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Transferability and utility of white oat (*Avena sativa*) microsatellite markers for genetic studies in black oat (*Avena strigosa*)

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ABSTRACT. Preservation and use of wild oat species germplasm are essential for further improvement of cultivated oats. We analyzed the transferability and utility of cultivated (white) oat *Avena sativa* (AACCDD genome) microsatellite markers for genetic studies of black oat *A. strigosa* (A_sA_s genome) genotypes. The DNA of each black oat genotype was extracted from young leaves and amplified by PCR using 24 microsatellite primers developed from white oat. The PCR products were separated on 3% agarose gel. Eighteen microsatellite primer pairs amplified consistent products and 15 of these were polymorphic in *A. strigosa*, demonstrating a high degree of transferability. Microsatellite primer pairs AM3, AM4, AM21, AM23, AM30, and AM35 consistently amplified alleles only in *A. sativa*, which indicates that they are putative loci for either the C or D genomes of *Avena*. Using the data generated by the 15 polymorphic primer pairs, it was possible to separate 40 genotypes of the 44 that we studied. The four genotypes that could not be separated are probably replicates. We conclude that *A. sativa* microsatellites have a

high transferability index and are a valuable resource for genetic studies and characterization of *A. strigosa* genotypes.

Key words: Cultivated oat; Wild oat; SSR; Breeding

INTRODUCTION

Wild species have great variability and are potential sources for novel genetic variation for crop improvement. The characterization of genetic variability in wild species and the development of tools to identify and transfer it into cultivated crops are important plant breeding goals. The preservation and use of the diverse germplasm of wild oat species are essential to further improvement of the cultivated oat and may help to safeguard genetic erosion due to biotic and abiotic stresses (Zeller, 1998). *Avena strigosa* Schreb (2n = 2x = 14) (A_sA_s genome) is a valuable source of new traits for oat breeding. Important traits already identified include pathogen resistance for stem rust (Dyck, 1966; Adhikari et al., 1999), powdery mildew (Herrmann and Roderick, 1996) and crown rust (Gregory and Wise, 1994; Wise et al., 1996) besides aluminum tolerance (Wight et al., 2006).

Alien introgression has been greatly facilitated by the identification of alien chromatin in the recipient progenies by molecular cytogenetic techniques (Heslop-Harrison et al., 1990; Mukai and Gill, 1991; Miller et al., 1995). Molecular markers have also been useful for identification of alien chromatin. Among various molecular markers available, microsatellites (Litt and Luty, 1989) or SSR (simple-sequence repeat) markers have shown more potential for this type of study due to their co-dominant nature and suitability for automation. Microsatellites can be identified using DNA database searches (Akkaya et al., 1992; Devos et al., 1995; Kantety et al., 2002), but this has not been feasible for *A. strigosa* where relatively few sequences are currently available. High development costs also make it impractical to develop them directly from wild species like *A. strigosa*. So for this species, it is necessary to study the transferability of microsatellite markers developed for related species.

Microsatellite transferability across related taxa depends on the conservation of flanking regions and primer binding sites in different taxa. A high conservation of microsatellite loci has been observed between tree taxa. This conservation has propitiated high transferability indexes between *Citrus* (Kijas et al., 1995), *Prunus* (Dirlewanger et al., 2002; Wunsch and Hormaza, 2002; Decroocq et al., 2003), *Vitis* (Sefc et al., 1999), *Elaeis* (Billotte et al., 2001), *Picea* (Hodgetts et al., 2001), *Pinus* (Shepherd et al., 2002), *Malus* and *Pyrus* (Yamamoto et al., 2001), and *Olea* (Rallo et al., 2003). Already in Gramineae, in the few accomplished studies, the transferability of microsatellite markers has been low (Brown et al., 1996; Bryan et al., 1997; Röder et al., 1998; Stephenson et al., 1998). Hernández et al. (2001), working with maize and *Miscanthos*, found high microsatellite marker transferability between these two species. This showed that the low transferability of microsatellite markers is not a rule among the Gramineae. Two studies were published describing the development of a larger number of microsatellite markers in *A. sativa* (Li et al., 2000; Pal et al., 2002). However, the transferability of these SSR markers for genetic studies in related species, such as *A. strigosa*, has not been verified.

The objective of the present study was to evaluate the transferability of the microsatellite markers developed from *A. sativa* to *A. strigosa* and determine their potential for genetic studies in *A. strigosa*.

MATERIAL AND METHODS

Plant material

Seeds of 44 genotypes of *A. strigosa* were provided by the Pro-Sementes Foundation (Passo Fundo, RS, Brazil). These genotypes are inbred lines of *A. strigosa* maintained in germplasm collections in southern Brazil.

DNA extraction

DNA was extracted from frozen young leaf tissue using the CTAB method (Murray and Thompson, 1980) with the following modifications. Two grams of fresh leaves was ground to fine powder in liquid nitrogen, followed by the addition of 600 µL preheated (65°C) extraction buffer with further grinding. The extraction buffer consisted of 1% (w/v) CTAB, 1.4 mM NaCl, 20 mM EDTA and 100 mM Tris-HCl, pH 8.0. The homogenates were incubated at 65°C for 60 min and extracted with a phenol chloroform:isoamyl alcohol (24:1) solution. DNA was precipitated in cold isopropanol and treated with RNase A. The concentration and quality of each sample were estimated by spectrophotometry (Spectronic Genesys).

PCR amplification and microsatellite analysis

Twenty-four microsatellite primer pairs developed from *A. sativa* (Li et al., 2000) were utilized (Table 1). Amplification reactions were carried out in 25 µL volumes containing 50 ng DNA, 1 U *Taq* DNA polymerase, 200 µM of each dNTP, 10 pmol of each primer, 2 mM $MgCl_2$, 50 mM KCl, and 10 mM Tris-HCl, pH 8.3. Depending on the annealing temperature of the primers used, amplification was performed in two "Touchdown" PCR profiles according to the method described by Li et al. (2000). Aliquots of each PCR (12 µL) were run on agarose gels (3%) in 0.5X Tris-borate EDTA and electrophoresed at 110 V for 2 h. The products were visualized by the ethidium bromide staining method. The size of each fragment was estimated with reference to a size marker of a 100-bp ladder (Invitrogen). DNA of *A. sativa* cultivar 'UFRGS 17' was used in all PCR amplifications as control. All amplifications were performed twice and independently to make sure that the results were correct.

Data analysis

The genetic similarity was estimated using the unweighted pair group method using arithmetic average (UPGMA) clustering algorithm (NTSYpc, version 2.1) with the data of 15 primer pairs that presented polymorphism.

RESULTS

Analysis of SSR markers

DNA from 44 genotypes of *A. strigosa* was tested with 24 *A. sativa* SSR primer pairs developed by Li et al. (2000) and the results are summarized in Table 1. Eighteen primer pairs

(75%) gave reproducible amplification products from *A. strigosa* DNA, and were therefore useful for genetic studies. Fifteen primer pairs showed polymorphism among the *A. strigosa* genotypes. The primer pair AM1 presented two loci, agreeing with the results found by Li et al. (2000). In *A. strigosa* the type of polymorphism found was the presence/absence of an allele (Figure 1). The absence of the alleles was consistent in all the repetitions; in this way, the evaluation was consistent in independent PCRs and evaluations were according to the presence or absence of the allele. Six primer pairs (25%), AM3, AM4, AM21, AM23, AM30, and AM35, did not show amplification in the *A. strigosa* genotypes analyzed, which indicates that probably they are not located on the A_sA_s genome.

Table 1. Characterization of *Avena sativa* microsatellite markers in *A. strigosa* genotypes.

SSR	Product size in *A. sativa*[1]	Product size in *A. strigosa*	SSR	Product size in *A. sativa*[1]	Product size in *A. strigosa*
AM1[2]	204	160/175	AM22	138	112
AM2	144	100	AM23	147	-
AM3	280	-	AM25	229	260
AM4	166	-	AM26	224	205
AM5	172	142	AM27	161	158
AM6	209	190	AM28	135	152
AM11	255	155	AM30	203	-
AM14	133	112	AM31	186	143
AM15	229	250	AM35	216	-
AM17	250	100	AM38	178	150
AM19	251	290	AM41	205	193
AM21	210	-	AM42	193	180

[1]From Li et al. (2000). [2]Two loci. - = no amplification; SSR = simple-sequence repeat markers.

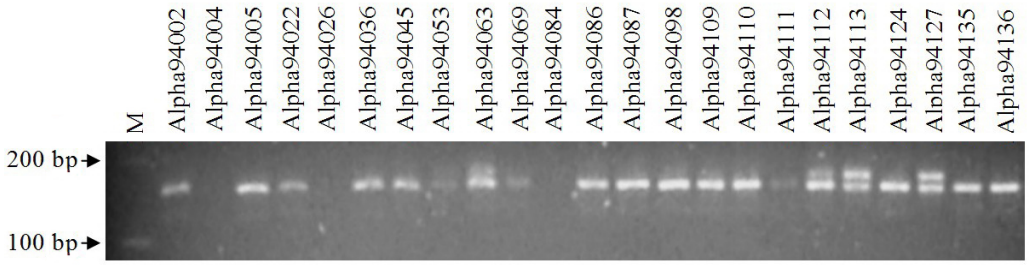

Figure 1. Pattern of amplification of the AM1 primer pairs showing amplification of two loci (genotypes Alpha 94063, 94112, 94113, and 94127) and absence of the alleles (genotypes Alpha 94004, 94026 and 94084). *Lane M* = DNA size markers.

Data analysis

With the data of 15 polymorphic primer pairs, a dendrogram of the 44 genotypes of *A. strigosa* was constructed (Figure 2). With these data it was possible to separate 40 genotypes of the 44 analyzed. Only the genotypes Alpha 94155, Alpha 94156, Alpha 94199, and Alpha 94231 were not individually identified (Figure 2).

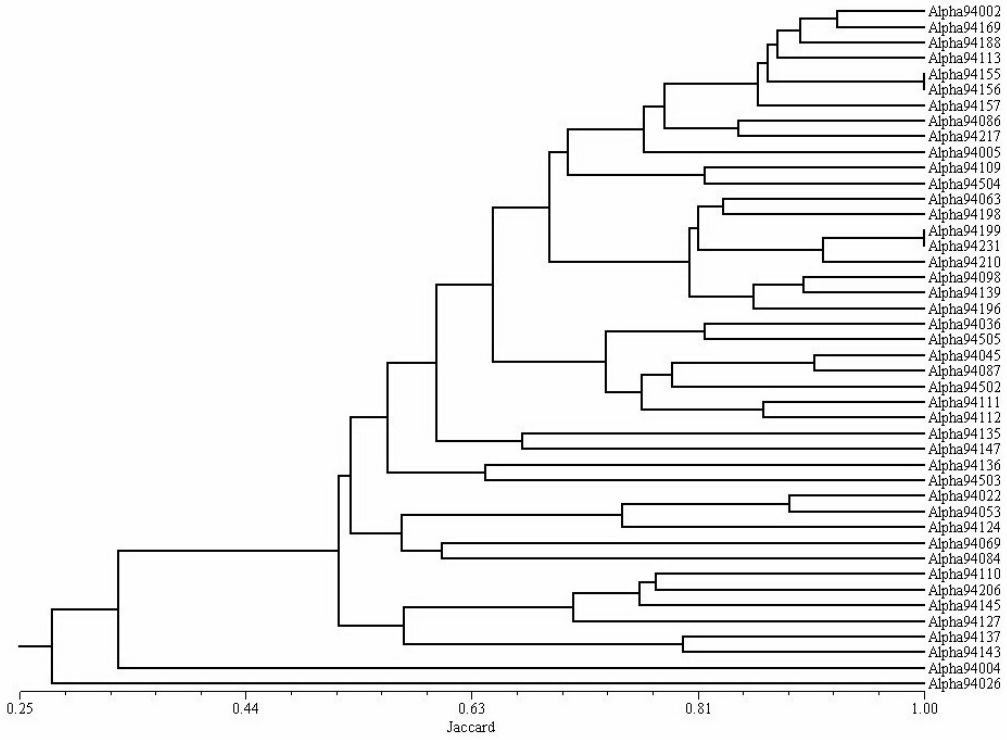

Figure 2. Dendrogram of the 44 *Avena strigosa* genotypes constructed using 15 polymorphism microsatellite primers previously developed in *A. sativa* and transferred by this study for *A. strigosa*.

DISCUSSION

SSR transferability

This study was carried out to determine the transferability of *A. sativa* SSR to *A. strigosa* and its potential for genetic analyses in the *A. strigosa* germplasm collection of southern Brazil. Previous studies have shown that SSR primer pairs developed for one species can be used in genetically related species (Peakall et al., 1998; Downey and Iezzoni, 2000; Yamamoto et al., 2001). This depends on the conservation of SSR flanking regions and primer binding sites in different taxa. Several studies have shown high conservation of these flanking regions in tree species, which explains the high tranferability of SSR among those species (Thomas and Scott, 1993; Kijas et al., 1995; Cipriani et al., 1999; Sefc et al., 1999; Billotte et al., 2001; Hodgetts et al., 2001; Yamamoto et al., 2001; Dirlewanger et al., 2002; Wunsch and Hormaza, 2002; Rosseto et al., 2002; Shepherd et al., 2002; Decroocq et al., 2003; Rallo et al., 2003). However, transferability in the Gramineae has generally been poor where this has been tested (Brown et al., 1996). This is illustrated by studies in hexaploid wheat where few SSR markers developed for related species, such as barley, would amplify (Bryan et al., 1997; Röder et al., 1998; Stephenson et al., 1998; Kuleung et al., 2004). In our study, 75% of the SSR

markers tested showed amplification product in *A. strigosa*. The results are in accordance with Hernández et al. (2001), who also found a high level (74.5%) of transferability of SSR markers between maize and *Miscanthus*. The most common type of polymorphism found is the presence of null alleles; this has been common in studies of this type. Gupta et al. (2003) analyzed the transferability of wheat EST-SSR for *Triticum-Aegilops* complex, rice, barley, rye, oats, and maize and verified that 45% of EST-SSR presented null alleles. The high frequency of null alleles may be either due to deletion and/or substitutions at the 5'-end of primer binding site in DNA that is being tested (Cordeiro et al., 2001).

Avena strigosa variability

SSR markers have been the most suitable for variability studies because of the high polymorphism information content. Li et al. (2000) analyzing 20 *A. sativa* cultivars with SSR markers, found that only 36% of the primers were polymorphic. To differentiate the 20 cultivars, the authors needed the information of 19 primer pairs. In our study, over 80% of the primer pairs were shown to be polymorphic among the genotypes studied and with 15 primer pairs it was possible to differentiate 40 genotypes of *A. strigosa*. The data obtained in the present study showed a higher level of polymorphism between genotypes of *A. strigosa* in comparison to *A. sativa* in accordance with the literature data when diploid and polyploidy species are compared.

Putative genome-specific markers

Pestsova et al. (2000) used a diploid progenitor of the D genome of wheat for identification of SSR markers specific to the genome for bread wheat. With these data, the authors confirmed that diploid progenitors have a high potential as a source of SSR markers specific to the genome for polyploid-derived species. The presence of SSR markers that do not amplify fragments in *A. strigosa* indicates that these are putative genome-specific markers for the C or D genomes. On the other hand, the SSR markers that amplified in *A. strigosa* are probably located in the A_s genome and might be useful as a tool to explore this genome in *Avena* species.

In conclusion, SSR markers developed in *A. sativa* presented great potential to be used for genetic studies in *A. strigosa*, and were able to detect high levels of genetic variability among the *A. strigosa* genotypes studied.

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