Optimizing the use of inter-simple-sequence repeat (ISSR) markers to detect diversity in *Andropogon virginicus*

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Abstract

Andropogon virginicus (broomsedge) is an indigenous grass of eastern North America that plays a significant role during succession in old fields throughout the mid-Atlantic region. It is introduced in California, where it is naturalizing in hydric soils of the Central Valley, and in Hawaii where it ranks as a serious invasive species. About 50% of native populations are infected with a smut fungus pathogen, but this disease is absent in the introduced ranges. We seek to characterize the population genetic structure of this grass in the context of its invasion and disease ecology, so we are optimizing the use of highly variable ISSR (inter simple-sequence-repeat) molecular markers for use with *A. virginicus.* To date, no molecular markers have been developed for use in this important species. We selected a group of 20 different ISSRs to screen, based on their published use in related grasses. We experimented with different PCR (polymerase chain reaction) conditions to try and produce clear, repeatable DNA bands that exhibit polymorphism among plants that we collected from a population in Tyler State Park, PA. We experimented with different combinations of PCR annealing temperatures (45 - 50°C) and particular ISSRs, with PCR products run through 1.0 – 1.5% agarose gels. Of the 20 ISSRs tested thus far, five show repeatable polymorphic bands. We aim eventually to use an expanded set of ISSR markers to compare the level of genetic variation among infected and uninfected native populations, and between native, naturalizing, and invasive populations.

Introduction

Andropogon virginicus (broomsedge) is a warm-season, C_4, perennial grass that dominates the vegetation during early old field succession in the eastern United States, particularly on poor soils. Our investigation of plants from the eastern United States (its native range), California (where it is naturalizing), and Hawaii (where it is invasive) suggests that only native populations become infected by *Sporisorium ellisii*, a specialist smut fungus. In both the native and exotic range, high or low levels of genetic diversity within host populations may limit or allow for, respectively, the successful establishment of the pathogen.

Population genetic studies have been carried out for many plant species using inter-simple sequence repeats (ISSRs), which are highly variable and reliable dominant markers. They are useful for characterizing genetic diversity within and among natural populations. However, the conditions for using the polymerase chain reaction (PCR) with ISSRs requires optimization of annealing temperatures and magnesium concentrations for each separate ISSR primer.

Eventually, identifying and optimizing repeatable and highly polymorphic molecular markers for *A. virginicus* will facilitate large screens of populations throughout its range, leading to conclusions about the relationship between population genetic diversity, plant invasion history, and pathogen infection rates.

Methods

Initial trials on 12 ISSR primers:

- Primer + *A. virginicus* DNA in at least two PCR reactions with different annealing temperatures (45°, 47°, 50°, 53°, or 55°C) to test for viability and repeatable, scoreable bands after agarose gel electrophoresis, using DNA samples from one Pennsylvania and one Hawaii plant.
- Rejected primers that failed to amplify ISSR markers and produce a clear banding pattern.
- Chose an ideal annealing temperature for each remaining, viable ISSR primer based on the clarity, strength, and number of bands present.

Magnesium concentrations:

- Altered the magnesium concentration for primers at its ideal annealing temperature, producing a gradient of PCR reactions that included 1.5, 1.9, 2.5, and 3.1 mM $MgCl_2$ with the same DNA sample.
- Rejected primers that produced highly dissimilar banding patterns along this gradient.
- Selected ideal $MgCl_2$ concentration based on the clarity, strength, and number of bands present.

Third stage trials on additional 8 ISSR primers:

- Primer + *A. virginicus* DNA in PCR reactions with different annealing temperatures (43°, 45°, 47°, or 50°) to test for viability and repeatable, scoreable bands following agarose gel electrophoresis (1.0 – 1.5%), using various DNA samples from Tyler State Park, PA.
- Rejected primers that failed to produce clear bands or had substantial DNA bands in water control lanes.

Results

Table 1. ISSR primers screened sequentially for optimal annealing temperature and magnesium concentration during PCR. Bolded temperatures and concentrations were considered ideal; other values were rejected. Shaded regions indicate that no action was taken for a primer at a given stage. DNA templates included samples from Hawaii and Pennsylvania populations.

Primer Name	Nucleotide Sequence 5'→3'	Annealing Temp. Trials (°C)	Reject? (X)	$[Mg^{2+}]$ Gradient (mM)	Reject? (X)	Polymorphism & Repeatability Test
ISSR1	aca cac aca cac aca ct	45 50 53 **55**		1.9 2.5 3.1 **3.1**		4 samples, each repeated once
ISSR2	gag aga gag aga gag ayc	50 **53** 55		**1.5** 1.9 2.5 3.1		4 samples, each repeated once
ISSR3	tgt gtg tgt gtg tgt gt	50 53	X	1.5		
ISSR3A	tgt gtg tgt gtg tgt gg	47 50 53	X	1.5		
ISSR4	gac aga cag aca gac a	**50** 53 55		**1.5** 1.9 2.5 3.1		4 samples, two replicates
ISSR5	gga tgg atg gat gga t	50 53	X	1.5		
ISSR6	gtg gtg gtg gtg gtg	50 53	X	1.5		
ISSR7	gtg tgt gtg tgt gtg t	47 53	X	1.5		
ISSR8	tgt ctg tct gtc tgt c	45 47 **50** 53 55		1.5 1.9 2.5 3.1	X	
ISSR9	cat cat cat cat cat	47 53	X			
ISSR10	aac aac aac aac aac aac aac aac	**45** 47 50 53 55		**1.5** 1.9 2.5 3.1		4 samples, two replicates
ISSR11	tcc tcc tcc tcc tcc	45 47 50 53 **55**		1.5 1.9 2.5 3.1	X	
ISSR 20	aga gag aga gag aga gt	45 50	X	1.5		
ISSR 21	gag aga gag aga gag ac	45 50	X	1.5		
ISSR 22	ctc tct ctc tct ctc tt	50	X	1.5		
ISSR 23	cac aca cac aca cac ag	**45** 47 50		1.5		7 samples, one replicate
ISSR 24	aga gag aga gag aga gcc	50	X	1.5		
ISSR 25	gat aga tag ata gat a	50	X	1.5		
ISSR 26	gga tgg atg gat gga t	45 47 50	X	1.5		
ISSR 27	gtg gtg gtg gtg gtg	50	X	1.5		

Andropogon virginicus population in North Carolina

Table 2. Ideal conditions for the final four primers; applied and tested for repeatability and polymorphism in third stage.

Primer Name	Annealing Temperature (°C)	Magnesium Concentration (mM)
ISSR1	55	3.1
ISSR2	53	1.5
ISSR4	50	1.5
ISSR10	45	1.5
ISSR 23	45	1.5

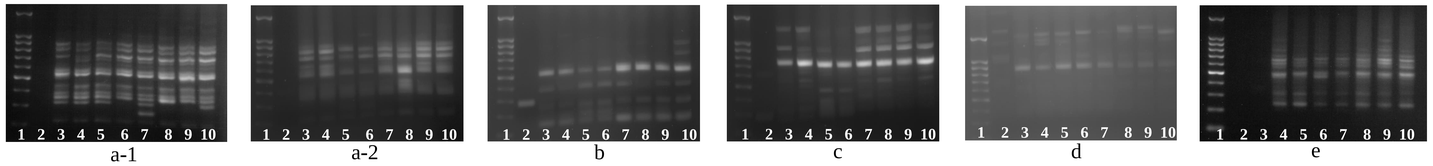

Figure 1 a-e. Agarose gel electrophoresis of ISSRs 1, 2, 4, 10, and 23. Lanes: 1=ladder; 2=neg. control; 3&4= Tyler sample 1; 5&6= Tyler sample 2; 7&8 = Tyler sample 3; 9&10= Tyler sample 4. a-1) Lanes: 1=ladder; 2=neg. control; 3-10=eight different Tyler samples. e) Lanes: 1=ladder; 2=blank; 3=neg. control; 4-10=seven different Tyler samples. b,d) Contamination of negative control lanes must be eliminated. a-2,e) Background amplification must be reduced, as well as further clarification and tightening of bands. b,c,d) Further clarification of weak bands or more specific amplification is necessary.

Discussion and Future Directions

ISSRs 1, 2, 4, 10 and 23 may be suitable for a large-scale screen of genetic markers for *A. virginicus* when used with the optimal PCR conditions we have identified. We found each of the final five ISSR primers to produce some polymorphic bands among the Tyler State Park, Pennsylvania samples, allowing us to distinguish genetic diversity between individuals. In all, these five primers amplified at least nine polymorphic bands among the individual plant samples. The differences between optimal PCR conditions (especially annealing temperature) found for each viable ISSR primer are due to differences in the biochemistry of reactions between primer molecules and *A. virginicus* DNA strands. Raising the annealing temperature to optimize some ISSR primers allowed us to reduce unwanted DNA amplification, or background amplification, and clarify useful marker bands. On the other hand, a low annealing temperature allowed some primers to anneal to DNA with less specificity, leading to a greater number of potential markers being amplified and visualized. Similarly, an increased magnesium concentration in a PCR reaction can increase the specificity of the reaction. At the same time, however, the amount of product formed can be reduced.

Future work should evaluate levels of polymorphism and repeatability for a greater sample size as well as a larger number of populations and population types (invasive vs. native, healthy vs. infected). Bands in negative water control lanes should be eliminated, and background amplification by ISSR primers should also be reduced. With population genetic analyses of *A. virginicus*, we hope to determine whether host plant genetic diversity is related to disease prevalence. In agricultural settings, the genetically uniform crops that typically are planted on modern farms often develop disease epidemics; host genetic diversity influences disease in natural populations.

Acknowledgements This project was funded by the Garden Club of America Summer Environmental Studies Scholarship and the Office of Academic Affairs at The College of New Jersey, through the Mentored Undergraduate Summer Experience (MUSE) program.