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ORIGINAL PAPER

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Construction of two BAC libraries from the wild Mexican diploid potato, *Solanum pinnatisectum*, and the identification of clones near the late blight and Colorado potato beetle resistance loci

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Abstract To facilitate isolation and characterization of disease and insect resistance genes important to potato, two bacterial artificial chromosome (BAC) libraries were constructed from genomic DNA of the Mexican wild diploid species, *Solanum pinnatisectum*, which carries high levels of resistance to the most important potato pathogen and pest, the late blight and the Colorado potato beetle (CPB). One of the libraries was constructed from the DNA, partially digested with *Bam*HI, and it consists of 40,328 clones with an average insert size of 125 kb. The other library was constructed from the DNA partially digested with *Eco*RI, and it consists of 17,280 clones with an average insert size of 135 kb. The two libraries, together, represent approximately six equivalents of the wild potato haploid genome. Both libraries were evaluated for contamination with organellar DNA sequences and were shown to have a very low percentage (0.65–0.91%) of clones derived from the chloroplast genome. High-density filters, prepared from the two libraries, were screened with ten restriction fragment length polymorphism (RFLP) markers linked to the resistance genes for late blight, CPB, *Verticillium* wilt and potato cyst nematodes, and the gene *Sr1* for the self-incompatibility *S*-locus. Thirty nine positive clones were identified and at least two positive BAC clones were detected for each RFLP marker. Four markers that are linked to the late blight resistance gene *Rpi1* hybridized to 14 BAC clones. Fifteen BAC clones were shown to harbor the PPO (polyphenol oxidase) locus for the CPB resistance by three RFLP probes. Two RFLP markers detected five BAC clones that were linked to the *Sr1* gene for self-incompatibility. These results agree with the library's predicted extent of coverage of the potato genome, and indicated that the libraries are useful resources for the molecular isolation of disease and insect resistance genes, as well as other economically important genes in the wild potato species. The development of the two potato BAC libraries provides a starting point, and landmarks for BAC contig construction and chromosome walking towards the map-based cloning of agronomically important target genes in the species.

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Introduction

Potato (*Solanum tuberosum* L.) is one of the world's most important food crops for humans and has a high value-added component. However, diseases and insects, such as late blight [*Phytophthora infestans* (Mont.) de Bary] and Colorado potato beetle (CPB; *Leptinotarsa decemlineata* Say) are the major biotic constraints in potato production. They have caused huge losses and present a constant threat to the potato industry worldwide (Niederhauser 1993; Guenthner et al. 2001). Although some R gene-based resistant cultivars are resistant to certain *P. infestans* strains, there is no genetic resistance to the new race of the pathogen in most potato cultivars (Ferro and Boiteau 1993; Inglis et al.1996; Douches et al. 1997). The Mexican wild diploid, self-incompatible species, *Solanum pinnatisectum* Dunal (a close relative of potato), was identified as a new source of resistance to the wide diversity of *P. infestans* and CPB (Douches et al. 2001; Kuhl et al. 2001; Chen et al. 2003). The Mexican wild species was also found resistant to *Verticillium* wilt, the root-knot nematode (*Meloidogyne hapla* Chitwood) and the potato cyst nematode (*Globodera rostochiensis* Wollenweber) (Bamberg et al. 1994). Introgression of the disease and insect resistance into the potato cultivars can be achieved via somatic hybridization (Austin et al. 1993;

Thieme et al. 1997). However, the development of commercially acceptable cultivars with late blight and CPB resistance has been very difficult due to the coincident introgression of undesirable characteristics from the wild species. Molecular genetic engineering of resistance genes has been proven an effective approach for improving potato disease and insect resistance (Hoy 1999; Coombs and Douches 2002).

Genetic studies indicated that late blight resistance in the Mexican wild species *S. pinnatisectum* is controlled by a single dominant locus designated *Rpi1* (Kuhl et al. 2001). The gene *Rpi1* was mapped in the central region of chromosome 7, and was flanked 5.2-cM proximally by the RFLP marker TG20 and 9.4-cM distally by CP56 (Kuhl et al. 2001). This region contains a rich cluster of disease resistance genes, such as the *Verticillium* wilt resistance gene *Ve* and the major gene *Gro1* that confers resistance to the potato cyst nematode (Barone et al. 1990; Juvick et al. 1991; Ballvora et al. 1995). Although the genetic mechanism for the CPB resistance present in the wild species *S. pinnatisectum* is not clearly understood, it is believed that leptine glycoalkaloids and glandular trichomes are two natural insect-host resistance mechanisms available in the wild potato (Sinden et al. 1980, 1986; Tingey 1984; Coombs and Douches 2002). It was found that polyphenol oxidase (PPO) in the trichome-mediated insect resistance is the major factor involved in the CPB resistance in wild potato (Tanksley et al. 1992; Bonierbale et al. 1994). The presence of different levels of PPO leads to entrapment and the death of small insects (Gregory et al. 1986), and reduces the survival and oviposition of CPB (Casagrande 1982; Wright et al. 1985; Yencho and Tingey 1994). Molecular cloning and characterization of these resistance genes or DNA segments would provide the means to develop resistant germplasm or cultivars through genetic transformation, and a molecular basis to develop efficient approaches to control the pathogens and pests.

However, for positional cloning and characterization of the disease and insect resistance genes present in the Mexican wild species *S. pinnatisectum*, libraries with large genomic DNA inserts are required (Martin et al. 1993; Tanksley et al. 1995). In recent years, the bacterial artificial chromosome (BAC; Shizuya et al. 1992) system has become an invaluable tool in genomic studies and has been widely used for large-insert library construction, because of its ability to stably maintain large DNA fragments, ease of manipulation and the low rate of clonal chimerism (Tao et al. 1994; Zhang et al. 1996; Song et al. 2000). To facilitate map-based cloning of late blight and CPB resistance genes, and to establish a general tool for marker-enhanced breeding, we are developing an integrated project based on exploiting wild potato gene pools for the improvement of disease and insect resistance in potato, and the construction of large-insert genomic BAC libraries to facilitate chromosome walking toward the target genes.

In this report, we describe the construction and analysis of two BAC libraries derived from genomic DNA of the multiple disease and insect resistant Mexican wild species *S. pinnatisectum*. Furthermore, we identified the BAC clones linked to late blight and CPB resistance genes, and the self-incompatibility *S*-locus by colony hybridization with RFLP (restriction fragment length polymorphism) markers on chromosomes 1, 7 and 8, where these genes have already been mapped with the RFLP markers (Gebhardt et al. 1991, 1994; Tanksley et al. 1992; Kuhl et al. 2001). The usefulness of the two BAC libraries in map-based cloning of these agronomically important genes, and construction of a physical map of the diploid potato genome, is discussed.

Materials and methods

BAC library construction

The BAC libraries were constructed according to Zhang (2000) with some modifications. The BAC vector, pECBAC1 (7.5 kb) (for restriction map and sequence, see http://hbz.tamu.edu), was used for the construction of the BAC libraries. The wild diploid potato species *S. pinnatisectum* previously obtained from the NRSP-6 Inter Regional Potato Introduction Station at Sturgeon Bay, Wisconsin, and verified to have the target resistances, was used as the source of high-molecular-weight (HMW) DNA. The plants of *S. pinnatisectum* were grown in a growth chamber at 17–18°C with a 12-h day/night light cycle. High-molecular-weight genomic DNA was isolated from leaf tissues that were collected from 2-month-old plants and then stored at −80°C following the procedure described by Zhang et al. (1995, 1996), with some modifications. Nuclei prepared from 50 g of leaf tissue were embedded in 2-ml low-melting-point (LMP) agarose plugs. Each 100-µl plug contained 5–10 µg of DNA. Partial digestion of the HMW DNA was carried out with either *Bam*HI or *Eco*RI and separated on an agarose CHEF gel. DNA fragments ranging from 150 to 400 kb was collected from the gel and then subjected to a second size selection. The compressed DNA bands from the second size selection were excised from the CHEF gel and used for ligation. About 100 ng of potato DNA were ligated to the linearized, de-phosphorylated pECBAC1 vector using a molecular weight ratio of 4 (potato DNA insert) to 1 (vector DNA), with 4 units of T4 DNA ligase at 16°C overnight. After ligation, 1 µl of the ligation mixture was added to 20 µl of the *Escherichia coli* strain ElectroMax DH10B cells for a single electroporation, using the BRL Gibco Cell-Porator and the Voltage Booster system. Then, transformed cells were immediately resuspended in 1 ml of the SOC medium and incubated at 37°C on a shaker for 1 h. The cells were then transferred onto LB plates containing 12.5 µg/ml of chloramphenicol, X-gal and IPTG, and incubated at 37°C for 24–48 h to allow the colony colors to fully develop. The recombinant clones (BACs) could be clearly identified by their white (recombinant) phenotype, while blue clones contained no inserts. White clones were transferred directly to 384-well microtiter plates containing 50 µl of LB freezing buffer plus 12.5 µg/ml of chloramphenicol (Zhang et al. 1996). The plates were incubated at 37°C for 24 h, and then stored at −80°C.

BAC insert sizing and library characterization

To check the insert sizes of the clones, BAC clones were inoculated onto 5-ml LB containing 12.5 µg/ml of chloramphenicol and incubated overnight at 37°C. The BAC DNA was isolated according to the modified standard alkaline lysis method (Zhang et al. 1996; Zhang 2000). The final DNA pellet was resuspended in 40 µl of TE. One quarter of the BAC DNA was digested with *Not*I for 3 h to release the insert DNA from the vector. The digested BAC DNA was separated on a 1% agarose CHEF (Bio-Rad, USA) gel with a 5- to 15-s linear ramp time at 6 V/cm, and 14°C in a 0.5×TBE buffer for 16 h.

Table 1 Positive BAC clones identified with different markers

Origin	Probe	Loci on chrom.	Linked genes	Number of hits	No. of positive clones (%) in:	
					*Bam*HI library	*Eco*RI library
RFLP	TG24	1 (P10)	*Sr*1	3	1/40 328	2/17 280
RFLP	CP108	1 (P14)	*Sr*1	2	2/40 328	0/17 280
RFLP	GP127	7 (P2)	*Rpi*1	6	2/40 328	4/17 280
RFLP	TG20	7 (P11)	*Rpi*1, Ve	2	1/40 328	1/17 280
RFLP	CP51	7 (P12)	*Rpi*1	2*	0/40 328	2/17 280
RFLP	CP56	7 (P1)	*Rpi*1, *Gro*1	4	0/40 328	4/17 280
RFLP	GP174	7 (P4)	–	5	3/40 328	2/17 280
RFLP	TG302	8 (P7)	PPO	4*	1/40 328	3/17 280
RFLP	TG624	8 (P9)	PPO	5	4/40 328	1/17 280
RFLP	TG181	8 (P15)	PPO	6*	1/40 328	5/17 280
Total	10	3	3	39	15/40 328	24/17 280
Chloroplast DNA	ndhA, RbcL, psbA			24	10/1,536 (0.65%)	14/1,536 (0.91%)

* A single BAC clone was found to carry the CP51, TG302 and TG181 markers

Preparation of a high-density filter and library screening by RFLP markers

High-density colony filters were prepared using the GeneTAC G3 Robotic Workstation (Genomic Solutions, Inc., USA). BAC clones were gridded in double spots using a 3×3 array, and 1,536 clones were spotted onto each 8×12-cm Hybond-N+ membrane filter. Colonies were grown at 37°C for 16 h before the filters were processed as described by Zhang et al. (1996) and baked at 80°C for 2 h. Colony filters were processed and hybridized using published protocols (Chang et al. 2001; Tao et al. 2001, 2002).

RFLP probes (Table 1) prefixed with CP and GP were potato probes provided by Dr. C. Gebhardt (Max-Planck-Institut für Züchtungsforschung, Köln, Germany), while probes prefixed with TG were tomato probes obtained from Dr. S. Tanksley (Department of Plant Breeding and Biometry, Cornell University, NY, Ithaca, USA). Four markers, TG20, CP56, CP51 and GP127, on chromosome 7 were used to isolate BAC clones linked with the new late blight resistance gene *Rpi1* (Kuhl et al. 2001). The gene *Rpi1* is flanked by the marker TG20 and CP56 (Kuhl et al. 2001). Marker GP127 was mapped in the same position as TG20, while marker CP51 was distal to CP56 and located in the interval between markers TG20 and CP56 in the potato/tomato map of Gebhardt et al. (1991). Another RFLP marker GP174 was located at the distal end of chromosome 7. Three barley chloroplast DNA probes, *ndhA, rbcL* and *psbA*, were also used to screen the two BAC libraries to detect chloroplast DNA inserts in the BAC libraries. Markers TG302, TG624 and TG181 on chromosome 8 are tightly linked to the PPO gene that is involved in the Colorado potato beetle resistance in the wild potato species *Solanum berthaultii* Hawkes (Tanksley et al. 1992; Bonierbale et al. 1994). The two markers, TG24 and CP108, are located on chromosome 1 and linked to the gene *Sr1*, which codes for one allele of the *S*-locus for self-incompatability in potato (Kaufmann et al. 1991). This gene was mapped without recombination with locus CP108 in Gebhardt et al. (1991), mapped to the F1840 population. This locus is located in a proximal position with respect to TG24 and was separated by 11.6 cM (Gebhardt et al. 1991).

Results

BAC library construction and characterization

To create a library that is truly representative of the complete genome, HMW potato DNA embedded in LMP agarose plugs was partially digested with the enzyme *Bam*HI or *Eco*RI. The optimal partial digestion conditions produced most DNA fragments in the range of 100–500 kb. The partially digested DNA was size-selected twice. The DNA fragments from the second size selection was isolated from the gel and ligated to the pECBAC1 vector. The ligation from the *Bam*HI partially digested DNA was transformed into *E. coli*, and produced about 500 white clones per µl of the ligation mixture. Analysis indicated that about 97% of the clones contained inserts. A total of 40,328 clones (105×384 well microtiter plates) were produced from this ligation. The estimation of the mean insert size for each ligation was based on pulsed-field gel analysis of 30 randomly chosen clones digested with the *Not*I restriction enzyme. Figure 1A shows the *Not*I restriction pattern of randomly picked BAC clones derived from the library on a CHEF gel. The common band in all the lanes (7.5 kb) is from the BAC vector and the remaining bands are potato DNA fragments. The insert sizes of these clones ranged from 90 to 160 kb with an average insert size of 125 kb.

The second BAC library was constructed from the *Eco*RI partially digested potato DNA fragments. After the second size selection, the compressed DNA bands were excised from the CHEF gel, ligated into the BAC vector and transformed into *E. coli*. Approximately 300 white clones from 1 microlitre of ligated DNA were obtained from a single electroporation. This ligation yielded a total of about 17,280 BAC clones (45×384 well microtiter plates). BAC DNA was isolated from randomly picked clones, digested with *Not*I and analyzed on a CHEF gel for insert size. Approximately 96% of the clones were recombinant with an average insert size of 135 kb, ranging from 95 to160 kb (Fig. 1B).

The insert size data for the 30 *Bam*HI BACs and the 30 *Eco*RI BACs are summarized in Fig. 3. The data shows that 86.7% of the *Bam*HI BACs (white columns) contained inserts larger than 100 kb; and 70% of *Eco*RI BACs (black columns) contain inserts that were greater than 110 kb, indicating that the libraries are suitable for potato genomic research. The average insert size was determined by the analysis of the randomly picked clones and gave an

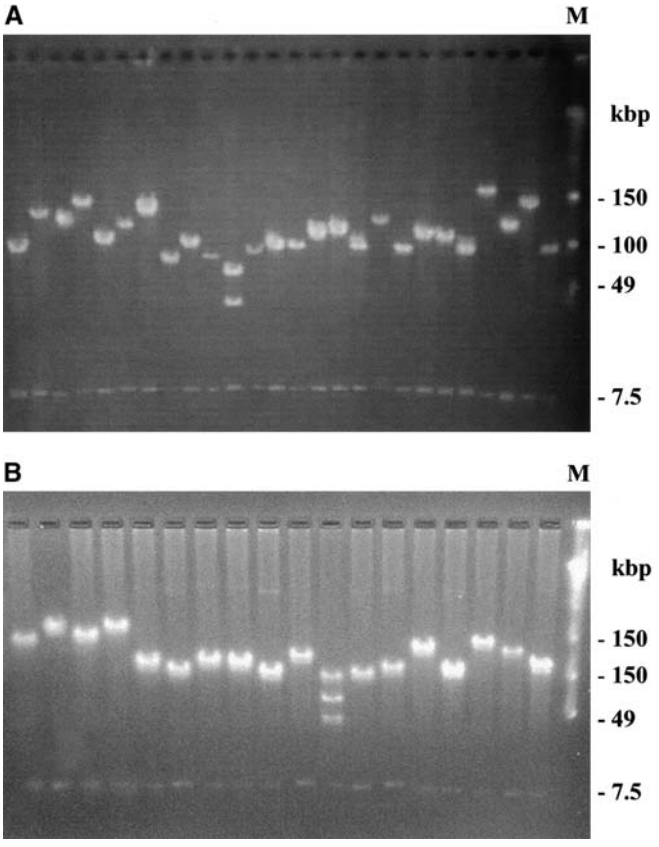

Fig. 1A, B Analysis of the insert size of random BAC clones from the two wild potato BAC libraries by pulsed-field gel electrophoresis (PFGE). The 7.5-kb common band is from the cloning vector pECBAC1. Molecular weight size markers (M) are lambda ladders (Sigma Chemical, St. Louis, Missouri). Insert sizes are given in kb. **A**: the *Bam*HI BAC library. **B**: the *Eco*R1 BAC library

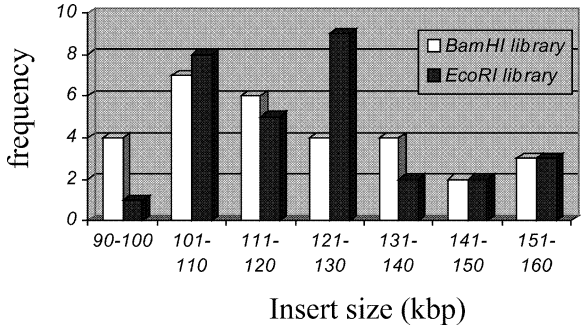

Fig. 2 Insert size distributions of the potato *Bam*HI (*white columns*) and *Eco*RI BAC (*black columns*) libraries. DNA sample of 60 clones randomly picked from the two BAC libraries were analyzed and grouped

average insert size of 130 kb for the two libraries (Fig. 2). A total of 57,408 BAC clones were collected for these two libraries, which represents approximately six equivalents of the wild potato haploid genome.

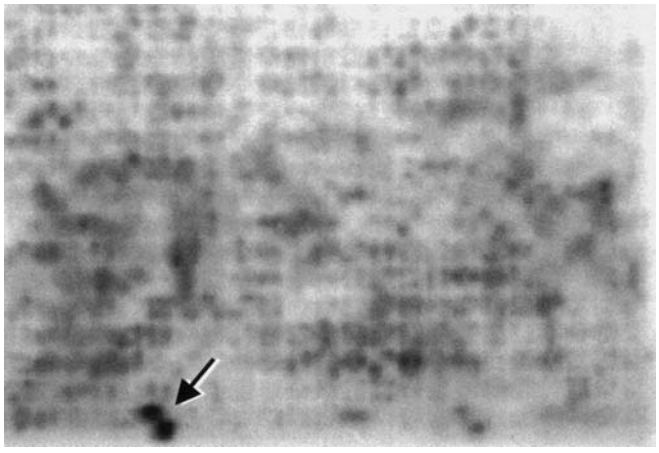

Fig. 3 Hybridization image of a high-density colony filter of the potato *Bam*HI BAC library using RFLP marker TG20, that co-segregated with late blight resistance gene *Rpi1*, as a probe. The *arrow* shows the positive clone. Each set of two strong points represent two copies of one BAC clone. The filter contains 1,534 double-spotted clones

Screening the library for chloroplast sequences

To investigate the occurrence frequency of BAC clones containing chloroplast DNA sequences in the libraries, three barley chloroplast DNA probes were used to screen a portion of the two libraries composed of 2×1,536 individual BAC clones (two 12×8-cm filters). With a probe mixture of the three chloroplast genes, ten BACs (0.65%) and 14 BACs (0.91%) out of 1,536 clones were detected in the *Bam*HI and *Eco*RI libraries, respectively, indicating that a very low percentage (<1%) of the clones were derived from the chloroplast DNA in the libraries (Table 1).

BAC library screening with RFLP markers

To validate of the utility of the libraries for positional cloning, the BAC clones of the libraries were gridded onto 37 high-density nylon membrane filters (1,536 double-spotted clones/12×8-cm filter), and probed with the RFLPs linked to several genes important to the potato.

Isolation of candidate BAC clones near a late blight resistance gene *Rpi1*

Five RFLP clones (CP51, CP56, TG20, GP127 and GP174) on chromosome 7 were used to screen the high-density filters; the first four markers are linked to the late blight resistance gene *Rpi1* on chromosome 7 of *S. pinnatisectum*. The five chromosome 7-specific RFLP markers identified 19 positive BAC clones from the two libraries. For each of these five RFLPs, at least two positive BAC clones were identified. The RFLP probe GP127 identified six corresponding BACs from the two

libraries (Table 1). Overall, an average of 3.8 BAC clones per marker was identified. Markers TG20 and CP56 co-segregated with the *Rpil* gene, which are located approximately 5.2-cM proximal to TG20 in the central region of chromosome 7 (Kuhl et al. 2001). When the whole *Bam*HI and *Eco*RI libraries were probed with these two markers, two and four positive BACs were identified for TG20 and CP56, respectively (Table 1). Figure 3 shows the hybridization image for a filter of the *Bam*HI library using the marker TG20 as a probe. Marker GP174, which is located in the distal region of chromosome 7, identified three BAC clones from the *Bam*HI libraries, and two BAC clones from the *Eco*RI library.

Isolation of the PPO gene candidate BAC clones

The high-density colony filters from the two wild potato BAC libraries were also screened with the three RFLP markers, TG302, TG624 and TG181, that are closely linked with the PPO locus (Table 1). When the two libraries were probed with the three markers, 4, 5 and 6 positive BACs were identified for TG302, TG624 and TG181, respectively. It was found that a single BAC clone carried the three markers, TG302, TG181 and CP51 (Table 1).

Screening the library with markers for the gene *Sr1*

The self-incompatibility gene *Sr1* was mapped without recombination with RFLP marker CP108, and is located 12.4 cM from TG24 on chromosome 1 in an F2 population consisting of 99 individuals (Gebhardt et al. 1991). The two probes CP108 and TG24 detected two and three BAC clones, containing the *Sr1* locus from the *Bam*HI library and the *Eco*RI library, respectively (Table 1).

Altogether, the ten RFLP markers detected 39 positive BAC clones from the two libraries. At least two BAC clones were identified for each probe. The hybridization data demonstrated the usefulness of our libraries for facilitating the isolation of the genes for disease and insect resistance, as well as other economically important genes in the species. BAC end-cloning (Chen and Gmitter 1999) was carried out on these BAC clones. BAC end clones will be used as probes to repeat the screening of the positive BAC clones and determine their order in the contig for a specific trait. However, it should be noted that for the *Bam*HI library, no positive BAC clones were found for two of the markers, while one probe failed to detect positive BAC clones in the *Eco*RI library (Table 1).

Discussion

We have been attempting to use a map-based cloning strategy to isolate the genes involved in disease and insect resistance from the Mexican wild diploid species *S. pinnatisectum*, especially those genes that confer resistance to late blight and the CPB. Late blight and the CPB are currently regarded as the two most destructive potato pests in the world (Niederhauser 1999; Guenthner et al. 2001). The construction of BAC libraries is an important component of this effort. As a first step toward map-based cloning of these resistance genes, we constructed two BAC libraries from the multi-disease and insect resistant Mexican wild species *S. pinnatisectum* (Chen et al. 2003). To achieve complete representation of the potato genome, two different restriction enzymes, *Bam*HI and *Eco*RI, were used in the construction of the libraries. The choice of the enzymes was based on their complementarity nucleotide sequences at their restriction sites, those of *Bam*HI being rich in G/C whereas those of *Eco*RI being rich in A/T. Therefore, these two BAC libraries are complementary in their coverage of the potato genome. The screening of the potato BAC libraries with ten RFLP markers revealed the presence of positive clones for every marker used in the two libraries. However, not all sequences tested were represented in each library. Two and one out of the ten markers were not detected in any of the BAC clones in the *Bam*HI library and the *Eco*RI library, respectively. This indicates that use of a variety of restriction enzymes in the construction of different sets of libraries is necessary to achieve a complete representation of the whole potato genome (Chang et al. 2001; Tao et al. 2001, 2002; Sun et al. 2003). Although BAC libraries have quickly become the most popular cloning systems and are widely used in genomic research for physical mapping, genome sequencing and positional cloning, the construction of a large-insert genomic BAC library is still a time-consuming endeavor. Good preparations of the vector and size-selected HMW DNA are important steps for constructing a high quality BAC library. The quality of the size-selected HMW DNA inserts is likely crucial to the success of the BAC library construction, and the DNA must be maintained in a condition that prevents shearing and degradation. Improvement in the methodologies facilitating the preparation of the vector and storage of the HMW DNA could save time and effort.

The haploid DNA content of the diploid potato genome is estimated to be about 800–930 Mbp (Arumuganathan and Earle 1991). In the present study, the total of 57,608 BAC clones were generated from two-enzyme ligations and arrayed into 150 384-well microtiter plates for the two libraries. About 96% of the two libraries contained nuclear DNA inserts (Fig. 2). The contamination with chloroplast clones was less than 1% for the two libraries. After subtracting the number of the insert-empty clones and organellar DNA clones, the *Bam*HI library contains 38,328 nuclear DNA-containing clones with an average insert size of 125 kb. The *Eco*RI library contains 16,422 nuclear DNA-containing clones with an average insert size of 135 kb. So, the two *S. pinnatisetum* BAC libraries collectively represent approximately six equivalents of the potato haploid genome. This means that there is an over 99% chance of obtaining a specific BAC clone during library screening for a specific sequence. A 6-fold

coverage of the potato genome provides reasonable assurance that all regions of the genome are likely to be represented in the libraries. Indeed, screening of the libraries using ten RFLP markers linked to the disease and insect resistance, and other economic important genes as probes, isolated two to six positive BAC clones for each marker (Table 1). Among the ten RFLP markers used in the screening of the libraries, four markers, TG20, CP56, CP51 and GP127, on chromosome 7 are linked to the late blight resistance *Rpi1* gene in *S. pinnatisectum* (Kuhl et al. 2001),while three probes, TG302, TG624 and TG181, were closely linked to the PPO locus involved in CPB resistance. Together the seven probes identified 29 positive BAC clones from the two libraries with an average of 4.1 BAC clones per marker. This demonstrated that these two libraries provide a valuable resource and a tool for potato genome research, and for map-based gene cloning of candidate gene isolations.

The availability of large-insert BAC libraries will facilitate chromosome walking and BAC contig construction for resistance gene regions, and greatly improves the feasibility of an undertaking of chromosome walking for resistance gene cloning. The new late blight resistance gene *Rpi1*, identified in *S. pinnatisectum*, has been mapped on potato chromosome 7 with RFLP markers. However, the precise physical position and structure of the *Rpi1* locus on the chromosome has not yet been identified. The *Rpi1* locus should be located somewhere between TG20 and CP56, because the genetic distance of the *Rpi1* gene is approximately 5.2 cM proximal from TG20 and the flanking loci CP56 (Gebhardt et al. 1991; Tanksley et al. 1992; Kuhl et al. 2001). The marker CP51 was also mapped between TG20 and CP56 on the RFLP map of Gebhardt et al. (1991). Chromosome walking will be initiated using the insert ends of these BAC clones. The ends of the positive BACs from the two libraries have been sequenced and will be used to develop new markers. Thus, by using the libraries, we will be able to construct a BAC contig of several hundred kilobases that cover the *Rpi1* region (Chen and Zhang, unpublished data). We will identify the *Rpi1* gene and the PPO locus containing BACs, and develop a high-density AFLP map surrounding the area using a mapping population of more than 500 plants that is segregating for the *Rpi1* gene and the PPO locus. The availability of a high-density AFLP map of these regions will facilitate the isolation of the late blight and CPB resistance genes.

Although these two BAC libraries were originally constructed for the positional cloning of genes involved in late blight and CPB resistance, we envisage many other uses for them. In addition to the late blight resistance gene, TG20 is also linked to several other resistance genes including *Ve*, a major gene responsible for *Verticillium* wilt resistance (Juvick et al. 1991), and *Gro1,* a major dominant gene conferring resistance to the potato cyst nematode (Barone et al. 1990; Ballvora et al. 1995). Gene *Gro1* co-segregates with the RFLP markers CP56 and CP51 (Barone et al. 1990). In genetic mapping, *Rpi1*, *Ve* and *Gro1* genes, are closely linked in a cluster of functional related resistance genes, which extends some 12 cM (Gebhardt et al. 1991; Tanksley et al. 1992; Kuhl et al. 2001). These resistance genes would be represented in the libraries produced in the present study according to their genome coverage. Another interesting trait in *S. pinnatisectum* is its self-incompatibility *S*-locus. The potato *S*-locus has been mapped proximally to the reference marker TG24 and co-segregates with CP108 on chromosome 1, which is similar to its chromosomal position in tomato (Tanksley and Louiza-Figueroa 1985). In the present study, the markers TG24 and CP108 detected five positive BACs from the two BAC libraries. Isolation of the *Sr1* gene, and an examination of its structure and gene products, may facilitate a better understanding of the gametophytic self-incompatibility system.

Several genes, such as *Rpi1*, *Ve*, *Gro1*, PPO and *Sr1,* have been mapped on chromosomes 7, 8 and 1, with the RFLP markers used in the present study. Therefore, these BAC landmarks can be used to initiate chromosome walks toward these genes. As the DNA can easily be isolated from BAC clones, all clones picked by colony hybridization will be examined by DNA fingerprinting with *Bam*HI and *Eco*R1 digestion. Fingerprinting of these BACs will initiate chromosome walking to build overlapping clones spanning these genes. In each case of library screening of the BAC insert ends, several hits with each were observed. Using the libraries, small contigs will be generated for the BAC clones, identified by RFLP markers linked with disease and insect resistance, and extended by chromosome walking. In order to facilitate investigation of the physical structure of the resistance gene cluster and to establish the order and spacing of the resistance genes in these regions, we will reconstruct the genomic context of these regions of the genes through the contig assembly of the resistance gene- and *S*-locus gene-hybridizing BAC clones on chromosomes 7, 8 and 1 from the wild potato libraries. This work will facilitate the saturation of a genomic target region with DNA markers that are physically linked to a specific gene (Vos et al. 1995; Zhang and Wing 1997). Since resistance genes tend to be clustered, a BAC contig that spans one resistance gene may also span others (Martin et al. 1993). In the cases of the *Rpi1,Ve* and *Gro1* resistance loci of potato, the genomic region harboring these loci have been enriched with AFLP markers, in addition to those already available RFLP markers (Ballvora et al. 1995).

The BAC clones identified in this study will also make it possible to use fluorescence in situ-hybridization (FISH) techniques to order the markers, and BAC clones on the chromosomes and to estimate the physical distances between them. FISH has been a powerful physical mapping tool for many animal and plant species. However, DNA probes as small as a few hundred base pairs, such as a majority of the RFLP probes used in genetic linkage mapping, cannot be consistently detected on plant chromosome preparations. The physical mapping by FISH using large genomic DNA BAC clones provides an alternative approach to map small DNA probes (Jiang

et al. 1995; Dong et al. 2000). The strategy is to screen a BAC library using a small DNA probe, such as an RFLP probe to isolate a large insert BAC clone, and then map the larger insert BAC clone on chromosomes using FISH. Using this technique, a physical map of potato involving late light and CPB resistance genes, as well as the *Sr1* gene, can be constructed using RFLP marker-targeted BAC clones as probes. Then, this physical mapping data could be correlated to the genetic linkage mapping data of potato (Gebhardt et al. 1991, 1994; Tanksley et al. 1992).

The construction of the BAC libraries that cover 6-fold of the wild potato haploid genome from *S. pinnatisectum* makes it possible to isolate the economically important genes, and provide sequence information for these disease-resistance-gene-rich regions and the self-incompatibility *S*-locus. Because of the nature of the DNA source used in the library construction, we expect that it should be of particular interest to potato researchers and those conducting research on disease and insect resistance genes and the *S*-locus. Together with the diploid potato *S. bulbocastanum* BAC library previously constructed (Song et al. 2000), the 6-fold wild potato libraries will be valuable long-term tools for potato genomic studies.

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