and 12 (13.3%) for fibre. Of the markers associated with Brix putative QTLs, 11 were also found associated with Pol with similar effect values and direction (i.e. increase or decrease in the effect).

In relation to fibre, 58% of the putative QTLs identified had a negative effect on this trait. The SCB27.3C marker was associated with a QTL with negative effect for fibre (reduction of -0.91% in fibre) and also to a QTL with positive effect (increase of 0.49 Pol%) for Pol.

Table 1—Single marker analysis for stalk height, stalk diameter (SD), stalk number (SN) and quality parameters (brix, Pol and fibre) in plant cane.

Markers	Homology	Height	SD	SN	Brix	Pol	Fibre
SCB40.4D2	Lamin	–	–	–1.23*	–	–	–
SCA47.1C	Aminotransferase protein	–	–	–1.49*	–	–	–
CV37.3C		–	–	1.69*	–	–	–
SCA61.1D2	Root specific protein ZRP3	–	–	–1.29*	–	0.33*	–
SCA44.1C	Ubiquitin-specific protease	–	–	1.99*	–	–	–
SCB130.1D1	Non-Phototropic hypocotyl protein	–	0.13***	–1.40*	–0.39*	–	–0.55*
SCB130.10D1	Non-Phototropic hypocotyl protein	–	0.14***	–	–	–	–
SCB307.4C		–	0.13**	–	–	–	–
14.1C	LRR transmembrane protein kinase	–	–0.13**	–	–	–	–
14.5C	LRR transmembrane protein kinase	–	–0.12**	–	–	–	–
SMC415MS.6D2		–	–	–	0.35*	0.35*	–
SMC415MS.5C		–	–	–	–	–	–0.85**
SCB82.1C	Peroxidase	–	–	–	–0.54*	–0.45*	–
SCC30.3D1	Putative mitochondrial uncoupling protein	–	–	–	–0.35*	–	–
SCB60.1D1	Conserved hypothetical protein AAF34431	–	–	–	–0.46**	–0.40*	–
SCB60.3D1	Conserved hypothetical protein AAF34431	–	–	–	–0.44*	–	–
CV38.14D2		–	–	–	0.42*	0.46**	–
CV38.15D2		–	–	–	0.42*	0.46**	–
CV38.16C		–	–	–	–	–	–0.86**
SCC33.2C	Putative protein	–	–	–	0.46*	–	–
7.3D1	NADPH HC toxin reductase	–	–	–	–0.47**	–	–
CIR1.5D2		–	–	–	–	0.39*	–
CIR 12.6C		–	–	–	–	0.37*	–
CIR 50.2C		–	–	–	–	0.44*	–
SMC31CUQ.6		–	–	–	–	0.36*	–
SMC2039.4C		–	–	–	–	–0.39*	–
SCB27.3C	Putative protein	–	–	–	–	0.49*	–0.91*
SCB55.1D1	Beta-adaptin-like protein A	–	–	–	–	–0.42*	–
SCB58.7D1	Myb-like protein	–	–	–	–	–	0.58*
CIR14.1D1		0.16***	–	–	–	–	–0.56*
CIR 18.3D1		0.12*	–	–	0.45*	–	0,58*
CIR 32.1D2		–	–	–	–0.42*	–0.35*	–
CIR 32.5D2		0.14***	–	7.48**	–	–	–
CIR 32.10D1		0.12*	–	–	–	–	–
CIR 35.1D2		–	–	–	–	–	0.55*
CIR 35.2C		–	–	2.00*	–	–	–0.79*
CIR 35.3D1		–0.12*	–	–	–	–	–
CIR 35.4D1		0.14**	–	–	–	–	–
CIR 67.1D2		–	–	–	–	–	–0.68*
CIR 67.3C		0.14*	–	–	0.57***	–	–
CIR 67.4D2		–	–	1.26*	–	–	–
CIR 67.5C		–0.12*	–	–1.55*	–	–	–
SMC236.2D1		0.12*	–	–	–	–	–
SMC236.3C		–	–	–	0.65***	0.50***	–
SMC1047HA5D2		0.13*	–	–	–	–	–
SMC1047HA7D2		0.12*	–	–	–	–	–
SMC1047HA9D1		–	–	–	–0.39*	–0.39*	–
SMC119CG.1D2		0.15**	–	–	–	–	–
SMC21AS.1D1		0.12*	–	–	0.42*	0.39*	–
SCB25.2D2	Protein kinase	0.15***	–	–	–	–	–
SCB118.1D1	Homeobox transcription factor GNARLY1	–0.11*	–	–	–	–	–
SCA53.1D2	Indeterminate spikelet 1	0.15**	–	–	–	–	–
SCA53.5C	Indeterminate spikelet 1	–	–	–	0.51*	0.52**	–
SCB43.5C	Putative pyrophosphate	–0.15*	–	–	–	–	–
SCB01.3C	Glycine-rich RNA binding protein	–0.13*	–	–	–	–	–
SCA17.1C	ESTsD41739	–0.22***	–0.18***	–	–	–	0.97*
SCA17.2D2	ESTsD41739	–	–	–	–	–	0.85***
SCB309.1C		0.13*	–	–	–	–	–
SMC280.8D2		–	–	–1.31*	–	–	–
SMC2039.4C		–	–	–1.69*	–	–	–
SMC1011HA.3C		–	–	1.84*	–	–	–
SMC863CG.2C		–	–	1.69*	–	–	–
SCA48.1D2	Phytoene synthase	–	–	–1.33*	0.43*	0.42*	–
Total		20 (3) (4)	6 (3) (3)	15 (1) (0)	18 (2) (2)	19 (3) (1)	12 (2) (1)

*Significance level at 5% (P<0.05). ** Significance level at 1% (P<0.01). *** Significance level at 0.5% (P<0.005). D1: Single dose in IACSP95-3018. D2: Single dose in IACSP93-3046. C: Single dose in both parents. In parenthesis number of marker trait associations detected at (P<0.01) and (P<0.005), respectively.

The same trend was observed for SCA48.1D2 marker that was not only associated with a QTL with a positive effect for Brix and Pol but also with a QTL with negative effect for stalk number.

Biochemical relationship between marker homology and putative QTL detected

The utilisation of molecular markers derived from ESTs allowed the investigation of the biochemical relationship between the role of the gene used as marker and the respective associated agronomic trait.

In our study, the relationship was investigated *a posteriori* as the EST-SSRs were chosen at random. Some of the putative QTL detected were associated with markers, which could be related with the phenotype evaluated.

For example, marker SCB58.7D1 having homology to Myb-like protein was associated with fibre. Some MYB genes have been shown to be involved in the control of phenylpropanoid metabolism involved in lignin production.

Marker SB130.1D1 associated with stalk diameter, stalk number and Brix has homology with a non-phototropic hypocotyl protein (NPH4) that encodes the auxin-regulated transcriptional activator ARF7 (AUXIN RESPONSE FACTOR 7), which is conditionally required for the modulation of differential growth of aerial tissues in *Arabidopsis* (Harper *et al.*, 2000).

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IDENTIFICATION DE MARQUEURS MICROSATELLITES ASSOCIÉS AUX COMPOSANTES DU RENDEMENT ET AUX PARAMÈTRES DE QUALITÉ CHEZ LA CANNE À SUCRE

Par

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MOTS-CLÉS: QTL, Marqueurs Moléculaires, Composantes de Rendement, Paramètres de Qualité.

Résumé

LA SÉLECTION assistée par marqueurs dépend d'une étroite association entre le marqueur et le caractère à l'étude. Des marqueurs fonctionnels (EST-SSRs) et génomiques (gSSRs) ont été utilisés dans cette étude pour déterminer d'éventuels QTLs associés aux composantes de rendement (nombre de tiges, diamètre et hauteur) et aux paramètres de qualité (Brix, Pol et fibre) chez la canne vierge. Une descendance de 200 individus issue d'un croisement bi-parental (IACSP95-3018 x IACSP93-3046) provenant du IAC Sugarcane Breeding Program a été utilisée pour l'étude cartographique. Étant donné que la carte génétique n'a pas encore été réalisée, une association de marqueurs à caractère simple (single marker trait association), basée sur le test du rapport de vraisemblance (likelihood ratio test), a été utilisée pour déterminer les QTLs. Des 215 marqueurs simplex (ratio 1:1 et 3:1) identifiés à l'aide de 43 amorces microsatellites (18 gSSRs et 25 EST-

SSRs), 90 (42%) étaient associés aux QTLs putatifs. Un total de 41 associations marqueur/caractère a été identifié pour les composantes du rendement canne: 20 pour la hauteur, 6 pour le diamètre, et 15 pour le nombre de tiges. Un marqueur EST-SSRs correspondant à une protéine hypoctyle 4 (NHP_4) non-phototropique a été associé à un QTL putatif ayant un effet positif sur le diamètre mais influençant négativement le nombre de tiges. En ce qui concerne les paramètres de qualité, 18 combinaisons marqueur/caractère ont été obtenues pour le Brix, 19 pour le Pol et 12 pour la fibre. Un total de 58% des QTLS évalués a démontré un effet négatif pour la fibre. Il a été démontré que certains marqueurs associés aux QTLs exerçant un effet négatif sur la fibre avaient un effet positif sur le Pol, confirmant la corrélation négative observée généralement entre ces deux caractères.

IDENTIFICACIÓN DE MARCADORES MICROSATÉLITES ASOCIADOS CON COMPONENTES DE RENDIMIENTO Y PARÁMETROS DE CALIDAD EN LA CAÑA DE AZÚCAR

Por

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PALABRAS CLAVE: QTL, Marcadores Moleculares, Components de Rendimiento, Parámetros de Calidad.

Resumen

LA SELECCIÓN asistida por marcadores depende de la identificación de una fuerte asociación de ligamiento entre marcadores y caracter de interés. En el presente trabajo, marcadores funcionales (EST-SSRs) y genómicos (gSSRs) fueron usados para detectar QTLs putativos para componentes de rendimiento de la caña de azúcar (número de tallos, diámetro y altura), así como para parámetros de calidad (Brix, Pol y fibra) en planta de caña de azúcar. La población a cartografiar (de 200 individuos) fue derivada de una cruza bi-parental (IACSP95-3018 × IACSP93-3046) del Programa de Fitomejoramiento de Caña de Azúcar del IAC. Ya que el mapa se encuentra bajo construcción, un análisis de asociación entre caracter y marcadores únicos fue efectuado para detectar los QTLs, basado en una prueba de probabilidad. De los 215 marcadores de una sola dosis (1:1 y 3:1), 90 (42%) fueron asociados con QTLs putativos involucrando 43 iniciadores microsatélite (18 gSSRs y 25 EST-SSRs). Para los components de rendiumiento, 41 asociaciones fueron encontradas entre marcadores y caracter: 20 para altura, 6 para diámetro y 15 para número de tallos. Un marcador EST-SSR con homología a la proteína 4 (NPH) del hipocotilo no fototrópico fue asociada con un QTL putativo de efecto positivo para el diámetro, así como con un efecto negativo para el número de tallos. En relación con los parámetros de calidad, 18 asociaciones entre marcadores y caracteres fueron encontrados para el Brix, 19 para el Pol y 12 para la fibra. Para la fibra, 58% de los QTLs detectados mostraron un efecto negativo en este caracter. Algunos marcadores asociados con un efecto negativo para la fibra mostraron un efecto positive para Pol, reflejando una correlación negativa generalmente observada entre ambos caracteres.