CHROMOSOME WALKING TOWARDS A MAJOR RESISTANCE GENE FOR COMMON RUST OF SUGARCANE

By

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Abstract

A major resistance gene for common rust (Puccinia melanocephala H. & P. Syd.) of sugarcane was identified in the cultivar R570 (2n = 115). Genetic mapping efforts surrounded the gene with markers, the closest being 2 cM on each side. Various BAC resources are being used to approach the gene: the R570 sugarcane BAC library, the rice Nipponbare BAC contigs, and sorghum BAC contigs. Progress on this work is reported as well as constraints imposed by the high ploidy and heterozygosity of sugarcane.

Introduction

Inheritance of resistance to common rust (Puccinia melanocephala H. & P. Syd.) of sugarcane was investigated using selfed progeny of cultivar R570 (2n = 115). A 3:1 segregation ratio, indicative of a dominant resistance gene, was observed (Daugrois et al., 1996). This is the first major gene identified in sugarcane. The International Consortium for Sugarcane Biotechnology was interested in exploring map-based cloning in sugarcane, a complex aneuploid polyploid. A BAC library was constructed using cultivar R570 (Tomkins et al., 1999). Development of a fine map in the gene’s region is the first step for map-based cloning. Two strategies were used: one based on synteny among grasses and the other on bulked segregant analysis using AFLP. This localised the gene at the extremity of one co-segregation group (homoeology group VII of the R570 map) and flanked the gene with markers. The closest were located at 2 cM on each side (Grivet et al., 1996; Asnaghi et al., 2000; Hoarau et al., 2001, and unpublished data).

We are now using various BAC resources developed at Clemson University Genomic Institute (CUGI), including the sugarcane R570 BAC library, a sorghum BAC library and a rice BAC library, to progress towards the gene.

Results and discussion

Exploitation of the sugarcane BAC library

The starting point was a set of AFLP markers closely linked to the rust resistance gene that were cloned and hybridized with the sugarcane BAC filters. Then, the following sequence was initiated:

- The BACs identified were end-cloned.
- The end-clones were tested to exclude those corresponding to repeated sequences.
- The different BACs were ordered by analysing the patterns of end-clone hybridisation to the respective BACs.

- The end-clones corresponding to unique sequences, and located at the edges of the contig, were hybridised onto whole sugarcane BAC library filters.

This sequence can be repeated to extend the contig and progress toward the gene.

On each side of the rust resistance gene, we have identified one contig which encompasses 50 and 14 overlapping BACs, respectively. The main drawback to this type of strategy is the presence of repeated sequences that preclude contig analysis. Indeed, in our case, 70% (20/29) of the end-clones already tested contained repeated sequences.

Exploitation of the rice BAC library

Rice is a diploid species with a DNA content per basic genome half that of sugarcane, and it has relatively simple synteny relationships with sugarcane (Glaszmann et al., 1997; Asnaghi et al., 2000). A partially ordered BAC library is available for rice. This library is used to accelerate our progress toward the target sugarcane gene. The sugarcane BAC end-clones were tested for hybridisation to rice DNA and those that did were then hybridised to the rice BAC library. The rice BACs detected allowed identification of rice BAC contigs thanks to the ongoing ordering process at Clemson University.

We have identified rice contigs on each side of the gene. On one side, the contig contains 228 BACs (corresponding to around 1270 kb) and has been assigned to rice chromosome 2. This chromosome is globally homoeologous to the sugarcane homoeology group (VII) bearing the rust resistance gene. On the other side of the gene, we have identified two contigs of 83 and 49 BACs (corresponding to around 530 and 360 kb, respectively) that were assigned to rice chromosomes 6 and 5, respectively. Confirmation of these results would mean that this rice chromosomal region differs from the homoeologous sugarcane region. This may complicate the use of rice as a
source of shortcuts in this area. One other limitation of this approach is the low percent of cross hybridisation between rice and sugarcane DNA with the BAC sub-clones we tested (22%, or 4/18).

**Exploitation of the sorghum BAC library**

Sorghum has clear and simple synteny relationships with sugarcane (Grivet *et al.*, 1994; Dufour *et al.*, 1996, 1997; Ming *et al.*, 1998). Although it has a DNA content per basic genome similar to that of sugarcane, it presents the advantage of being a diploid species. There also exists a partially ordered BAC library. Similarly to rice, we are using the sorghum BAC library to accelerate our progress towards the sugarcane resistance gene. Sorghum BAC contigs have been identified on both sides of the gene.

On one side, we have identified a contig of 38 overlapping BACs. On the other side, we have identified two small contigs of eight BACs each. The sorghum contigs identified so far are smaller than the rice ones due to a less advanced BAC ordering process. However, the percent of cross hybridisation (61%, or 11/18) observed between sorghum and sugarcane DNA is much greater than with rice. This, and the fact that sorghum exhibits clear and simple synteny relationships with sugarcane, should facilitate the exploitation of the sorghum contigs.

**REFERENCES**


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**MARCHÉ CHROMOSOMIQUE POUR LE CLONAGE D'UN GÈNE MAJEUR DE RÉSISTANCE À LA ROUILLE COMMUNE CHEZ LA CANNE À SUCRE**

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**Résumé**

Un gène majeur de résistance à la rouille commune (*Puccinia melanocephala* H & P Syd) a été identifié chez la canne à sucre dans le cultivar R 570 (2n = 115 chromosomes). Différentes techniques ont été utilisées pour placer des marqueurs liés au gène recherché, le plus proche se situant à environ de 2 cM de chaque côté. Plusieurs banques de clones de BAC (cultivar R 570, Nipponbare contigs BAC pour le riz et les contigs BAC pour le sorgho) sont utilisées afin de se rapprocher du gène.

Le progrès accompli ainsi que les difficultés rencontrées en raison de la plioïdie élevée et de l'hétérozygotie de la canne à sucre sont rapportés dans ce papier.

**Mots clés:** Saccharum, cartographie physique, banque de BAC (bacterial artificial chromosome), sorgho, riz, polyploïde.
HACIA UN GEN MAYOR DE RESISTENCIA A LA ROYA COMÚN DE LA CAÑA DE AZÚCAR CAMINANDO SOBRE EL GENOMA

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Resumen

Un gen mayor de resistencia a la roya común (Puccinia melanocephala H. & P. Syd) de la caña de azúcar se identificó en el cultivar R570 (2n ≈ 115). El mapeo alrededor del gen con marcadores, permitió llegar a 2 cM en cada lado del gen. Varias fuentes de BAC se están utilizando para acercarse al gen: la librería BAC de caña de azúcar de R570, y los bordes de BAC de arroz Nipponbare, y de sorgo. Se presentan los progresos alcanzados y las limitaciones producidas por la alta ploidia y heterocigosidad de la caña de azúcar.

Palabras claves: Saccharum spp., mapeo físico, cromosoma bacteriano artificial (BAC), sorgo, arroz, poliploide.