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Construction of a BAC Library for a Defoliating Insect-Resistant Soybean and Identification of Candidate Clones Using a Novel Approach

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Abstract Positional cloning of an insect-resistance quantitative trait locus (QTL) requires the construction of a large-insert genomic DNA library from insect-resistant genotypes. To facilitate cloning of a major defoliating insect-resistance QTL on linkage group M of the soybean genetic map, a bacterial artificial chromosome (BAC) library for PI 229358 was constructed and characterized. The *Hin*dIII BAC library contains 55,296 clones with an average insert size 131 kb. This library represents a 6-fold soybean haploid genome equivalents, allowing a 99.8% probability of recovering any specific sequence of interest in soybean. BAC filters were screened with a genomic DNA probe Sat_258sc2 obtained through genome walking from flanking sequences of a simple sequence repeat (SSR) marker, Sat_258, which links to the insect-resistance QTL. Thirteen BAC clones were identified positive for Sat_258sc2, and two of them were confirmed to carry Sat_258. The results suggest that this library is useful in positional cloning of the major insect-resistance QTL, and the approach presented here can be used to screen a BAC library for a SSR marker without requiring the creation of BAC pools.

Keywords BAC library · Genome walking · Insect-resistance QTL · Soybean

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Introduction

Conventional breeding for defoliating insect resistance in soybean [*Glycine max* (L.) Merr.] has been unsuccessful due to the quantitative nature of the resistance, its associated linkage drag, and the limited understanding of resistance mechanisms (Lambert and Kilen 1984; Lambert and Tyler 1999; Rufener et al. 1989). One major source of soybean resistance to defoliating insects in soybean breeding programs is the Japanese plant introduction (PI) PI 229358, which was discovered in the early 1970s (Van Duyn et al. 1971). This PI was initially identified as highly resistant to Mexican bean beetle [*Epilachna varivestis* (Mulsant)], and later, its resistance was demonstrated against a number of other defoliating insects (Lambert and Kilen 1984; Van Duyn et al. 1971). Therefore, several efforts have been initiated to better characterize and eventually clone the major gene(s) responsible for insect resistance in soybean.

Rector et al. (1998, 2000) used restriction fragment-length polymorphism markers to identify a major quantitative trait locus (QTL) controlling both antixenosis (negative influence on insect preference and/or feeding) and antibiosis (adverse effects on one or more biological systems of insects) on linkage group M. This QTL, named QTL-M,

was confirmed in different soybean backgrounds (Komatsu et al. 2008; Walker et al. 2004; Zhu et al. 2006, 2008). Zhu et al. (2006) fine-mapped QTL-M to an approximately 0.5-cM interval flanked by Sat_258 and Satt702 through genotyping and phenotyping recombinant substitution lines identified from advanced backcross progenies. These mapping results have provided the necessary information to initiate the physical mapping and map-based cloning of the insect-resistance QTL-M.

Positional cloning of QTLs usually entails the construction of large-insert genomic DNA libraries (Tanksley et al. 1995). Nine bacterial artificial chromosome (BAC) libraries have been developed for six soybean genotypes, including "Williams 82" (Marek and Shoemaker 1997; Salimath and Bhattacharyya 1999), "Faribault" (Danesh et al. 1998), PI 437654 (Tomkins et al. 1999), "A3244" (Tomkins et al. 2000), "Forest" (Meksem et al. 2000; Wu et al. 2004), and "Misuzudaizu" (Xia et al. 2005). These soybean BAC libraries have been constructed for the purposes of cloning disease-resistance loci or for general genomic research, but none of the source genotypes are insect resistant. Therefore, these soybean BAC libraries cannot be used for the cloning of QTLs or genes for insect resistance.

As a first step toward positional cloning of QTL-M, we constructed a BAC library from PI 229358. The next step was the identification of the BAC clones carrying the simple sequence repeat (SSR) markers flanking the 0.5-cM interval containing QTL-M. Multidimensional pools are often used to identify BAC clones containing specific polymerase chain reaction (PCR)-based markers (Yim et al. 2007). However, it is not economically practical to establish a pooling system for a BAC library that will not be used repeatedly. Here, we present an effective approach to screen a BAC library for a PCR-based SSR marker without the use of BAC pools and show it to be useful with soybean, despite the highly repetitive nature of its genome (Shoemaker et al. 2006).

Materials and Methods

Plant Materials and BAC Cloning Vector

PI 229358 was grown in a greenhouse for 2 weeks at 25°C with a 14-h photoperiod. Two-week-old seedlings were held in complete darkness for 48 h before collecting young leaves. PI 229358 is a Japanese soybean cultivar, originally known as "Sodendaizu" (Komatsu et al. 2004), and carries the resistance allele of QTL-M for defoliating insect resistance (Rector at al. 1998, 2000).

The single-copy BAC vector pIndigoBac536 was obtained from H. Shizuya and M. Simon (Caltech, Pasadena, CA, USA) and prepared as described by Luo et al. (2001).

BAC Library Construction

Nuclei were isolated from fresh leaves, and megabase-size DNA was embedded in low-melting-point (LMP) agarose plugs as described by Zhang et al. (1995) with some modifications. Briefly, about 50 g ground tissue was suspended in 500 ml prechilled nuclei isolation buffer (NIB) [10 mM Tris–HCl pH 8.0; 10 mM ethylenediamine tetraacetic acid (EDTA) pH 8.0; 100 mM KCl; 500 mM sucrose; 4 mM spermidine; 1 mM spermine; 2% (*w*/*v*) polyvinylpyrrolidinone K-30; 0.2% (*v*/*v*) β-mercaptoethanol (BME)]. After being filtered through two layers of cheesecloth and two layers of Miracloth (Calbiochem/EMD, San Diego, CA, USA), chloroplasts were lysed by adding 1/20th volume of 10% prechilled Triton-X100 in NIB and incubating for 12 min in ice swirling every 2 min. The nuclei were consolidated by centrifuging at 1,800×*g* for 15 min at 4°C and washed with NIB without BME. An equal volume of 1.5% LMP agarose (45°C) was added to the 2-ml prewarmed, pelleted nuclei. Plugs of 100 µl each were made and incubated in ten volumes of EPS buffer [0.5 M EDTA pH 9.2; 1 mg ml^{-1} proteinase K; 1% (*w*/*v*) sarcosyl; 0.2% (*v*/*v*) BME] for 24 h at 50°C. The plugs were washed with 20 volumes of 1 mM phenyl methane sulfonyl fluoride in $T_{10}E_{10}$ (10 ml 1 M Tris–HCl; 100 ml 0.5 M EDTA) twice, 1 h each, and then washed with 20 volumes of $T_{10}E_1$ (10 ml 1 M Tris–HCl; 10 ml 0.5 M EDTA) twice, 1 h each.

Partial digestion, size selection, and elution of DNA were performed as described by Tomkins et al. (1999) with some modifications. A pilot partial digestion was performed at 37°C for 20 min using different *Hin*dIII (Promega, Madison, WI, USA) concentrations to determine the optimum concentration for obtaining 100 to 350 kb fragments. Chopped plugs were aliquoted in 100 µl and incubated on ice for 30 min with 14 µl 10× *Hin*dIII buffer, 14 µl 40 mM spermidine, and 1.4 µl 10 mg ml^{-1} bovine serum albumin. After another 30 min incubation with the predetermined concentration (two to three units) of *Hin*dIII, digestions were performed at 37°C for 20 min and stopped by adding 20 µl of 0.5 M EDTA. The partially digested DNA fragments were first size-selected by pulsed-field gel electrophoresis (PFGE) using CHEF MAPPER™ system (Bio-Rad, Hercules, CA, USA) in a 1% agarose gel in 12°C 0.5× Tris base–boric acid–EDTA buffer using 0.75 l min^{-1} pump speed for 18 h with 1 s initial switching, 40 s final switching, 6 V cm^{-1}, and 120° included field angle. Three fractions between 100 and 350 kb were cut from the gel and subjected to the second size selection in 1% LMP agarose gel under the same conditions as the first size-selection, except 3 s initial switching and 5 s final switching. High molecular weight insert DNA was electro-eluted at 12 mA $tube^{-1}$ for 3 h from the gel slices using the Electro-Eluter 422 system (Bio-Rad).

Ligation, desalting, and transformation were conducted as described by Tomkins et al. (1999) with some modifications. Ligation was performed in a 150-µl reaction using 75 ng vector, 750 ng insert DNA, and nine units of T4 ligase (Promega), and allowed to proceed for 20 h at 16°C. The ligation mix was desalted by dialysis against 5% polyethylene glycol 8000 on a piece of 0.025 µm Millipore nitrocellulose filter for 1.5 h. Transformation was performed by electroporation in a cell-porator with voltage booster (Gibco/BRL, Carlsbad, CA, USA) using 320 V at a resistance of 4 kΩ and using 2 µl ligation (about 2 ng ligated DNA) and 20 µl electro-competent DH10B cells (about 1.0×10^9 cfu/µg for pUC19; Gibco/BRL). Transformants were immediately transferred to 0.5 ml of Super Optimal broth with Catabolite repression (SOC) medium and shaken at 250 rpm for 1 h at 37°C. They were then plated on Luria–Bertani (LB) medium containing 12.5 µg ml^{-1} chloramphenicol, 90 µg ml^{-1} isopropyl β-D-1-thiogalactopyranoside, and 90 µg ml^{-1} 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside (X-gal) and were incubated for 20 h at 37°C. White colonies were transferred using a Qbot robot (Genetix Ltd., Dorset, UK) to 384-well microtiter plates containing 50 µl of LB freezing broth (Woo et al. 1994). The plates were incubated at 37°C overnight and stored at −80°C.

General BAC Library Characterization

A set of 192 randomly picked clones was selected. The clones were cultured and shaken at 325 rpm for 18 h at 37°C in 96-deepwell plates with each well containing 0.9 ml Terrific Broth (Invitrogen, Carlsbad, CA) and 12.5 µg ml^{-1} chloramphenicol to check the average insert size of the library. The automated BAC DNA minipreps were performed using a MICROLAB® 4000 Robot (Hamilton, Reno, NV, USA) according to a standard alkaline-lysis protocol (Sambrook et al. 1989). The BAC DNA was digested with ten units of *Not*I (Promega) at 37°C for 4 h. The digested DNA was resolved by PFGE under the same conditions as used for the size selections, except that 5 s initial switching, 15 s final switching, and a run time of 15 h were used (Fig. 1).

BAC clones containing chloroplast DNA were identified by using five soybean chloroplast clones containing *rpl23*, *ndhB*, *rrn16*, *psbB*, and *rps2* gene sequences as probes to screen the BAC filters. These sequences are spaced equidistantly around the 152 kb soybean chloroplast genome (Saski et al 2005; NC_007942) and can be obtained from the BAC/EST Resource Center (www.genome.clemson.edu).

Screening BAC Filters

High-density BAC filters for hybridization-based screening of the library were prepared with the Genetix QBot. Clones were double-spotted using a 4×4 array on 22.5×22.5 cm^2 Hybond N^+ nylon filters (Amershan Biosciences, Bucks, UK). This gridding pattern allows 18,432 clones to be represented per filter. Treatment and hybridization of BAC colony filters and radio-labeling (^{32}P) of DNA probes followed standard techniques (Sambrook et al. 1989).

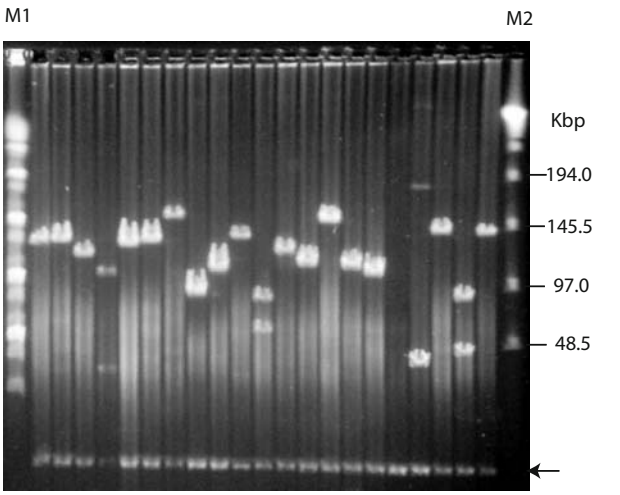

Fig. 1 The pulsed-field gel electrophoresis analysis of some BAC clones digested with *Not*I randomly selected from the PI 229358 library. The PFGE gel (5–15 s switch time, 6 V/cm, 12°C, and 18 h) showing insert DNA above the common 7.5-kbp pIndigoBac536 vector band indicated by the *arrow*. *M1* is MidRange I PFG marker, and *M2* is lambda ladder PFG marker (New England BioLabs, Ipswich, MA, USA)

Identification of Single- or Low-Copy, Nonrepeat Genomic Sequences Associated with an SSR Marker

The first nonrepeat sequence flanking Sat_258's core AT repeat was identified as follows. Using primer 3 (Rozen and Skaletsky 2000), a pair of primers (5′-CTT GAC ATC GAC CAA CAA CG-3′ and 5′-TTT GAC AAG TTT AAT CTT TGA CCT C-3′) was designed from the original DNA sequence (GenBank #: CC453793) that was used to develop the SSR marker Sat_258. The primers were used to amplify the genomic sequence 3′ flanking Sat_258's core AT repeat. The PCR amplicon was named as Sat_258sc1 (Fig. 2) and checked on a Southern blot of *Hin*dIII-digested PI 229358 genomic DNA to determine if single- or low-copy number of bands was produced before screening BAC filters (Fig. 3b).

The second flanking sequence was identified using the GenomeWalker™ Universal Kit (BD Biosciences Clontech, Palo Alto, CA, USA). Briefly, genomic DNA with minimum smearing (bigger than 50 kb) was isolated from PI 229358 lyophilized leaves (Sambrook et al. 1989). The DNA was digested with four different restriction enzymes (*Dra*I, *Eco*RV, *Pvu*II, and *Stu*I) to form four libraries following

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Sat_258      ATATATATAT ATATATATAT ATATATATAT ATATATATAT ATATATATAT ATATATATAT    60
Sat_258sc1   ---------- ---------- ---------- ---------- ---------- ----------     0
Sat_258sc2   ---------- ---------- ---------- ---------- ---------- ----------     0

Sat_258      ATATATATTA TATTCAAAAG CTTGACATCG ACCAACAACG TAGACTGACA TGTGTATCAC   120
Sat_258sc1   ---------- ---------- CTTGACATCG ACCAACAACG TAGACTGACA TGTGTATCAC    40
Sat_258sc2   ---------- ---------- ---------- ---------- ---------- ----------     0

Sat_258      TCGTCTATTT GATCTTGTTT TCACTTTCCC ---------- ---------- ----------   150
Sat_258sc1   TCGTCTATTT GATCTTGTTT TCACTTTCCC CGTGATTTCC TATTGTCAAT ATTCTGGAAG   100
Sat_258sc2   ---------- GATCTTGTTT TCACTTTCCC CGTGATTTCC TATTGTCAAT ATTCTGGAAG    50

Sat_258      ---------- ---------- ---------- ---------- ---------- ----------   150
Sat_258sc1   TGTTATACAT ATTTTAAGAG GTCAAAGATT AAACTTGTCA AA-------- ----------   142
Sat_258sc2   TGTTATACAT ATTTTAAGAG GTCAAAGATT AAACTTGTCA AAGACTCAAA AATAAAATAA   110

Sat_258      ---------- ---------- ---------- ---------- ---------- ----------   150
Sat_258sc1   ---------- ---------- ---------- ---------- ---------- ----------   142
Sat_258sc2   AAGTCCATTC AAAGAAATAA AATGATGTTT TAGTTATGCA TACTAATTTT TTATTATTAA   170

Sat_258      ---------- ---------- ---------- ---------- ---------- ----------   150
Sat_258sc1   ---------- ---------- ---------- ---------- ---------- ----------   142
Sat_258sc2   TATTCAAATT AGATATTTTT TACCACAAGT GCCTTTTTAT ATTTTTCATT TTAATTACTT   230
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Fig. 2 Sequence comparison of PI 229358 Sat_258, Sat_258sc1, and Sat_258sc2. Sat_258 of PI 229358 is 223 bp long (only 150 bp shown), and Sat_258sc1 is 142 bp long, while Sat258sc2 is 1,635 bp long (only 230 bp shown). The 20-bp sequence shared by Sat_258, Sat_258sc1, and Sat_258sc2 is *highlighted*. The *dash* means bps missing in the corresponding sequences

the procedure recommended by the kit manufacturer. The libraries were purified following the procedure suggested by the manufacturer, except using 24:1 chloroform/isoamyl alcohol instead of phenol. The resulting DNA was ligated to the GenomeWalker™ adaptor following the exact procedure provided by the manufacturer. The primary genome walking and secondary genome walking PCRs were conducted following the instructions, except a different *Taq* DNA polymerase (Promega) was used. Two Sat_258-specific primers (5′-GAC ATC GAC CAA CAA CGT AGA CTG ACA-3′ and 5′-GAT CTT GTT TTC ACT TTC CCC GTG ATT-3′) were designed using the sequence of Sat_258sc1 (Fig. 2). After the secondary genome walking, the PCR amplicons were separated on a LMP agarose gel. The major band was excised from the gel and purified with Zymoclean Gel DNA Recovery Kit (Zymo Research, Orange, CA, USA). If one end sequence of the amplicon matches the original sequence of Sat_258sc1, the amplicon is named as Sat_258sc2 and checked on a Southern blot as described previously before screening BAC filters (Figs. 2 and 3c).

Confirmation of Positive Clones

The BAC clones identified through hybridization were cultured in individual tubes containing 5 ml LB broth with 12.5 μg ml^{-1} chloramphenicol and incubated at 37°C for 16 h while shaking at 280 rpm. The BAC DNA was isolated with the standard alkaline lysis method (Sambrook et al. 1989). To confirm the positive clones, first the BAC DNA was checked by amplifying Sat_258 from it using the protocol described by Li et al. (2002) and then confirmed by comparing the SSR amplicon sequence from the BAC DNA with the SSR sequence amplified from PI 229358.

Results

Construction and Characterization of the BAC Library

A BAC library was constructed that consists of 55,296 clones and is arrayed in 144 384-well micro-titer plates. The library has been made available to the public through the Clemson University Genomics Institute (www.genome.clemson.edu, verified May 2008). One hundred and ninety-two BAC clones were randomly sampled from the library and analyzed with PFGE (Fig. 1). The insert sizes of these clones range from 30 to 320 kb, with an average of 131 kb (Fig. 4). More than 84% of the clones have inserts larger

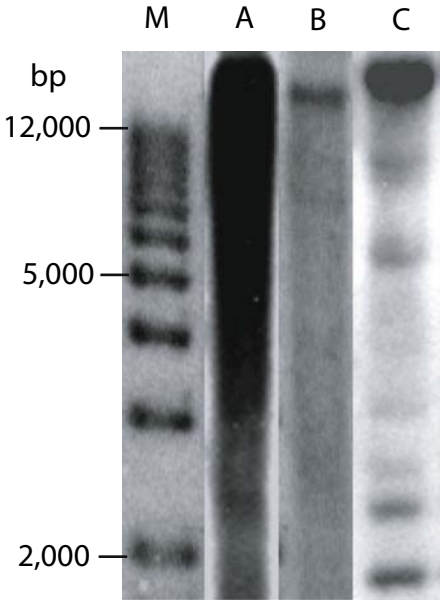

Fig. 3 Southern blots of *Hin*dIII-digested PI 229358 DNA hybridized with different probes. *A* Sat_258 PCR amplicon as the probe, *B* Sat_258sc1, and *C* Sat_258sc2. *M* is 1 kb+ lambda ladder

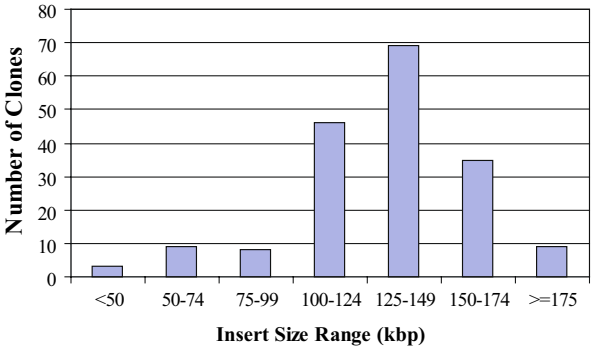

Fig. 4 Insert size distribution of BAC clones from the PI 229358 BAC library. Data from 192 randomly sampled BAC clones subjected to *Not*I digestion and pulsed-field gel analysis

than 100 kb, indicating that the two rounds of size selection were effective. Seven (about 4%) clones have no inserts.

All clones were double-spotted on high-density BAC filters for hybridization-based screening of the BAC library. The entire library was represented by three filters. When hybridized with the chloroplast DNA-derived probes, positive hybridization signals were observed for 2,322 clones or 4.2% of clones in the library.

Identification of BAC Clones Carrying a SSR

No BAC pools for the PI 229358 library were made; however, the high-density BAC filters carrying the whole library are available as indicated above. Probing a Southern blot of *Hin*dIII-digested PI 229358 genomic DNA with the PCR amplicon from an SSR marker Sat_258 (SoyBase http://soybase.org, verified May 2008) resulted in a smear (Fig. 3a), indicating the core AT repetitive motif of the Sat_258 is too common in the soybean genome for the Sat_258 amplicon to serve as a useful probe. Therefore, hybridization of the BAC filters with the amplicon from Sat_258 as a probe was not an option for identification of BAC clones carrying the SSR marker.

Accordingly, Sat_258sc1, a nonrepeat genomic sequence of 142 bp from the right side flanking Sat_258's AT repeats, was identified (Fig. 2). Hybridization of Sat_258sc1 with a Southern blot produced a single band (Fig. 3b). Therefore, the BAC filters were probed with Sat_258sc1. Two clones on the BAC filters hybridized to the probe; however, none of them contains Sat_258. Given it was impossible to obtain a correct amplicon using these BAC clones as template and the original primers for Sat_258 (SoyBase http://soybase.org, verified may 2008), it was necessary to identify another nonrepeat sequence from further right side flanking the Sat_258sc1 for use as a probe.

In the end, Sat_258sc2, a second nonrepeat genomic sequence of 1,635 bp, was identified by genome walking. Sequence comparison between Sat_258sc1 and Sat_258sc2 indicated that the 1,635-bp sequence from the right side truly flanks Sat_258 AT repeats (Fig. 2). The sequence produced a dark band and a few lighter bands on a Southern blot (Fig. 3c). Thus, the sequence was used as a probe, and 13 BAC clones were identified. Two of them (GM_PIBd070C19 and GM_PIBd118A03) were confirmed to carry the SSR marker, Sat_258 from PI 229358, by running PCR on the BAC DNA with the original Sat_258-specific primers (Fig. 5) and by comparing the SSR amplicon sequence from the BAC DNA with the SSR sequence amplified from PI 229358 DNA. The remaining clones that hybridized to Sat_258sc2 do not carry Sat_258.

Discussion

Given the soybean haploid genome size of 1.12×10^9 bp (Arumuganathan and Earle 1991) and after excluding the clones without inserts (2,211) or with chloroplast DNA (2,322), the rest of clones in the library represent about a 6-fold equivalent of the soybean haploid genome. The depth of coverage gives a 99.8% probability of finding any soybean sequence of interest, as determined by using the formula described by Clarke and Carbon (1976). Thus, the quality of this BAC library is comparable to that of the other available soybean BAC libraries (Marek and Shoemaker 1997; Meksem et al. 2000; Tomkins et al. 1999; Wu et al. 2004; Xia et al. 2005). Moreover, this soybean BAC library is unique because it is the only one derived from a soybean genotype that carries defoliating insect-resistance alleles. It will be useful for the map-based cloning of the major insect-resistance QTL, QTL-M, and other insect-resistance QTLs from PI 229358 as well.

One of our goals was to identify a BAC clone containing Sat_258, a SSR marker flanking QTL-M (Zhu et al. 2006), which could be used towards QTL cloning. Because this library was made with the intent of just cloning a maximum

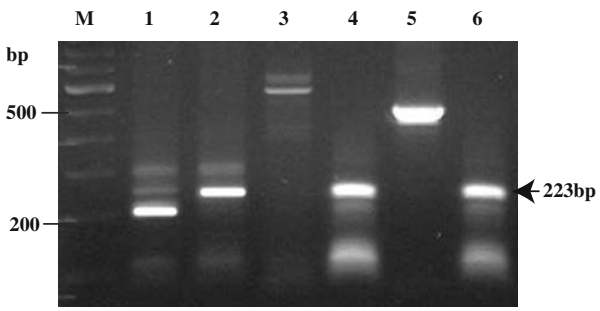

Fig. 5 PCR confirmation of BAC clones with a SSR marker Sat_258. *Lane 1* soybean cultivar "Benning"; *lane 2* PI 229358; *lanes 3* and *5*: GM_PIBd065C05 and GM_PIBd068D21, respectively, not carrying Sat_258 but a homologous sequence of Sat_258; and *lanes 4* and *6*: GM_PIBd070C19 and GM_PIBd118A03, respectively, carrying 223 bp of Sat_258 from PI 229358. *M* 100 bp DNA ladder

of three QTLs for defoliating insect resistance, a multidimensional pooling strategy is not economically practical to identify BAC clones containing SSR markers associated with the trait. BAC pools are not available for the PI 229358 library, requiring the use of an alternative strategy that could identify a sequence of interest in a BAC library made from a complex and highly duplicated genome (Shoemaker et al. 2006).

Since the amplicon from Sat_258 of PI 229358 contains 41 AT repeats (partly shown in Fig. 2), its use as a probe would produce numerous hits on PI 229358 BAC filters. The impossibility of finding a BAC clone carrying a SSR marker by directly using the PCR amplicon from the SSR marker as a probe prompted us to find a single- or low-copy, nonrepeat DNA sequence flanking the SSR marker.

Neither of the two BAC clones identified by hybridizing Sat_258sc1 on the Southern blot of *Hin*dIII-digested PI 229358 genomic DNA contained the target SSR marker, Sat_258. Similarly, 11 of the 13 clones detected by hybridization with Sat_258sc2 did not carry Sat_258, either. These false-positive clones likely carry a sequence homologous to Sat_258sc1 or Sat_258sc2 because soybean genome is highly duplicated (Shoemaker et al. 2006). Therefore, it is necessary to use a strategy that can identify enough single- or low-copy DNA for use as a probe to identify a BAC clone containing an otherwise highly repeated motif in the genome.

Genome walking has been used previously to clone 5′-flanking regions, i.e., promoters of genes (Wu et al. 2001; Oleksiak and Crawford 2002). This strategy was used here to successfully find a low-copy DNA sequence flanking Sat_258, which was then used as a probe to successfully find BAC clones carrying Sat_258. These results suggest that this approach can be used to screen a BAC library for other PCR-based markers as well without depending on BAC multidimensional pools, which are expensive and time-consuming to create. This approach is novel for this purpose and could be useful to those organisms in which whole genome sequence is not available.

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References

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218. doi:10.1007/BF02672069

Clarke L, Carbon J (1976) A colony bank containing synthetic Col El hybrid plasmids representative of the entire *E. coli* genome. Cell 9:91–99. doi:10.1016/0092-8674(76)90055-6

Danesh D, Penula S, Mudge J, Denny RL, Nordstrom H, Martinez JP, Young ND (1998) A bacterial artificial chromosome library for soybean and identification of clones near a major cyst nematode resistance gene. Theor Appl Genet 96:196–202. doi:10.1007/s001220050727

Komatsu K, Okuda S, Takahashi M, Matsunaga R (2004) Antibiotic effect of insect-resistant soybean on common cutworm (*Spodoptera litura*) and its inheritance. Breed Sci 54:27–32. doi:10.1270/jsbbs.54.27

Komatsu K, Takahashi M, Nakazawa (2008) Antibiosis resistance of QTL introgressive soybean lines to common cutworm (*Spodoptera litura* Fabricius). Crop Sci 48:527–532. doi:10.2135/cropsci2006.12.0770

Lambert L, Kilen TC (1984) Influence of three soybean plant genotypes and their F1 intercrosses on the development of five insect species. J Econ Entomol 77:622–625

Lambert L, Tyler J (1999) Appraisal of insect-resistant soybeans. In: Wiseman BR, Webster JA (eds) Economic, environmental, and social benefits of resistance in field crops. Thomas Say Publications, Entomological Society of America, Lanham, MD, pp 131–148

Li Z, Wilson RF, Rayford WE, Boerma HR (2002) Molecular mapping genes conditioning reduced palmitic acid content in N87-2122-4 soybean. Crop Sci 42:373–378

Luo M, Wang Y, Frisch D, Joobeur T, Wing RA, Dean RA (2001) Melon bacterial artificial chromosome (BAC) library construction using improved methods and identification of clones linked to the locus conferring resistance to melon *Fusarium wilt* (*Fom-2*). Genome 44:154–162. doi:10.1139/gen-44-2-154

Marek LF, Shoemaker RC (1997) BAC contig development by fingerprint analysis in soybean. Genome 40:420–427. doi:10.1139/g97-056

Meksem K, Zobrist K, Ruben E, Hyten D, Quanzhou T, Zhang HB, Lightfoot DA (2000) Two large-insert soybean genomic libraries constructed in a binary vector: applications in chromosome walking and genome wide physical mapping. Theor Appl Genet 101:747–755. doi:10.1007/s001220051540

Oleksiak MF, Crawford DL (2002) 5′ Genomic structure of human Sp3. Mol Biol Evol 19:2026–2029

Rector BG, All JN, Parrott WA, Boerma HR (1998) Identification of molecular markers linked to quantitative trait loci for soybean resistance to corn earworm. Theor Appl Genet 96:786–790. doi:10.1007/s001220050803

Rector BG, All JN, Parrott WA, Boerma HR (2000) Quantitative trait loci for antibiosis resistance to corn earworm in soybean. Crop Sci 40:233–238

Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics methods and protocols: methods in molecular biology. Humana, Totowa, NJ, pp 365–386

Rufener GKII, St Martin SK, Cooper RL, Hammond RB (1989) Genetics of antibiosis resistance to Mexican bean beetle in soybean. Crop Sci 29:618–622

Salimath SS, Bhattacharyya MK (1999) Generation of a soybean BAC library, and identification of DNA sequences tightly linked to the Rps1-k disease resistance gene. Theor Appl Genet 98:712–720. doi:10.1007/s001220051125

Sambrook J, Frisch E, Maniatus T (1989) Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Saski C, Lee SB, Daniell H, Wood TC, Tomkins J, Kim HG, Jansen RK (2005) Complete chloroplast genome sequence of *Glycine max* and comparative analyses with other legume genomes. Plant Mol Biol 59:309–322. doi:10.1007/s11103-005-8882-0

Shoemaker RC, Schlueter J, Doyle JJ (2006) Paleopolyploidy and gene duplication in soybean and other legumes. Curr Opin Plant Biol 9:104–109. doi:10.1016/j.pbi.2006.01.007

Tanksley SD, Ganal MW, Martin GB (1995) Chromosome landing: a paradigm for map-based gene cloning in plants with large

genomes. Trends Genet 11:63–68. doi:10.1016/S0168-9525(00)88999-4

Tomkins JP, Mahalingham R, Miller-Smith H, Goicoechea JL, Knapp HT, Wing RA (1999) A soybean bacterial artificial chromosome library for PI 437654 and the identification of clones associated with cyst nematode resistance. Plant Mol Biol 41:25–32. doi:10.1023/A:1006277417789

Tomkins JP, Frish DA, Byrum JR, Wing RA (2000) Construction and characterization of a BAC library for the soybean cultivar A3244. Soybean Genet. Newsletter 27, http://www.soygenetics.org. Cited 20 May 2008

Van Duyn JW, Turnipseed SG, Maxwell JD (1971) Resistance in soybeans to the Mexican bean beetle. I. Sources of resistance. Crop Sci 11:572–573

Walker DR, Narvel JM, Boerma HR, All JN, Parrott WA (2004) A QTL that enhances and broadens Bt insect resistance in soybean. Theor Appl Genet 109:1051–1057. doi:10.1007/s00122-004-1714-9

Woo S, Jiang J, Gill B, Paterson A, Wing R (1994) Construction and characterization of a bacterial artificial chromosome library of Sorghum bicolor. Nucleic Acids Res 22:4922–4931. doi:10.1093/nar/22.23.4922

Wu Q, Andolfatto P, Haunerland NH (2001) Cloning and sequence of the gene encoding the muscle fatty acid binding protein from the desert locust, *Schistocerca gregari*. Insect Biochem Mol Biol 31:553–562. doi:10.1016/S0965-1748(00)00158-2

Wu C, Springman R, Santos FA, Nimmakayala P, Meksem K, Scheuring C, Lightfoot DA, Zhang HB (2004) Construction and characterization of a soybean bacterial artificial chromosome library and use of multiple complementary libraries for genome physical mapping. Theor Appl Genet 109:1041–1050. doi:10.1007/s00122-004-1712-y

Xia Z, Sato H, Watanabe S, Kawasaki S, Harada K (2005) Construction and characterization of a BAC library of soybean. Euphytica 141:129–137. doi:10.1007/s10681-005-6380-8

Yim YS, Moak P, Sanchez-Villeda H, Musket TA, Close P, Klein PE, Mullet JE, McMullen MD, Fang Z, Schaeffer ML, Gardiner JM, Coe EH, Davis GL (2007) A BAC pooling strategy combined with PCR-based screenings in a large, highly repetitive genome enables integration of the maize genetic and physical maps. BMC Genomics 8:47. doi:10.1186/1471-2164-8-47

Zhang HB, Zhao X, Ding X, Paterson AH, Wing RA (1995) Preparation of megabase-size DNA from plant nuclei. Plant J 7:175–184. doi:10.1046/j.1365-313X.1995.07010175.x

Zhu S, Walker DR, Boerma HR, All JN, Parrott WA (2006) Fine mapping of a major insect resistance QTL in soybean and its interaction with minor resistance QTLs. Crop Sci 46:1094–1099. doi:10.2135/cropsci2005.06-0109

Zhu S, Walker DR, Boerma HR, All JN, Parrott WA (2008) Effects of defoliating insect resistance QTLs and a *cry1Ac* transgene in soybean near-isogenic lines. Theor Appl Genet 116:455–463. doi:10.1007/s00122-007-0682-2