TECHNICAL NOTE

Intersimple sequence repeat (ISSR) polymorphisms as a genetic marker system in cotton

BAO LIU and JONATHAN F. WENDEL
Department of Botany, Bessey Hall, Iowa State University, Ames IA 50011, USA

Abstract

We studied the applicability of intersimple sequence repeat (ISSR) polymorphism in cotton. We found that: (i) the resolving power of agarose gels is poor relative to that provided by sequencing gels; (ii) fluorescent labelling of ISSR amplification primers produced numerous scorable bands; (iii) primer mixing (double priming) generated more bands than the sum of fragments resulting from two single primers, although an unexplained disappearance of several larger fragments also reproducibly occurred; (iv) ISSR fingerprinting patterns are highly heritable; and (v) double priming ISSR is an easy and informative genetic marker system in cotton for revealing both inter- and intraspecific variations.

Keywords: cotton, fluorescent-labelling, Gossypium, intersimple sequence repeat (ISSR) polymorphism, primer mixing

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Microsatellites or simple sequence repeats (SSR) are short DNA sequence stretches consisting of motifs of one to six bases that are tandemly repeated. Owing to their ubiquity, hypervariability, abundance and genome-wide distribution, SSR loci represent a new generation of powerful genetic markers for eukaryotes. Use of this marker system, however, is hampered by the requirement for sequence information from flanking regions, from which primers are designed for polymerase chain reaction (PCR) amplification. Discovery and characterization of a large number of SSRs is therefore time-consuming and expensive for many taxa.

A recently developed modification of SSR-based marker systems, i.e. ISSR (intersimple sequence repeat) analysis, circumvents this requirement for flanking sequence information, and thus has found wide applicability in a variety of plants. Popularized largely by A. Wolfe and colleagues (Wolfe et al. 1998), ISSR analysis involves PCR amplification of genomic DNA using a single primer that targets the repeat per se, with 1–3 bases that anchor the primer at the 3′ or 5′ end. In addition to freedom from the necessity of obtaining flanking genomic sequence information, ISSR analysis is technically simpler than many other marker systems. The method provides highly reproducible results and generates abundant polymorphisms in many systems.

Most applications to date have used either agarose gel electrophoresis with ethidium bromide detection (Nagaoka & Ogihara 1997; Joshi et al. 2000) or polyacrylamide gel electrophoresis (PAGE) separation combined with either silver staining or isotopic detection (Blair et al. 1999).

Cotton (Gossypium) provides the world’s most important textile fibre. Genetic diversity in cultivated cotton has been studied using several approaches (Wendel et al. 1992; Brubaker & Wendel 1994; Pillay & Myers 1999; Liu et al. 2000). A consensus reached by these studies is that genetic variation among cotton cultivars is very low. Thus, more efficient marker systems are needed for both breeding applications and basic evolutionary/ecological studies.

A recent modification of ISSR analysis utilizes fluorescein-labelling of amplification primers and automated detection on sequencing gels (Huang & Sun 2000). Here we extend these modifications and in addition demonstrate the flexibility of ISSR analysis to incorporate data on genome size and relative SSR frequency into experimental design.

To assess the efficiency of ISSR markers in revealing polymorphisms in cotton, nine accessions of Gossypium hirsutum and two accessions of G. barbadense were selected (Fig. 1c,d). These same accessions were used in earlier studies of allozyme and restriction fragment length polymorphism (RFLP) variation, permitting a comparison of techniques. To test whether ISSR markers were stably
files were captured and analysed with Rox) was added to each sample and gel images and trace and band sizing, an internal size standard (GeneScan-500 ABI Prism 373 DNA sequencer. For automated detection 3:1, FMC) and automated sequencing gels, the latter on an were electrophoretically separated by both agarose (NuSieve (2% formamide) was included. Amplification products synthesized, each based on the dinucleotide GA as a core inherited and to evaluate the effects of primer mixing, a synthetic allohexaploid (G350) between G. anomalum (G29) and G. hirsutum (G173) and its parental lines were used.

Genomic DNA was isolated from expanded leaves of individual plants using the Nucleon Plant DNA isolation & Purification Kit (Amersham). Three ISSR primers were synthesized, each based on the dinucleotide GA as a core sequence and terminated for 3' anchoring: (GA)T, (GA)C and (GA)A. The choice of the dinucleotide GA was based on its relative abundance anchoring: (GA)9C, (GA)9T, (GA)9A, (GA)9C + (GA)9T, (GA)9C + (GA)9A, and (GA)9T + (GA)9A of band differences and percentages (in parentheses). The data shown summarize results from six amplifications using different primers or primer combinations: (GA)9C, (GA)9T, (GA)9A, (GA)9C + (GA)9T, (GA)9C + (GA)9A, and (GA)9T + (GA)9A.

**Table 1** Number of polymorphic intersimple sequence repeat (ISSR) bands detected on automated sequencing (above diagonal) and agarose (below diagonal) gels. Total number of bands per accession are shown along the diagonal for automated sequencing and agarose (in brackets) gels. Intra- and interspecific polymorphisms are shown for each pairwise comparison among accessions, in terms of number of band differences and percentages (in parentheses). The data shown summarize results from six amplifications using different primers or primer combinations: (GA)9C, (GA)9T, (GA)9A, (GA)9C + (GA)9T, (GA)9C + (GA)9A, and (GA)9T + (GA)9A.

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molecular weight region (Fig. 1b,d; Table 1). Presumably this is due to the occurrence of two (GA)$_n$ loci that are flanked by different nucleotides at the 3' end. A similar phenomenon was recently observed in sweet potato (Huang & Sun 2000), suggesting that primer mixing may generally provide enhanced ISSR sensitivity.

An unexpected observation was the disappearance of several larger fragments when double primers were used (Fig. 1a,b). Neither fragment cloning and sequencing, or alterations of amplification conditions and constituent concentrations yielded insight into the cause of this phenomenon. Nonetheless, double-priming is clearly advantageous,
as ISSR patterns so produced are reproducible with a gain in fragment number that far exceeds the few bands that inexplicably vanish (Table 1).

Analysis of a synthetic allohexaploid between G. hirsutum and G. anomalum along with its parents, using all single and double primers, showed that ISSR bands were additive in the synthetic allopolyploid relative to its parents, indicating Mendelian inheritance (Fig. 1a,b).

As a preliminary assessment of the utility of ISSR analysis for detecting polymorphism in cotton, we selected nine representative accessions of G. hirsutum and two accessions of G. barbadense. These accessions were studied previously using allozyme and RFLP markers (Wendel et al. 1992; Brubaker & Wendel 1994), and collectively exhibit low levels of genetic variation. We subjected these materials to ISSR analysis using all six primer combinations described above. Few fragment differences were observed using agarose gels, and one pair of accessions was indistinguishable (TX21/TX94). Using sequencing gels, however, polymorphism levels were higher, with three times as many polymorphic bands in all pairwise comparisons than observed with agarose gels (Table 1, Fig. 1c,d). In all cases, more polymorphic bands were detected in inter- than in intraspecific comparisons (Table 1); G. hirsutum and G. barbadense were distinguished by approximately 13% of the fragments scored, whereas mean intraspecific polymorphism levels were about 6%. These observations are congruent with both established biological context and previous results based on allozyme and RFLP analysis (Wendel et al. 1992; Brubaker & Wendel 1994). The relatively high level of ISSR polymorphism observed in both cotton species indicates that relative to other existing technologies, ISSR methods represent a cost-effective and efficient means of generating molecular markers in cotton.

An especially attractive attribute of ISSR analysis is its flexibility in terms of experimental design. For example, the number of amplicons generated may be optimized by primer design (e.g. the number of anchored bases) and by judicious combining of primers. Hence, the strategy we outline here may have broad applicability across plants and animals, regardless of genome size and the abundance and distribution of SSR loci. Fluorescein-based sequencing gel ISSR analysis, particularly with double priming, appears to be an easy and informative genetic marker system for diverse applications.

Acknowledgements
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References