EVALUATION OF FUNCTIONAL MICROSATELLITE MARKERS FOR SUGARCANE POLYCROSS PATERNITY ANALYSIS

By

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Abstract

POLYCROSSES allow different cross combinations among elite parents to be evaluated in a single family. However, the male parent of the progeny is unknown. Microsatellites are considered the marker of choice for fingerprinting and paternity analysis. In this work, the potential of seven microsatellites derived from expressed sequence tags (EST-SSRs) and one genomic (gSSRs) were evaluated to identify the male parent of four sibs selected from one polycross involving nine different male parents. The four sibs were selected based on their high productivity of sugarcane per hectare at plant cane in the year of 2008 at the Sugarcane Breeding Station of Ribeirão Preto, SP, Brazil. The products of the PCR reactions were separated on 6% denaturing polyacrilamide gels and silver stained. The eight SSRs screened over the female parent, the nine male parents and the four selected sibs produced a total of 62 alleles with an average of 7.75 alleles/locus. The genetic similarity (Jaccard coefficient) was calculated among all the materials evaluated. The average genetic similarity among the female parent and the nine male parents (GS = 0.547) was similar to that among the nine male parents (GS = 0.534) and also to that obtained among the four sibs and the nine male parents (GS = 0.539). For each locus, the female parent alleles that were present in each sib were excluded from the analysis to enable us to identify the origin of the male parent alleles present in the sibs. The male parent was identified excluding, for each locus at time, all the female parent alleles that were present in each sib, enabling the identification of the male parent with the alleles present in the sibs. The male parent identified with this approach showed the highest GS with the respective sib in pair wise comparisons among all male parents and sibs. The microsatellites used in the present work were used to successfully identify the male parent of the sibs evaluated.

Introduction

Sugarcane is an allogamous plant vegetatively propagated through stem cuttings. However, sexual reproduction is used by breeders to generate genetic variability for selection through crosses involving two (bi-parental crosses) or more parents (polycrosses).

Although different cross combinations among elite parents can be evaluated in polycrosses in a single family, the male parent of the progeny is unknown.

Nowadays, molecular markers have proven to be an important tool in breeding programs (Eathington, 2007). Among the different types of molecular markers, microsatellites are considered the marker of choice for sugarcane fingerprinting and paternity analysis (Cordeiro, 2001).

In the present study, the potential of seven EST-SSRs and one gSSRs were evaluated to identify the male parent of four sibs selected from one polycross involving nine different male parents genetically related.

Material and methods

Four sibs (clones) derived from one polycross involving the variety IACSP98-6209 (female parent) and nine male parents (SP84-7017, IACSP95-3028, IACSP94-2111, IACSP94-2094, IACSP97-3313, IACSP97-6671, IACSP99-3012, CTC-1, IACSP96-2036) were selected based on their high productivity of sugarcane per hectare at plant cane in the year 2008 at the Sugarcane Breeding Station (Centro de Cana). Seven EST-SSRs (ESTB312, ESTC05, ESTC48, ESTB130, ESTB82, ESTB07, ESTA48) (Oliveira *et al*., 2009) and one gSSRs (SMC1047HA) were used. These microsatellites were chosen based on the high quality of the amplification products. PCR reactions and separation of the amplified products were performed according to Oliveira *et al*. (2009). Markers were scored based on their presence/absence and used to estimate the genetic similarity between all the materials evaluated, adopting the Jaccard similarity coefficient using the NTSYS-PC software, version 2.0 (Exeter Software, NY, USA; Rohlf, 1993).

Results and discussion

The eight SSRs screened over the materials produced a total of 62 alleles (markers) ranging from 2 (ESTB130) to 13 (ESTA48) with an average of 7.75 alleles/locus. The pair wise genetic similarity among all the materials was moderate (0.603) and that among the nine candidate male parents (0.534) was slightly inferior to the average value estimated among the female parent and the nine candidate male parents (0.547).

The identification of the most probable male parent was done for each locus at time, excluding all female parent markers present in the sibs (clones) and analysing only the candidate male parent markers (Table 1).

Table 1—Identification of the probable male parent based on the exclusion of the female parent markers.

Markers	**Female**	P1	P2	P3	P4	**P5**	P6	P7	P8	P9	**Clone 17**
ESTC05.2	0	0	0	0	0	**1**	0	0	0	0	1
ESTC48.4	0	1	0	1	1	**1**	1	1	1	0	1
ESTB07.2	0	1	1	1	1	**1**	0	1	0	0	1
ESTA48.7	0	0	0	0	0	**1**	0	0	0	0	1
Total	0	2	1	2	2	**4**	1	2	1	0	4
	Female	P1	P2	**P3**	P4	P5	P6	P7	P8	P9	**Clone 19**
ESTB312.1	0	1	1	**1**	1	0	0	0	0	0	1
ESTB312.8	0	0	0	**1**	0	0	1	1	0	0	1
ESTC05.5	0	0	0	**1**	0	0	0	0	0	0	1
ESTC48.1	0	0	0	**1**	0	0	0	0	0	1	1
SMC1047HA.5	0	0	0	**1**	0	0	0	0	0	0	1
ESTA48.3	0	0	0	**1**	0	0	0	0	1	1	1
ESTA48.12	0	1	1	**1**	1	1	1	1	1	1	1
Total	0	2	2	**7**	2	1	2	2	2	3	7
	Female	P1	P2	P3	**P4**	**P5**	P6	P7	P8	P9	**Clone 21**
ESTB312.9	0	1	0	0	**1**	0	1	0	0	1	1
ESTB07.2	0	1	1	1	**1**	1	0	1	0	0	1
ESTA48.8	0	1	0	0	**1**	0	1	0	0	1	1
ESTA48.11	0	0	0	0	**1**	0	0	1	0	0	1
Total	0	3	1	1	**4**	1	2	2	0	2	4
	Female	P1	P2	P3	P4	P5	**P6**	P7	P8	P9	**Clone 30**
ESTB312.5	0	0	0	0	0	0	**1**	0	1	0	1
ESTB312.8	0	0	0	1	0	0	**1**	1	0	0	1
ESTC48.4	0	1	0	1	1	1	**1**	1	1	0	1
ESTB82.2	0	0	1	0	0	0	**1**	1	1	0	1
1047HA.11	0	0	1	1	0	0	**1**	0	0	1	1
ESTA48.12	0	1	1	1	1	1	**1**	1	1	1	1
Total	0	2	3	4	2	2	**6**	4	4	2	6

Female: IACSP98-6209. Candidate male parents: P1:SP84-7017, P2: IACSP95-3028, P3: IACSP94-2111, P4: IACSP94-2094, P5: IACSP97-3313, P6: IACSP97-6671, P7: IACSP99-3012, P8: CTC-1, P9: IACSP96-2036.

With this approach, it was possible to assume that the clones 17, 19, 21 and 30 have, as the most probable male parent, the sugarcane varieties IACSP97-3313, IACSP94-2111, IACSP94-2094 and IACSP97-6671 respectively. Indeed, exclusion of female parent markers constitutes a successful strategy in paternity identification (Buteler *et al.,* 1997) being able, in our work, to indicate the most probable male parent.

As shown in Table 1, the male parent of clone 17 was easily identified, as the EST derived markers ESTC05.2 and ESTA48.7 were exclusively from the variety IACSP97-3313. The same was observed for clone 19, for the markers ESTC05.5 and SMC1047HA.5 that were exclusively from the variety IACSP94-2111.

As expected, the most probable male parent identified by the exclusion of the female parent markers approach, showed the highest genetic similarity with the respective clone (Table 2).

Table 2—Pair wise genetic similarity for female parent, clones and candidate male parents estimated based on 62 microsatellite markers.

Clone	Female	P1	P2	P3	P4	P5	P6	P7	P8	P9
17	0.765	0.512	0.526	0.512	0.579	**0.722**	0.512	0.475	0.610	0.524
19	0.649	0.432	0.475	**0.610**	0.452	0.575	0.465	0.463	0.558	0.548
30	0.514	0.487	0.500	0.564	0.556	0.487	**0.706**	0.486	0.590	0.500
21	0.676	0.595	0.528	0.442	**0.727**	0.553	0.553	0.556	0.500	0.525

Female: IACSP98-6209. Candidate male parents: P1:SP84-7017, P2: IACSP95-3028, P3: IACSP94-2111, P4: IACSP94-2094, P5: IACSP97-3313, P6: IACSP97-6671, P7: IACSP99-3012, P8: CTC-1, P9: IACSP96-2036.

Although the 8 microsatellites used in the present work were able to identify the male parent, other polycrosses are being evaluated with this same set of microsatellites to verify their potential in polycross paternity identification.

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ÉVALUATION DES MARQUEURS MICROSATELLITES FONCTIONNELS POUR L'ANALYSE DE LA PATERNITÉ DANS LES POLYCROISEMENTS DE LA CANNE À SUCRE

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MOTS CLÉS: Polycroisements, Microsatellites, Vérification de Paternité.

Résumé

LES POLYCROISEMENTS permettent les combinaisons de croisements parmi les parents élites pour être évalués comme une seule famille. Cependant, le parent mâle des progénitures est inconnu. Les microsatellites sont considérés comme les marqueurs de choix pour établir l'empreinte génétique pour l'analyse de la paternité. Le potentiel de sept microsatellites dérivés des étiquettes de séquences exprimées (EST-SSRs) et un microsatellite génomique (gSSRs) ont été évalués dans cette étude pour identifier le parent mâle de quatre progénitures. Celles-ci ont été sélectées d'un polycroisement impliquant neuf différents parents mâles. Les quatre progénitures ont été choisies en 2008 au Sugarcane Breeding Station de Ribeirão Preto, SP, Brésil sur la base de leur rendement élevé à l'hectare en vierge. Les produits PCR ont été séparés sur gel dénaturant de polyacrilamide de 6% suivi de la coloration au nitrate d'argent. Les huit SSRs criblés par rapport au parent femelle, les neuf parents mâles et les quatre progénitures ont produit au total 62 allèles avec une moyenne de 7.75 allèles/locus. La similarité génétique (coefficient de Jaccard) a été calculée parmi le matériel évalué. La similarité génétique moyenne parmi les parents femelles et les neuf parents mâles (GS = 0.547) était similaire à celles des neuf parents mâles (GS = 0.534) et également à celle obtenue pour les progénitures et les neufs parents mâles (GS = 0.539). Pour chaque locus, les allèles des parents femelles qui étaient présents dans chaque progéniture étaient exclus de l'analyse pour permettre d'identifier l'origine des allèles des parents mâles présents dans les progénitures. Le parent mâle a été identifié en excluant, pour chaque locus, les allèles transmis par le parent femelle et qui étaient présents dans les progénitures, permettant ainsi l'identification du parent mâle. Le parent mâle identifié grâce à cette approche a démontré que le GS est le plus élevé avec sa progéniture respective dans des comparaisons par paires parmi tous les parents masculins et progénitures. Les microsatellites utilisés dans cette étude ont été capables de mener à bien l'identification du parent mâle des progénitures évaluées.

EVALUACIÓN DE LOS MARCADORES FUNCIONALES MICROSATÉLITES PARA ANALISIS DE PATERNIDAD EN POLICRUZAMIENTOS EN CAÑA DE AZÚCAR

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PALABRAS CLAVE: Policruzamientos, Microsatélites, Verificación Paternal.

Resumen

LOS POLICRUZAMIENTOS permiten diferentes cruzas entre parentales élites para evaluar en familias, aunque el parental masculino es desconocido. Los microsatélites son considerados marcadores útiles para descifrar su identidad genética, así como para el análisis de paternidad. En este trabajo se evalúa el potencial de siete microsatélites derivado de secuencias expresadas (EST-SSRs) y un genómico (gSSRs), para identificar el parental masculino de cuatro hermanos seleccionados de un policruzamiento que tiene nueve padres masculinos diferentes. Los cuatro hermanos fueron seleccionado basándose en su alta productividad de azúcar por ha en caña planta en el 2008, evaluados en la Estación de Mejoramiento de Ribeirão Preto, SP. Brasil. Los productos de la reacción de PCR fueron separados en un gel de poliacrilamida al 6% y luego teñido con plata. Los ocho SSRs identificados en el parental femenino, nueve del parental masculino y cuatro de los hermanos produjeron un total de 62 alelos con un promedio de 7.75 alelos/locus. Se calculó la similaridad genética (Coeficiente de Jaccard) entre los materiales evaluados. El promedio de similaridad genética entre el parental femenino y los nueve masculinos (GC = 0.547), fue similar al de los nueve parentales masculinos (GS = 0.534), así como el obtenido entre los cuatro hermanos y los nueve parentales masculinos (GS = 0.539). Para cada locus, el alelo del parental femenino que estuvo presente en cada hermano se excluyó del análisis para que permita identificar el origen de los alelos del parental masculino presente en los hermanos. El parental masculino se identificó luego de excluir todos los alelos al mismo tiempo del parental femenino presente en cada uno de los hermanos, para permitir la identificación de los alelos del parental masculino en los hermanos. El parental identificado con este sistema mostro el más alto valor de GC relación a su respectivo hermano en comparaciones pareadas entre parentales y hermanos. Por tanto, los microsatélites usados en este trabajo fueron usados en forma efectiva para identificar el parental masculino del grupo de hermanos evaluados.