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Abundance and characterization of simple-sequence repeats (SSRs) isolated from a size-fractionated genomic library of *Brassica napus* L. (rapeseed)

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Abstract A size-fractionated library of *Brassica napus* L. (rapeseed), composed of 15000 clones, was screened for the presence of GA-, CA-, and GATA-simple-sequence repeats (SSRs). GA-SSRs were four- and five-fold more abundant than CA- and GATA-SSRs, respectively, and present at a frequency of approximately one SSR for every 100 kb of DNA. Following the sequencing of 124 positive clones, primer pairs were designed and evaluated for seven selected SSRs. Products were amplified in an array of individuals of *B. napus*, *B. oleracea* and *B. rapa*, demonstrating that the seven SSRs were conserved among species. Two SSRs were polymorphic. Among 11 accessions, the dinucleotide (GA)-repeat, B.n.9A, yielded 12 fragments, while the tetranucleotide-repeat (GATA), B.n.6A2, revealed two fragments. Automated, fluorescence-based detection of polyacrylamide gels has been employed to simultaneously increase throughput, reduce unit cost, improve analytical resolution, and expedite data acquisition of SSR analysis. Though initial financial investment and technical capabilities may prevent some from directly employing our documented approach, SSR analysis warrants further investigation as a tool in genetic studies for enhancing both the conservation and utilization of genetic resources.

Key words Genetic analysis · Fluorescence-based detection · STR · Microsatellite DNA · Multiplex PCR

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Introduction

Effective conservation of plant genetic resources requires a comprehensive understanding of genetics. This understanding attempts to integrate organizational levels (molecules, chromosomes, cells, individuals, populations, species, and ecosystems) as well as to firmly establish the relationship of genotype to phenotype. With increasing demand by scientists for ready access to useful and representative genetic resources, the present is a challenging yet opportune time to enhance our knowledge of the genetics and genome organization of crop species for improving conservation planning and practice. In particular, curators of plant genetic resources require a more accurate measurement of the following genetic parameters: (1) identity: the determination that an accession in the genebank is catalogued correctly and true to type; (2) relationship: the degree of relatedness among genotypes in an accession or among accessions in a collection; (3) structure: the amount of genetic variation present, and how it is partitioned among individuals, accessions, and collections; and (4) location: the presence of a desired gene/gene complex in a specific accession and of a the mapped site of a desired DNA sequence on a particular chromosome in an individual (Kresovich et al. 1992).

The development and application of molecular genetic markers provide the opportunity to reveal DNA sequence polymorphisms useful to discriminate genetic variation among individuals and within populations. As with applications in plant breeding (Ragot and Hoisington 1993), not all molecular markers are suitable for use in all applications of plant genetic resources conservation. Both restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNA polymorphisms (RAPDs) have been used extensively to characterize plant genetic resources (Tanksley et al. 1989; Rafalski et al. 1991). Though useful in selected applications, both RFLPs and RAPDs exhibit limitations and can be misused, misrepresented, or misinterpreted. For example, in certain situations it may be difficult to infer molecular genotype from

phenotype, to detect the state (heterozygotic or homozygotic) at a locus, or to identify allelic relationships. Also, the feasibility of employing either technique to resolve questions that require a large number of samples (>1 000) to be profiled is questionable based on technical (discriminatory ability, sensitivity, reproducibility, and need for further genetic analysis and diagnostics development) as well as operational (protocol characteristics, time, and cost) considerations.

To examine the use of molecular genetic markers for improved conservation of plant genetic resources, we have focused our research efforts on marker development, integration of advanced technology, and improved analysis of generated data. This effort has been founded on: (1) the application of polymerase chain reaction (PCR)-based DNA amplification, (2) the utilization of repetitive sequence DNA as markers, and (3) fluorescence-based automated DNA detection (Ziegle et al. 1992). Among the classes of repetitive DNA sequences that have proven amenable for PCR amplification is the simple-sequence repeat (SSR) (Weber and May 1989), a variety of di-, tri-, tetra-, and penta-nucleotide tandem repeats that are dispersed throughout the genome. Based on studies in humans, the advantages of an SSR locus include: (1) its great abundance and distribution, (2) its ability to be "tagged" in the genome, (3) its high level of polymorphism (Fregeau and Fourney 1993; Kimpton et al. 1993), (4) its clearly defined genetic definition (Queller et al. 1993), and (5) its ease of detection via automated systems (Morgante and Olivieri 1993; Rafalski and Tingey 1993). However, technical drawbacks that currently restrict the use of this type of marker on a large scale are also apparent. Cregan (1992) highlighted technical concerns about the development and application of SSR markers, including the costly and time-consuming nature of the identification of polymorphic loci and the possible detection problems incurred when alleles differ only slightly in length.

At present, information on SSR markers in plant species is limited (Condit and Hubbell 1991; Akkaya et al. 1992; Lagercrantz et al. 1993; Senior and Heun 1993; Thomas and Scott 1993; Thomas et al. 1993; Saghai-Maroof et al. 1994; Wang et al. 1994; Rongwen et al. 1995). Moreover, primary applications of SSR markers have focused on the use of these landmarks for genome mapping. Like other investigators, we have been concerned about the potentially great investment needed for the generation of 100s–1 000s of SSR markers needed for mapping. However, the utilization of SSR markers to resolve curatorial needs may be far less ambitious in both scope and size, e.g., a goal of 20–30 highly polymorphic loci (with polymorphism information content values of 0.5) per crop species. The objective of this initial phase of research was (1) to establish measures of abundance, and (2) to characterize selected di- and tetra-nucleotide SSRs, isolated from a size-fractionated genomic library of *Brassica napus* L., for ultimate use in resolving genetic identity, relatedness, and structure.

Materials and methods

Construction of small insert genomic libraries

Genomic DNA was isolated from leaf tissue of *B. napus* L. 'Jet Neuf' using a modified CTAB technique with subsequent purification employing a cesium chloride gradient (Sambrook et al. 1989). Following isolation, DNA was digested with *Taq*I and electrophoresed on a low-melting-temperature agarose gel (Nusieve GTG, FMC). The 100–500-bp fragments were excised and isolated from the gel via agarase digestion (Dumais et al. 1987). Selected fragments were ligated to *Cla*I-digested pGEM-7Zf(+) (Promega) that had been dephosphorylated with calf intestinal alkaline phosphatase (Sambrook et al. 1989). Recombinant clones were transformed in *E. coli* JM109 cells (Promega) using a standard protocol provided by the supplier.

Screening of libraries for clones containing selected di- and tetra-nucleotide repeats

Colonies were transferred onto Duralose UV filters (Stratagene) following protocols provided by the supplier. Two dimeric repeats, $(GA)_{10}$ and $(CA)_{10}$, and a single tetrameric repeat, $(GATA)_6$, oligonucleotide (supplied by Applied Biosystems, Inc.) were labeled with ^{32}P by a 5′ Terminus Labeling System (Gibco, BRL). The selection of a core repeat sequence was based on surveys of databases for the abundance and informativeness of loci in human and plant species, particularly *B. napus*. The oligonucleotides were used as probes to screen 15 000 clones each from the *B. napus* genomic library, according to the protocol supplied by Stratagene. Hybridizations and subsequent washes were conducted at 45 °C under high stringency. Each filter was screened twice and only positives colonies detected in both screens were sequenced.

Sequencing of positive clones

Plasmid DNA of GA-, CA-, and GATA-positive clones was isolated using a modified phenol-chloroform protocol supplied by Applied Biosystems, Inc. Clones were sequenced from M13 sequencing primer sites of the pGEM 7Zf(+) using either the *Taq* DyeDeoxy Terminator Cycle Sequencing Kit or the Prism Sequenase Terminator Double-Stranded DNA Sequencing Kit on an upgraded ABI model 370 DNA Sequencing System (Applied Biosystems, Inc.).

Primer design

Primers complementary to the flanking regions of the repeats (Table 1) were designed using Designer PCR (Research Genetics). Two key criteria in primer pair formulation included a primer T_m of 65–66 °C and a primer pair T_m variance of 1.0 °C. These stringent criteria were employed to preclude problems with spurious banding patterns generated during amplification. Because one of our operational goals is to perform multiplex PCRs with SSRs, a uniform annealing temperature across all SSRs was essential. Also, primer pairs were generated to produce amplified DNA fragments between approximately 96 and 347 bp in length. Primers were synthesized by Operon Technologies, Inc. or on an ABI model 392 nucleic-acid synthesizer and were purified by either HPLC or by OPC purification cartridges (Applied Biosystems, Inc.), respectively.

PCR profiling

A test array of 11 *Brassica* accessions was selected to represent a range of diversity within the U.S. National Plant Germplasm System *Brassica* collections. Three species, *B. napus*, *B. oleracea*, and *B. rapa*, were represented in the array (Table 2). Ten plants of each accession were grown in the greenhouse for DNA isolation. Tissue

Table 1 Sequences of *Brassica* SSR primer pairs

Locus	5′ Flanking sequence	Repeat sequence	3′ Flanking sequence
B.n.6A2	5′CTTTGTGTGGACTTTTAGAACTTTA	$(GATT)_4$	5′CGCAGCTTTTGGCCCACCTG
B.n.6A3	5′GCTACCCACTCATGTCCTCTG	$(CATAT)_6$	5′CCAAGCTTATCGAATCTCAGGTA
B.n.9A	5′GAGCCATCCCTAGCAAACAAG	$(GA)_{27}$	5′CGTGGAAGCAAGTGAGATGAT
B.n.16A	5′CGACCGTGGAAGCAAGTGAG	$(CT)_{28}$	5′CCATGATTACGCCAAGCTATTTA
B.n.18A1	5′TCAATCCCACCAACCAGACAAA	$(CT)_{15}$	5′TAAGACAGGTAAGGTTTGGCCC
B.n.20A	5′GACAATCAATCCCACCAACCAG	$(GA)_{28}$	5′TAAAAGAAGAGTGCCAATCCCAT
B.n.25A	5′CACGTGGTATGTTGGTATTGGG	$(CT)_{10}$	5′TGATTCTCCTCCGACGCATGC

Table 2 *Brassica* spp. test array

Accession	Taxon	Source
Jet Neuf	*napus* var. *olifera*	Belgium
Westar	*napus* var. *oleifera*	Canada
Laurentian	*napus* var. *radicola*	Canada
Wisconsin Golden Acre	*oleracea* var. *capitata*	USA
Jersey Wakefield	*oleracea* var. *capitata*	USA
Couve Nabica	*oleracea* var. *costata*	Portugal
Westland Winter	*oleracea* var. *selenesia*	Netherlands
Span	*rapa* var. *oleifera*	Canada
Tatsoi	*rapa* var. *narinosa*	Japan
Michihili	*rapa* var. *pekinensis*	USA
Purple Top White Globe	*rapa* var. *rapifera*	USA

from each plant was subsequently harvested and freeze-dried. DNA from an individual plant of each accession was extracted by a modified CTAB extraction according to Colosi and Schaal (1993). PCRs were carried out in a 20-µl solution containing 30 ng of DNA template, 1.0 µM of each primer, 0.25 mM of dNTPs, 3.125 mM $MgCl_2$, 1×Perkin Elmer Cetus reaction buffer, and 1.0 unit of Perkin Elmer Cetus Ampli*Taq* DNA polymerase. After one denaturing step of 2 min at 94 °C, a "touchdown" amplification profile was utilized (Mellersh and Sampson 1993). This profile included a denaturing step of 60 s at 94 °C and an extension step of 45 s at 72 °C. The initial annealing step was 30 s at 68 °C for two cycles and subsequently was dropped by 1 °C every two cycles until a final temperature of 58 °C was reached. The annealing temperature of 58 °C was employed for the last 20 cycles of the amplification. Loading dye was added to the reaction mixtures and the amplified products were electrophoresed at 50 V for 18 h on a 4% FMC NuSieve 3:1 agarose gel with 1×TBE buffer. Following completion of the run, the gel was stained with ethidium bromide (0.10 µg/ml), visualized with UV light, and photographed. Subsequent generation and detection of fluorescently labeled amplification products was accomplished following the protocols of Ziegle et al. (1992).

Results

Sequencing of positive clones

Sequence data were obtained for a total of 124 size-fractionated clones. This total represented 51 GA-, 42 CA-, and 31 GATA-positive inserts. Our subsequent description of the repeat units among the positive clones follows the initial definitions as proposed by Weber and May (1989)

Table 3 Characterization of positive GA, CA, and GATA clones

Clone	GA	CA	GATA
Number	51	42	31
Repeat type			
Perfect	18	3	3
Imperfect	7	2	5
Compound	5	5	0
None/Other	21	32	23

and is highlighted in Table 3. We were surprised by the low number of CA-clones with perfect repeats of any length. The GA-, CA-, and GATA-positive clones with uninterrupted motifs averaged 12 (ranging from 3 to 28), six (ranging from 3 to 11), and four (ranging from 3 to 5) repeats, respectively.

Primer design

Primer pairs were designed for seven sequences that included five long (core repeat number of 10–28), perfect dinucleotide repeats and two more complex, tetra- and pentanucleotide repeats (see Table 1). As expected, amplifications utilizing 'Jet Neuf' genomic DNA generated products in the range of 96–347 bp for the seven SSRs.

PCR Profiling

A summary of the PCR profiling of the selected SSRs is presented in Table 4. With the exception of SSR B.n.9A, primer pairs, produced an amplified product(s) with every genomic DNA from the test array. However, three of the four *B. oleracea* accessions ('Wisconsin Golden Acre', 'Jersey Wakefield' and 'Westland Winter') failed to produce a product at this locus. Amplification of products in all species provided evidence that these seven SSRs were conserved among the three species of *Brassica*. Though resolution was limited by ethidium-bromide staining of agarose, two SSRs were observed to be polymorphic. The other SSRs appeared monomorphic. However, because only a limited number of individuals were typed, further

Table 4 Characteristics of *Brassica* SSR primer-pair assays

Locus	Expected product size in "Jet Neuf" (bp)	Range of product sizes (bp)	Conservation of locus among *Brassica* spp. (yes/no)	Polymorphic in test array (yes/no)
B.n.6A2	96	92–96	Yes	Yes (2 fragments)
B.n.6A3	347	–	Yes	No
B.n.9A	184	180–260	Yes	Yes (12 fragments)
B.n.16A	267	–	Yes	No
B.n.18A1	161	–	Yes	No
B.n.20A	215	–	Yes	No
B.n.25A	123	–	Yes	No

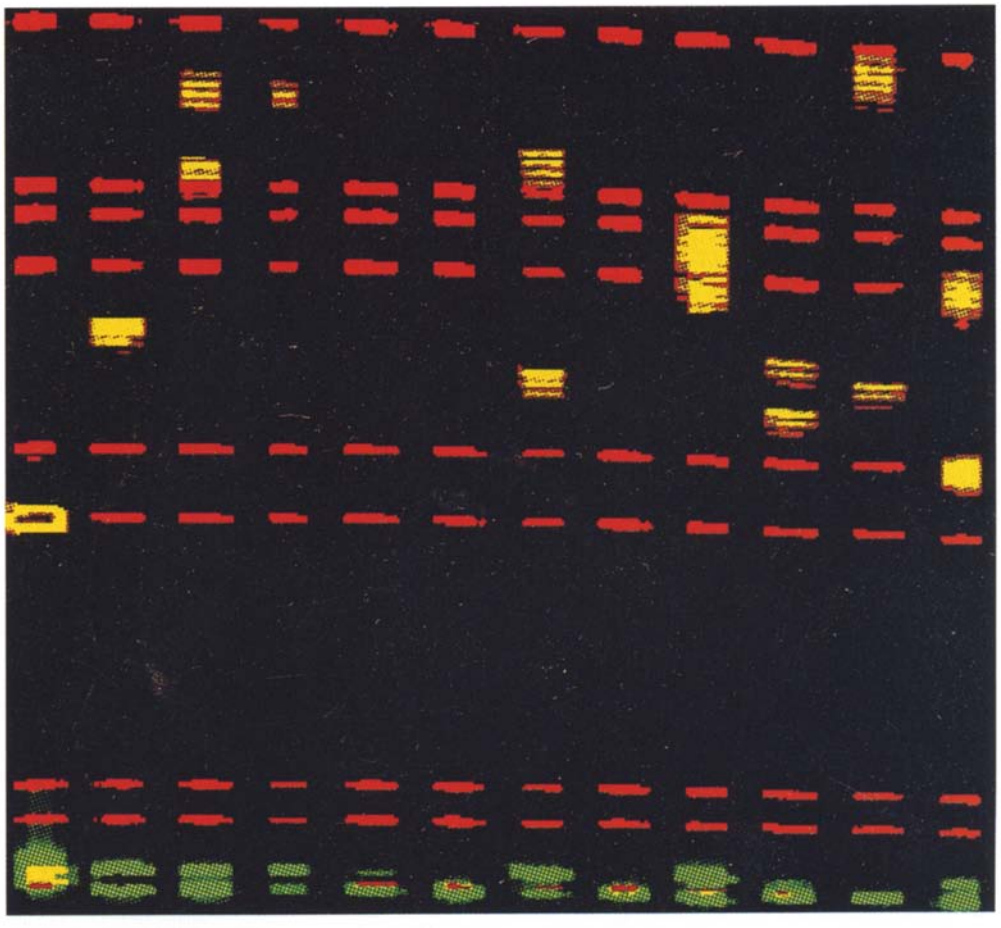

Fig. 1 Multiplex PCR of B.n.6A2 (*green* fluorescent fragments) and 9A (*yellow* fluorescent fragments). *Lane 1* is clone DNA. *Lanes 2–4*, *5–8*, and *9–12* include accessions of *B. napus*, *B. oleracea*, and *B. rapa*, respectively. *Red fragments* are internal lane size-standards

testing of these SSRs for variants with more accessions would be justified before discarding them as uninformative. Another option would be to re-design primer pairs for these uninformative SSRs. Subsequent characterization of variant SSRs B.n.6A2 and B.n.9A employing fluorescent-based detection proved highly informative (Fig. 1, accompanying electropherogram not shown). With the tetranucleotide SSR B.n.6A2, seven plants (among a total of 11) were putatively heterozygotic. Similarly, among plants which produced products (a total of eight) with the dinucleotide SSR B.n.9A, six were putatively heterozygotic. Within the 11-plant array, two fragments (92 and 96 bp) were observed at B.n.6A2, while 12 fragments (ranging in size from approximately 180 to 260 bp) were observed at B.n.9A.

Discussion

The attributes of SSRs in *B. napus* L. and related *Brassica* species may ultimately establish these types of molecular genetic markers as valuable tools for use in the character-

ization of plant genetic resources. SSR markers are abundant and relatively easy to isolate and characterize.

On the basis of the number of clones exhibiting some type of SSR per amount of genome covered by the 15 000 clones (assuming an average insert size of 300 bp), a GA-, CA-, and GATA-SSR can be estimated to be present every 100 kb, 440 kb, and 560 kb of DNA, respectively. These values for GA- and CA-SSRs correspond well with the estimates of 125 kb and 350 kb, respectively, of Lagercrantz et al. (1993). Therefore, our data corroborate the hypothesis of Lagercrantz et al. (1993) and others (Morgante and Olivieri 1993; Wang et al. 1994) that in absolute values, dinucleotide SSRs may be approximately three- to fivefold less abundant in plants than in vertebrates (estimated to be about one dinucleotide repeat every 6 kb in the human genome). However, it must be reiterated that data in plant species are still scant in terms of the number of species evaluated as well as the amount of genome surveyed or the technique employed for library construction. In the present case, we examined approximately 4 500 kb, amounting to about 0.5% of the haploid genome of *B. napus*.

The abundance of the GATA-SSRs in *B. napus* was significantly higher than one might expect based on previous database surveys. Our survey suggested that this tetranucleotide repeat was approximately equal in abundance to the CA-SSRs. Perhaps previous estimates of the abundance of this tetranucleotide repeat have been biased by database surveys (EMBL and GenBank) limited to only DNA sequences associated with coding regions of the genome (Lagercrantz et al. 1993; Morgante and Olivieri 1993; Wang et al. 1994) or else our results were biased by the amount of genome examined or the library construction techniques. Because a tetranucleotide core repeat permits easier allelic size discrimination than a dinucleotide repeat, a more extensive effort to isolate and characterize more GATA-SSRs may be warranted. Though employed in a different manner, the classical hybridization-based DNA profiling strategy of Poulsen et al. (1993) in plants in general, and in *Brassica* spp. in particular, also adds value to the ready access and use of this specific tetranucleotide repeat.

Like the findings of Lagercrantz et al. (1993), we noted that each of the seven designed primer pairs amplified a product (or products) in each of the three *Brassica* species typed. It has been hypothesized (U 1935) that *B. oleracea* and *B. rapa* are the progenitors of the amphidiploid, *B. napus*. If so, it may not be unexpected that the SSRs evaluated were conserved among these closely related species. However, we were surprised by the results at B.n.9A where three of the four *B. oleracea* accessions exhibited no product. These results may reflect alterations (as a result of an insertion, deletion or single-basepair change) in the nucleotide sequence of the flanking regions of this site. This empirical evidence supports the hypothesis that there exists a possibly greater divergence between *B. oleracea* and the other two *Brassica* species. However, further investigation with more molecular markers is needed to support this hypothesis.

From a perspective of the utility of SSR primer pairs, our results were encouraging because they provide further evidence that a high degree of conservation exists among plant species in a genus, and potentially among genera in a family. If so, primer-pair sharing among researchers working within plant families may be highly desirable and fruitful. In preliminary work, we have found selected pairs of oligonucleotide primers of *Arabidopsis thaliana* (Bell and Ecker 1994) to amplify DNA sequences in the three species of *Brassica* tested in this effort. This ability to cross-amplify may be expected based on the relatively high levels of macro- and micro-synteny noted between *B. oleracea* and *A. thaliana* (Kowalski et al. 1994). Sharing of oligonucleotide primer pairs will potentially reduce the cost of SSR marker analysis in plant species and unify maps across taxa of a family.

Among the seven SSRs assayed for polymorphism, B.n.6A2 and B.n.9A were polymorphic among the array of *Brassica* entries typed. The dinucleotide repeat B.n.9A exhibited a higher amount of polymorphism than the tetranucleotide repeat B.n.6A2. This comparative difference is consistent with findings in other organisms that dinucleotide SSRs are more polymorphic than either tri- or tetranucleotide repeats (Cooper 1992). This result may be correlated with greater slippage rates and instability associated with dinucleotide motifs (Economou et al. 1990). Therefore, in addition to their abundance, the higher levels of information obtained on a per locus basis suggests why dinucleotide loci, particularly CA-SSRs, have served as the markers of choice for human genome mapping. Though GA-SSR markers are less abundant than AT-SSRs in plants (Condit and Hubbell 1991; Lagercrantz et al. 1993; Morgante and Olivieri 1993; Wang et al. 1994), it is possible that GA-SSR markers may serve as the dinucleotide repeat candidates for use because of their relative ease of isolation and characterization when compared to AT-SSRs. However, this suggestion will require further scrutiny as information becomes available across additional plant taxa.

The remaining five SSRs were monomorphic across the *Brassica* test-array typed. This proportion (5 of 7) of invariant SSRs was surprising and discordant with other literature on plants (Akkaya et al. 1992; Lagercrantz et al. 1993; Senior and Heun 1993; Thomas and Scott 1993; Thomas et al. 1993; Rongwen et al. 1995). Moreover, *Brassica* species are primarily allogamous, insect-pollinated taxa and a certain level of polymorphism at given locus would be expected. Therefore, whether the relatively low amount of polymorphism detected can be attributed to the limited array of genotypes profiled or to some other factor(s) may only be established as more individuals and SSRs are characterized.

Our ultimate goal has been to develop higher-quality plant genetic resource collections through the application of molecular analyses of identity, relatedness, and structure. Though initial financial investment and technical capabilities may now currently prevent some from directly employing the strategy and technology we have utilized, the output from our problem-oriented research is made

available to allow users of genetic resources more ready access to a broader range of appropriately defined materials for both present and future needs.

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References

Akkaya MA, Bhagwat AA, Cregan PB (1992) Length polymorphisms of simple sequence repeat DNA in soybean. Genetics 132:1131–1139

Bell CJ, Ecker JR (1994) Assignment of thirty microsatellite loci to the linkage map of *Arabidopsis*. Genomics 19:137–144

Colosi JC, Schaal BA (1993) Tissue grinding with ball bearings and vortex mixer for DNA extraction. Nucleic Acids Res 21:1051–1052

Cooper, NC (ed) (1992) Los Alamos science. Los Alamos National Laboratory, Los Alamos, California

Condit R, Hubbell SP (1991) Abundance and sequence of 2-base repeat regions in tropical tree genomes. Genome 34:66–71

Cregan PB (1992) Simple-sequence repeat DNA length polymorphisms. Probe 2:18–22

Dumais MM, Nochumson S (1987) Small DNA fragment separation and M13 cloning directly in remelted NuSieve GTG agarose gels. BioTechniques 5:62–67

Economou EP, Bergen AW, Warren AC, Antonarakis SE (1990) The polydeoxyadenylate tract of Alu repetitive elements is polymorphic in the human genome. Proc Natl Acad Sci USA 87:2951–2954

Fregeau CJ, Fourney RM (1993) DNA typing with fluorescently tagged short tandem repeats: a sensitive and accurate approach to human identification. BioTechniques 15:100–118

Kimpton CP, Gill P, Walton A, Urquhart A, Millican ES, Adams M (1993) Automated DNA profiling employing multiplex amplification of short tandem-repeat loci. PCR Methods Appl 3:13–22

Kowalski SP, Lan TH, Feldmann KA, Paterson AH (1994) Comparative mapping of *Arabidopsis thaliana* and *Brassica oleracea* chromosomes reveals islands of conserved organization. Genetics 138:499–510

Kresovich S, Williams JGK, McFerson JR, Routman EJ, Schaal BA (1992) Characterization of genetic identities and relationships of *Brassica oleracea* L. via a random polymorphic DNA assay. Theor Appl Genet 85:190–196

Kresovich S, Lamboy WF, Szewc-McFadden AK, McFerson JR, Forsline PL (1993) Molecular diagnostics and plant genetic resources conservation. AgBiotech News Info 5:555–558

Lagercrantz U, Ellegren H, Andersson L (1993) The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. Nucleic Acids Res 21:1111–1115

Mellersh C, Sampson J (1993) Simplifying detection of microsatellite length polymorphisms. BioTechniques 15:582–584

Morgante M, Olivieri AM (1993) PCR-amplified microsatellites as markers in plant genetics. Plant Jour 3:175–182

Poulsen GB, Kahl G, Weising K (1993) Abundance and polymorphism of simple repetitive DNA sequences in *Brassica napus* L. Theor Appl Genet 85:994–1000

Queller DC, Strassmann JE, Hughes CE (1993) Microsatellites and kinship. Trends Ecol Evol 8:285–288

Rafalski JA, Tingey SV (1993) Genetic diagnostics in plant breeding: RAPDs, microsatellites and machines. Trends Genet 9:275–279

Rafalski JA, Tingey SV, Williams JGK (1991) RAPD markers – a new technology for genetic mapping and plant breeding. AgBiotech News Info 3:645–648

Ragot M, Hoisington DA (1993) Molecular markers for plant breeding: comparisons of RFLP and RAPD genotyping costs. Theor Appl Genet 86:975–984

Rongwen J, Cregan PC, Akkaya MS, Bhagwat AA, Lavi U (1995) The use of simple sequence repeat DNA markers for soybean genotype identification. Theor Appl Genet 90:43–48

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual (2nd ed.) Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Saghai-Maroof, MA, Biyashev RM, Yang GP, Zhang Q, Allard RW (1994) Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations, and population dynamics. Proc Natl Acad Sci USA 91:5466–5470

Senior ML, Heun M (1993) Mapping maize microsatellites and polymerase chain reaction confirmation of the targeted repeats using a CT primer. Genome 36:884–889

Tanksley SD, Young ND, Paterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding: new tools for an old science. BioTechniques 7:257–264

Thomas MR, Scott NS (1993) Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequence-tagged sites (STSs). Theor Appl Genet 86:985–990

Thomas MR, Matsumoto S, Cain P, Scott NS (1993) Repetitive DNA of grapevine: classes present and sequences suitable for cultivar identification. Theor Appl Genet 86:173–180

U N (1935) Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jpn J Bot 7:389–452

Wang Z, Weber JL, Zhong G, Tanksley SD (1994) Survey of plant short tandem repeats. Theor Appl Genet 88:1–6

Weber JL, May PE (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44:388–396

Ziegle JS, Su Y, Corcoran KP, Nie L, Mayrand PE, Hoff LB, McBride LJ, Kronick MN, Diehl SR (1992) Application of automated DNA sizing technology for genotyping microsatellite loci. Genomics 14:1026–1031