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Transferability of SSR markers among wheat, rye, and triticale

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Abstract Simple sequence repeat (SSR) markers are a valuable tool for many purposes, such as mapping, fingerprinting, and breeding. However, they are only available in some economically important crops because of the high cost and labor intensity involved in their development. Comparative mapping reveals a high degree of colinearity between closely related species, which allows the exchange of markers between them. Our objective was to examine the transferability of SSR markers among wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.), and triticale (X *Triticosecale* Wittmack). One hundred forty-eight wheat and 28 rye SSR markers were used to amplify genomic DNA extracted from five lines each of wheat, rye, and triticale. Transferability of wheat SSR markers to rye was 17%, whereas 25% of rye markers were amplifiable in wheat. In triticale, 58% and 39% transferability was achieved for wheat and rye markers, respectively. Wheat markers gave an average of 2.6, 2.7, and 2.4 polymorphic bands in wheat, rye, and triticale, respectively, while rye markers gave an average of 2.0 in rye and none in wheat and triticale. These transferable markers can now be exploited for further genetic and breeding studies in these species.

Introduction

Triticale (× *Triticosecale* Wittmack) is a synthetic hybrid derived by crossing wheat (*T. durum* L. or *T. aestivum* L.) and rye (*Secale* sp.). Triticale contains genomes from wheat (AABB or AABBDD) and rye (RR). Most commonly grown triticales are hexaploids (AABBRR). Octoploid triticales (AABBDDRR) are less common. Other ploidy levels, such as decaploid and tetraploid are rarer. Most triticale cultivars are derived from crossing two triticale parents or from crossing wheat with triticale.

Scientists and producers are interested in triticale because it is well adapted to harsh environmental conditions of high elevation, acid soil, salinity and aluminum toxicity, drought, and waterlogged soils. Triticale also has greater tolerance to common wheat diseases than does wheat (Horlein and Valentine 1995). Triticale grain also is high in essential amino acids, which makes it more nutritionally valuable than wheat, although the baking quality is inferior to that of bread wheat (Horlein and Valentine 1995). Therefore, triticale is a promising crop and a valuable genetic resource for transferring desirable genes, particularly disease-resistance genes, from rye to wheat.

DNA-based molecular markers are powerful tools used for gene mapping, DNA fingerprinting, and studying population structure and genetic diversity. Simple sequence repeat (SSR) markers, DNA fragments containing tandem repeats of a short sequence (2–6 nucleotides), are widely used because of their abundance, codominance, high polymorphism, and ease of assay by polymerase chain reaction (PCR). The primary disadvantage of SSRs as molecular markers is the cost and research effort required for their development. SSR markers have found broad utility in studies of relationships among closely related plant species, as well as among subpopulations of a single species (Bowcock et al. 1994).

SSR markers are currently used for genetic mapping in rice (*Oryza sativa* L., Temnykh et al. 2001), soybean (*Glycine max* L., Cregan et al. 1999), barley (*Hordeum vulgar* L., Liu et al. 1996), and maize (*Zea mays* L., Sharopova et al. 2002), and for fingerprinting and characterization in rye (Saal and Wricke 1999), sugarcane (*Saccharum* sp., Cordeiro et al. 2000), wheat (Röder et al. 1998; Song et al. 2002), and for genetic analysis in barley (Hernández et al. 2002). SSRs serve as a tool for the identification of genotypes, tagging for important traits, and in population genetic studies (Gupta and Varshney 2000; Hernández et al. 2002). Plant SSRs are reported to

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exhibit high levels of polymorphism, with as many as 37 alleles at an individual locus in barley (Saghai-Maroof et al. 1994) and 26 alleles in soybean (Rongwen et al. 1995). In many plant species, the level of polymorphism has been shown to be up to ten times higher than RFLP markers (Akkaya et al. 1992; Senior and Heun 1993).

Recently, comparative genetics revealed that gene content and order are highly conserved among closely related species. Colinearity of common markers illustrated by comparative maps suggests that a marker of one genus/species will be present in another related genus/species (Van Deynze et al. 1998; Tikhonov et al. 1999). Sequence data obtained from several crop plants indicate sufficient homology existing between genomes in the regions flanking the SSR loci. Thus, primer pairs designed on the basis of the sequence obtained from one species could be used to detect SSRs in related species. Therefore, application of SSR markers developed from one species to another, called "transferability," has been proposed and successfully demonstrated in many species. Examples include species in the genera *Glycine* (Peakall et al. 1998), *Prunus* (Dirlewanger et al. 2002), and *Lycopersicon* (Alvarez et al. 2001), and between genera *Aegilops* and *Triticum* (Sourdille et al. 2001). Comparative mapping between wheat and rye genomes shows consensus markers mapped on the correspondent sites with mostly conserved order (Devos and Gale 1993; Devos et al. 1993). Additionally, Caudrado and Schwarzacher (1998) report that chromosomal organization of SSR motifs, which mainly distribute in intercalary sites, is very similar in both wheat and rye. Their intergenomic relationship allows exchange of genetic material between them. Wheat and rye SSR markers are available, but there are no SSR markers yet available for triticale. Therefore, our objective was to examine the transferability of SSR markers among wheat, rye, and triticale.

Materials and methods

Plant materials

Five lines of wheat ('Newton', 'Sullivan', 'MV-Matador', 'Bobwhite', and 'Arapahoe'), rye ('Emory', 'Aiita', 'Kaltenberger', 'Blanco', and 'Langauer taner'), and triticale (CIxt23, PI422264, PI355948, PI611791, and PI611793) were used to examine transferability of wheat and rye SSR markers.

DNA extraction

Genomic DNA of each line was isolated by a sap-extraction method from 100 mg of fresh tissues. Leaves of 2-week-old seedlings were placed between the two rollers of a sap-extraction apparatus (Ravenel Specialities, Seneca, S.C.), and 1 ml of extraction buffer (50 mM Tris-HCl, 25 mM EDTA, 1 M NaCl, 1% CTAB, 1 mM 1, 10-phenathroline, and 0.15% 2-mercaptoethanol) was slowly added to the rollers, immediately mixing with the sap for collection in 1.5-ml microcentrifuge tubes. The extract was incubated at 60°C for 1 h, and then mixed with equal volume of chloroform-isoamyl alcohol (24:1). After centrifuging at 12,000 rpm, the supernatant was transferred to a new tube and isopropanol added for a 30-min incubation to precipitate the DNA. The pellet was dried, resuspended in 200 μl of TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) plus 20 μg of RNase, and then incubated at room temperature overnight. The DNA solution was mixed with 20 μl of 8 M ammonium acetate and 400 μl of cold absolute ethanol for 30 min, centrifuged for 10 min, and air dried at room temperature. The DNA was then resuspended in 200 μl of TE buffer, and DNA concentration was quantified by spectrophotometry (TKO100 Fluorometer, Hoefer Scientific Instruments, San Francisco).

PCR amplification

One hundred forty-eight wheat (Röder et al. 1998; US Wheat and Barley Scab Initiative 2003) and 28 rye SSR markers (Saal and Wricke 1999) were screened for amplification and polymorphism in triticale. The PCR reaction mixture (25 μl total) consisted of 50 mM KCl and 10 mM Tris-HCl (pH 8.8), 2 mM $MgCl_2$, 125 μM of dNTP, 50 ng of each primer, 1.0 unit of *Taq* polymerase (Promega), and 20 ng of genomic DNA. Amplification was carried out in an MJ Research PTC-100 (Programmable Thermal Controller, MJ Research), using a program that consisted of initial denaturation for 1 min at 94°C, followed by 32 cycles of 30 s at 94°C, 50 s at 53°C, 50 s at 72°C, and final extension for 5 min at 72°C. The amplified PCR products were gel fractionated on 12% polyacrylamide gel (37 acrylamide:1 bis-acrylamide) in a TAE buffer (40 mM Tris-HCl, 20 mM sodium acetate, 1 mM EDTA), using a Hoefer vertical gel apparatus (SE600) at 300 V for 3 h at 20°C, using a circulating bath temperature set to 20°C. Gels were stained in ethidium bromide (1 μg/ml) for 20 min, destained in deionized water for 1 h, and photographed under the Gel Doc2000 (Bio-Rad).

Scoring and amplification analysis

The scored fragments were identified by fragment sizes that were similar to expected size. The amplified fragments were classified into four classes based on the signal intensity and ease of scoring: (1) strong signal and easy to score; (2) weaker signal, but able to score; (3) very weak signal and difficult to score; and (4) no signal. Classes 1 and 2 were considered positive amplification, while classes 3 and 4 were considered negative for amplification. To determine positive amplification of a SSR marker in a species, at least three out of five lines must be positively amplified. The number of amplified fragments in a species was counted regardless of polymorphism.

$$\text{Amplification } (\%) = (\text{number of amplified markers} \times 100)/\text{total number of markers} \quad (1)$$

Percentage of transferability was percentage of amplification of the SSR markers amplified in nondonor species.

Results and discussion

This study was carried out to determine the transferability of wheat and rye microsatellites to triticales. Of 176 SSR markers, 124 (111 wheat and 13 rye) markers were amplified in at least one species of wheat, rye, and triticale. Due to the limited number of public rye microsatellites, only 28 primer pairs were used, compared to 148 from wheat. Of these amplified markers, 19 wheat and six rye markers could be amplified in all species (Fig. 1), indicating some relationship among their genomes. Several investigators (Brown et al. 1996; Van Deynze et al. 1998; Tikhonov et al. 1999) have also shown that microsatellite primer pairs developed for one species can amplify DNA of close relatives. Transferability of

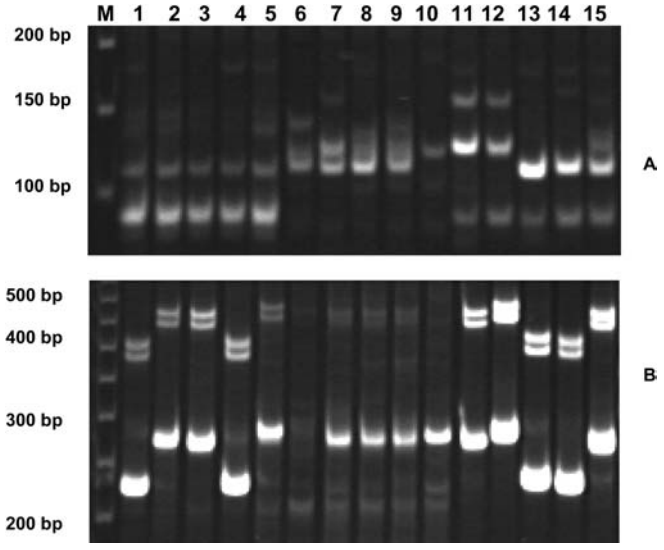

Fig. 1A, B PCR amplification of SSR markers. **A** SCM120 and **B** BARC176 and genomic DNA of wheat (*lanes 1–5* represent Newton, Sullivan, MV-Matador, Bobwhite, and Arapahoe, respectively), rye (*lanes 6–10* represent Emory, Aiita, Katenberger, Blanco, and Langauer taner respectively), and triticale (*lanes 11–15* represent accessions CIxt23, PI422264, PI355948, PI611791, and PI611793, respectively). *Lane M* contains the DNA size markers. The amplified DNA samples were fractionated in 12% nondenaturing acrylamide gels stained with ethidium bromide

maize microsatellites developed from genomic libraries was 74.5% in sugarcane (Hernandes et al. 2001). Twenty-one wheat and two rye markers could be amplified only in their own species. Twenty-five (17%) wheat markers gave amplification products in rye, while seven (25%) rye markers were amplified in wheat (Table 1). The amplified fragments of the target species were usually weaker than those of the donor species. Korzun et al. (2001) similarly reported that approximately 27% of wheat SSR markers were amplified in rye, whereas Röder et al. (1995) observed high transferability of wheat SSR markers to rye (60%), but most amplified products were weak. Transferability of plant SSR markers was usually less effective when compared to RFLP markers (Korzun et al. 1998; Van Deynze et al. 1998). However, this is not always the case. Dirlewanger et al. (2002) reported high transferability of SSR markers in peach (*Prunus* spp).

Of 176 tested markers, 85 (57%) wheat and 11 (39%) rye markers were amplified in triticale (Table 1). Eighty-two wheat and six rye markers were genome specific; they amplified only within their own species or triticale. These genome-specific markers would be useful for identifying DNA fragments introgressed into another species. Banding pattern and size of the markers amplified in triticale were usually similar to those of the donor species, suggesting that they were derived from the same loci and that these allelic regions of the primer binding sites are conserved. Surprisingly, many markers could not be amplified, although triticale contains both wheat and rye genomes. This degree of site loss is likely reflective of the genomic divergence in triticale subsequent to its hybrid formation. Rapid genomic modification commonly occurs in early generations of a newly formed polyploid to harmonize coexistence of different genomes in the same nucleus (Soltis and Soltis 1999; Ozkan et al. 2001). Additionally, as expected, most markers located in the wheat D genome were not amplified in triticale because some triticale lines do not contain the D genome. Although 96 markers were amplified in triticale, many markers produced several minor bands. Modifications of annealing temperature and $MgCl_2$ concentration did not always improve the amplification outcome.

Of the amplified markers, 60, 6, and 46 polymorphic markers were observed in wheat, rye and triticale, respectively. Only two rye markers showed polymorphism in rye, but none was observed in wheat and triticale. Although donor source of the markers influences level of polymorphism, generally reflecting to genetic distance, other factors such as ploidy level and mutation may complicate the relationship of transferability and genetic distance (Dirlewanger et al. 2002).

SSR markers were informative, even though only five lines of each species were examined. Polymorphic bands for a wheat marker varied from two to five bands in wheat and triticale, and two to four bands in rye, with an average of 2.6, 2.7, and 2.4 bands in wheat, rye and triticale, respectively. An average of 2.0 polymorphic bands was observed in rye using rye markers. The polymorphism levels were lower than in previous studies of wheat (4.6 alleles/locus, Röder et al. 1995; 7.4 alleles/locus, Prasad et al. 2000; 4.8 alleles/locus, Stachel et al. 2000) and in rye (5.9 alleles/locus, Saal and Wricke 1999; 3.0 alleles/locus, Hackauf and Wehling 2002). This is likely due to the small number of line used in this study.

It is useful to know at this point that if SSR primers are available from closely related species, then screening a high number of primers is required in order to identify a set that can be used as reliable polymorphic markers in a species of interest. Our results did not reveal high marker transferability among wheat, rye, and triticale. The number of transferred markers would likely improve by using markers developed from expressed sequences. However,

Table 1 Amplification of wheat and rye SSR markers in wheat, rye, and triticale

Donor species of markers	Number of markers	Number of amplified markers (percentage of amplification) in		
		Wheat	Rye	Triticale
Wheat (*Triticum aestivum* L.)	148	105 (70.9%)	25 (16.9%)	85 (57.4%)
Rye (*Secale cereale* L.)	28	7 (25.0%)	12 (42.9%)	11 (39.3%)

several markers gave good amplification in triticale and, thus, could be useful for genetic and breeding studies of triticale. The availability of more than 750 public wheat microsatellite primer pairs would constitute an efficient and cost-effective source of molecular markers for use in triticale breeding and genetics.

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