Short Communication

The use of ISSR markers to identify Texas bluegrass interspecific hybrids

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With 1 figure and 1 table

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Abstract

Seventeen ISSR primers were screened on Texas bluegrass (Poa arachnifera), Kentucky bluegrass (P. pratensis), Canadian bluegrass (P. compressa), Argentine bluegrass (P. ligularis), cv. ‘Sherman’ (P. secunda), putative Texas × Kentucky (TK) (P. arachnifera × P. pratensis) hybrids and hybrids involving Texas, Canadian, and Sherman bluegrass [(P. arachnifera × P. compressa) × (P. arachnifera × P. secunda)], to determine whether they could be used to produce robust and reproducible DNA fingerprints and identify interspecific hybrids. Nine of the 17 primers consistently produced robust fingerprints and nine 2-way primer combinations were also selected. DNA fingerprints were highly reproducible and the majority of the selected primers (16/18) amplified hybrid profiles using two putative TK full-sib hybrids. Combined with a rapid DNA extraction protocol, the ISSR technique enabled a fast and practical way to detect F1 interspecific hybrids early in the breeding programme and could also be useful for other applications that require DNA-based markers.

Key words: Poa arachnifera — PCR — DNA fingerprint

Texas bluegrass is a cool-season, sexual, dioecious species. Interspecific hybrids can be created by controlled movement of pollen from other bluegrass species onto female Texas bluegrass plants. The first Texas × Kentucky (TK) hybrids were created in 1908 by George W. Oliver and then again in 1934 by E. Marion Brown (Vinall and Hein 1937). ‘Reveille’ was the first TK cultivar released (Read et al. 1999). Texas × Kentucky hybrids can reproduce apomictically and have the potential to produce turf-type material with a wider geographical range of adaptation than pure Kentucky bluegrass. Hybrids are generally identified visually after they are established, based on inheritance of morphological/physiological traits from both parents. DNA markers could be a useful tool to aid in hybrid identification at the seedling stage. Marker systems differ in their cost and complexity to run, equipment required to view them, requirements for prior genome sequence data, degree of polymorphism, and reproducibility. Although random amplified polymorphic DNA (RAPD) markers (Williams et al. 1990) are based on a standard polymerase chain reaction (PCR) and do not require prior sequence data, they are not always reproducible. Amplified fragment length polymorphism (AFLP) markers (Vos et al. 1995) are highly informative and reproducible without prior sequence information, although they are more complex to generate and view. Microsatellites or simple sequence repeats (SSRs) are informative and based on a standard PCR. However, without prior sequence information they are time-consuming and costly to develop. The inter simple sequence repeat (ISSR) technique (Zietkiewicz et al. 1994) is a PCR-based method that involves amplification of a DNA segment present at an amplifiable distance between two identical microsatellite repeat regions oriented in opposite directions. ISSRs have high reproducibility, possibly because of the use of longer primers (16–25 mers), which permits the subsequent use of a high annealing temperature (45–60°C) leading to higher stringency (Reddy et al. 2002). ISSR markers can be highly polymorphic and combine many of the advantages of SSRs and AFLP with the universality of RAPDs (Reddy et al. 2002). RAPDs and AFLPs have been used for diversity and mapping studies with Texas bluegrass (Renganayaki et al. 2001, 2005). Abraham et al. (2005) developed species-specific sequence characterized amplified region markers derived from RAPD analysis of TK hybrids and backcross generations that was useful for hybrid identification.

Based on common primer selections reported for other plant species (Matos et al. 2001, Fernandez et al. 2002, Carvalho et al. 2005, Pharmawati et al. 2005), a group of 17 ISSRs from UBC9 were selected for evaluation. The University of British Columbia Biotechnology Laboratory set nine (UBC9) contains 100 ISSR primer sequences. The objective of this study was to determine whether a set of ISSR primers could be used to produce robust, reproducible DNA fingerprints useful for interspecific hybrid identification.

Materials and Methods

ISSR polymorphism was screened on Texas bluegrass (Poa arachnifera), Kentucky bluegrass (P. pratensis), Canadian bluegrass (P. compressa), Argentine bluegrass (P. ligularis), cv. ‘Sherman’ (P. secunda), putative TK (P. arachnifera × P. pratensis) hybrids and hybrids involving Texas, Canadian, and Sherman bluegrass [(P. arachnifera × P. compressa) × (P. arachnifera × P. secunda)]. A rapid DNA extraction method that does not require grinding and produces no hazardous waste, modified from Xin et al. (2003), was used. A single tender young leaf was cut into six to eight small (ca. 2 mm²) pieces and placed in a 0.2 ml thin-wall PCR tube. Fifty microlitres of 100 mM NaOH was added and the tubes were incubated at 70°C for 20 min or 40 min), thawed at room temperature, and 50 µl 100 mM HCl in 1x TE was added to each sample. Samples were pulse centrifuged and incubated at 70°C for 15 min and used immediately for PCR or frozen at –20°C for later use. Seventeen UBC9 ISSR primers (807, 808, 810, 811, 815, 817, 818, 823, 834, 835, 840, 841, 845, 848, 856, 857, 873) were initially screened for

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their ability to produce robust, reproducible, DNA fingerprints with all species. The total PCR volume was 10 l containing 1 l 10x buffer containing 1.5 mM MgCl2 from Qiagen (Valencia, CA, USA), 2 l DNA template, 0.2 mM each dNTP, 0.4 l M primer, 0.1% (v/v) BSA from New England Biolabs (Beverly, MA, USA), 100x stock used for restriction digests, 1% (w/v) polyvinylpyrrolidone (PVP-40) and 0.375 units Hot-Star Taq DNA polymerase (Qiagen). PCR was performed in a PTC100 or PTC200 thermal cycler (MJ Research, Waltham, MA, USA). The initial step of 95°C for 15 min was followed by 40 cycles of 94°C for 45 s, 52°C for 45 s, and 72°C for 2 min, and a final cycle at 72°C for 7 min. Primers selected from the initial group of 17 were tested in all two-way combinations on Texas bluegrass genotypes for their ability to produce fingerprints different than either primer alone. PCR reactions that contained two ISSR primers were set up the same way using 0.4 l M of each primer. PCR products were analysed by electrophoresis on a 1.5% (w/v) low EEO agarose gel (Fisher Scientific, Fairlawn, NJ, USA) in 1x TAE, stained with ethidium bromide, digitally photographed, and converted to a negative image. To demonstrate ISSRs ability to detect TK hybrids, two putative full-sib TK hybrids and their parents were screened with all selected primers and unique Texas and Kentucky parental bands that amplified in the hybrids were scored. PCRs were repeated two or more times starting from DNA extraction for the initial screening to select reproducible polymorphic ISSRs. PCRs were repeated twice and only reproducible medium–dark bands were counted while screening TKs and parents with selected primers.

Results and Discussion

The rapid DNA extraction method produced PCR ready DNA in about 45 min and was suitable for ISSR amplification. Nine of the 17 ISSR primers and nine 2-primer combinations consistently produced reproducible polymorphic fingerprints with a 52°C annealing temperature (Table 1, Fig. 1a). These primers produced a minimum of three bands with all species tested and in some cases greater than 10 bands depending on the genotype and primer(s) used. The nine combinations selected were based on their ability to detect polymorphisms between pure Texas genotypes (Fig. 1b) which were also useful

![Fig 1: ISSR fingerprints of Texas bluegrass (TX) (Poa arachnifera), Kentucky bluegrass (KY) (P. pratensis), Canadian bluegrass (CA) (P. compressa), Argentine bluegrass (ARG) (P. ligularis), cv. Sherman’ (P. secunda), putative Texas × Kentucky (TK) (P. arachnifera × P. pratensis) hybrids and hybrids involving Texas, Canadian, and Sherman bluegrass (TC × TS) [(P. arachnifera × P. compressa) × (P. arachnifera × P. secunda)] Poa species evaluated with UBC 857 (a), an assortment of pure TX genotypes evaluated with UBC 811 × 841 (b), a complex hybrid involving TX, CA and Sherman evaluated with UBC 807 × 841 (c), Parents and putative TK hybrids UBC 811 × 841, 811, 841, 807 × 811, 826 (d–h). L = 100 bp DNA Ladder N3231L (New England Biolabs, Beverly, MA, USA). TK3 and TK9 fingerprints were scored for Table 1]
for hybrid identification (Table 1). The ability of certain ISSR primer pairs to generate informative banding patterns different from either ISSR used alone was similar to the findings of Cekic et al. (2001). The selected ISSR primers were used for the assessment of two putative TK full-sib hybrids. Two single primers (817, 818) and one combination (808 · 857) were unable to confirm the hybrid origin of one or both genotypes. As all primers were selected using the same annealing temperature, they can all be run at the same time. Therefore, gel data can be obtained from a seedling or other tender leaf source shortly after tissue collection (ca. 5 h), the majority of which is hands-off time. It is likely that other ISSR primers not selected for this study or non-selected primers in this study, and screened under different PCR conditions could be useful. More concentrated gels or other resolving methods such as polyacrylamide gel electrophoresis or a fluorescent dye-based fragment analyzer could enable a greater number of reproducible polymorphic fragments to be detected. Future plans include determining whether the selected ISSRs can be used for determining mode of reproduction (sexual/apomictic) in elite hybrids prior to field establishment and whether any marker/trait associations can be detected. In conclusion, the set of ISSR primers described here can be a useful tool in a Texas bluegrass breeding programme.

References


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