Theor Appl Genet (2004) 108:720–724
DOI 10.1007/s00122-003-1472-0

ORIGINAL PAPER

D. MacRitchie · G. Sun

Evaluating the potential of barley and wheat microsatellite markers or genetic analysis of *Elymus trachycaulus* complex species

Received: 23 May 2003 / Accepted: 2 September 2003 / Published online: 11 October 2003

Abstract The potential of barley and wheat microsatellite markers for genetic analysis of *Elymus trachycaulus* complex species was evaluated. A set of 25 barley and 3 wheat microsatellite markers were tested for their ability to cross-amplify DNA from four accessions of *E. trachycaulus* and two accessions *Pseudoroegneria spicata*. Thirteen barley (52%) and two (68%) wheat primer pairs successfully amplified consistent products from both *E. trachycaulus* and *P. spicata* species. Four of the 15 successful primer pairs produced visible polymorphisms among the accessions tested. A higher successful rate of cross-species amplification of barley and wheat microsatellite markers in *E. trachycaulus* and *P. spicata* was found in this study. These primer pairs are now available for use as markers in genetic analysis of *E. trachycaulus* complex species. Our results suggest that publicly available wheat and barley microsatellite markers are a valuable resource for the genetic characterization of wild Triticeae species.

Introduction

Elymus trachycaulus (Link) Gould ex Shinners is a tetraploid that combines the genomes of a *Pseudoroegneria* species and a wild *Hordeum* species (Dewey 1968, 1975) and is the most morphologically and geographically diverse species of *Elymus* in North America (Dewey 1982). The range of *E. trachycaulus* extends from Eurasia through Alaska to Newfoundland and south to Mexico along the Rocky Mountains. It usually grows in open forests and along roadsides (Hitchcock 1951). *E. trachycaulus* is used for restoration of disturbed lands (Brown et al. 1978) and also contains genes used to improve barley and wheat resistance to Russian wheat aphid (Aung 1991). Taxa within the *E. trachycaulus* complex are circumscribed morphologically, but several of the traits used vary continuously and some are at least partially under environmental control. Although there are some relatively distinct entities in local areas, when the complex as a whole is considered, these are often linked by an uncomfortably large number of morphologically intermediate plants that may be derived from intraspecifc hybridization (Barkworth 1994). Although *E. trachycaulus* is known for its geographic and morphological diversity, it is not known whether these are attributable to genetic variation, physiological and morphological plasticity, hybridization, or a combination of these factors. Without an understanding of a species' genetic diversity it is difficult to plan for the efficient utilization and conservation of this diversity.

Previous studies have shown that microsatellite markers are a very useful tool for the analysis of genetic diversity in *Elymus* species (Sun et al. 1998c, 1999, 2001, 2002). Within the *Elymus* genus, several microsatellite markers have been developed from genomic DNA libraries from *E. caninus* L. and *E. alaskanus* (Scrib. ex Merr.) Löve (Sun et al. 1998a, 1998b). However, no microsatellite markers are available for *E. trachycaulus* complex species. Previous studies have shown that a few *E. caninus* and *E. alaskanus* microsatellites are transferable across species by amplification in *E. trachycaulus* (Sun et al. 1998a, 1998b). In the present study, microsatellite markers from barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) were tested to determine their potential for the genetic analysis of *E. trachycaulus* complex species.

Communicated by H.F. Linskens

D. MacRitchie · G. Sun (✉)
Biology Department,
Saint Mary's University,
Halifax, Nova Scotia, B3H 3C3, Canada
e-mail: genlou.sun@smu.ca

Materials and methods

Plant materials

The plant materials used in this study include one *Elymus trachycaulus* species, three *E. trachycaulus* subspecies, and two accessions of diploid *Pseudoroegneria* species. The diploid *Pseu-*

Table 1 *Elymus* and *Pseudoroegneria* species used in this study, together with their accession numbers, genomic constitution, and origin. The accession no. refers to the United State Department of Agriculture (USDA) collection number

Species	Accession no.	Genome	Origin
E. trachycaulus (Link) Gould ex Shinners	531690	SH	Utah, USA
E. trachycaulus ssp. *subsecundus* (Link) A. Löve & D. Löve	236685	SH	Canada
E. trachycaulus (Link) Gould ex Shinners ssp. *trachycaulus*	537323	SH	Utah, USA
E. trachycaulus ssp. *violaceus* (Link) A. Löve & D. Löve	276712	SH	Leningrad, Russian Federation
P. spicata (Pursh) A. Löve	232140	S	USA
P. spicata (Pursh) A. Löve	286198	S	USA

Table 2 Barley and wheat microsatellite primers used in this study. This table was modified based on Liu et al. 1996 and Röder et al. 1995

SSR	Primer sequence (5′→ 3′)	Repeat	PCR
HVM2	CAGGTGTCTAGTGGGTGCCTTT ACATCCAAGGAGCAATCCC	$(GA)_{11}$	1
HVM3	ACACCTTCCCAGGACAATCCATTG AGCACGCAGAGCACCGAAAAAGTC	$(AT)_{29}$	1
HVM4	AGAGCAACTACCAGTCCAATGGCA GTCGAAGGAGAAGCGGCCCTGGTA	$(AT)_9$	1
HVM5	AACGACGTCGCCACACAC AGGAACGAAGGGAGTATTAAGCAG	$(GT)_6(AT)_{16}$	1
HVM7	ATGTAGCGGAAAAAATACCATCAT CCTAGCTAGTTCGTGAGCTACCTG	$(AT)_7$	1
HVM11	CCGGTCGGTGCAGAAGAG AAATGAAAGCTAAATGGGCGATAT	$(GGA)_3(GGA)(GAA)_2$	3
HVM15	TCATAACCACGGCGTCCT CGTGACTGGAAACCCTGC	$(GA)_8$	1
HVM20	CTCCACGAATCTCTGCACAA CACCGCCTCCTCTTTCAC	$(GA)_{19}$	1
HVM22	TTTGGGGGATGCCTACATA TTTCAAATGGTTGGATTGGA	$(AC)_{13}$	1
HVM23	TCGGTGAAGAAATACGAGGC TCTTTGTGACCTACCGGTCC	$(GA)_9$	2
HVM26	GGCTATCACATTTGGTACCATC GCATGTGTAGGTGTTGGTGG	$(CA)_{11}$	2
HVM31	CGGTTTCTGGTTGCTTGG CGAAGGTCTCAGGCTTCATG	$(AC)_9$	1
HVM33	ATATTAAAAAAGGTGGAAAGCC CACGCCCTCTCCCTAGAT	$(CA)_7$	1
HVM40	CGATTCCCTTTTCCCAC ATTCTCCGCCGTCCACTC	$(GA)_6(GT)_4(GA)_7$	1
HVM44	AAATCTCAGGTTCGTGGGCA CCACGGAGACCACCTCACTT	$(GA)_8$	1
HVM51	TCTAAATTACCTTCCCAGCCA AAGCAGACATGTAGGAGGTCA	$(GA)_3(GGGA)_3$ $(GA)_8$	1
HVM54	AACCCAGTAACACCGTCCTG AGTTCCCTGACCCGATGTC	$(GA)_{14}$	1
HVM60	CAATGATGCGGTGAACTTTG CCTCGGATCTATGGGCCTT	$(AG)_{11}(GA)_{14}$	1
HVM63	CGCGCAAGCATGAATACTC ACTCACAAGTGGCGCGTAC	$(GA)_9$	1
HVM64	GATGTGAAGGCTGCCTG ACACGCCCTATTACCCAGTG	$(GA)_4(GT)_7(CT)_2(GT)_4(GA)_{48}$	1
HVM65	AGACATCCAAAAAATGAACCA TGGTAACTTGTCCCCCAAAG	$(GA)_{10}$	1
HVM67	GTCGGGCTCCATTGCTCT CCGGTACCCAGTGACGAC	$(GA)_{11}$	1
HVM68	AGGACCGGATGTTCATAACG CAAATCTTCCAGCGAGGCT	$(GA)_{22}$	1
HVM70	CCGCCGATGACCTTCTC ACCCACGACCTATGGCAC	$(CA)_8$	3
HVM77	GAAATTTGGTGTATGATGGTT CAAATCTTAAATCTCTCTGTTT	$(CA)_7$	1
WMS37	ACTTCATTGTTGATCTTGCAT CGACGAATTCCCAGCTAAAC	$(AG)_8GG(AG)_{21}$	4
WMS65	–	–	4
WMS165	–	–	4

doroegneria is one of the genomic donor species of *Elymus.* The accession numbers, genomic constitution and origins are given in Table 1.

DNA isolation

Leaf tissue samples were collected from these species and subspecies, frozen in liquid nitrogen, and kept at −80°C. DNA was extracted according to the method described by Junghans and Metzlaff (1990).

PCR amplification and microsatellite analysis

The primer pairs used in this study were purchased from ResGen/Invitrogen. Twenty-five barley and three wheat primer pairs were tested. PCR primer sequences from barley (Liu et al. 1996) and bread wheat are listed in Table 2 (Röder et al. 1995). Amplification reactions were carried out in 20 μl volumes, each containing 30 ng DNA, 200 μM of each dNTP, 0.5 U of *Taq* DNA polymerase (Rose Company), 2 μl 10x reaction buffer with 20 mM $MgCl_2$, and 0.1 μM of each primer. Depending on the primer pairs used, amplification was performed using one of the following four PCR conditions. The first profile was a "touchdown" protocol consisting of ten cycles of 94ºC for 1 min and 72ºC for 1 min. The annealing (30 s) temperatures for these initial cycles were progressively decreased by 1ºC every cycle from 64ºC to 55ºC. The reaction was then continued for 30 additional cycles of 94ºC for 1 min, 55ºC for 1 min and 72ºC for 1 min, and finished with a 10 min extension at 72ºC. The second profile was a similar "touchdown" protocol, except that the annealing temperatures were decreased from 69ºC to 60ºC over the first ten cycles. The third PCR profile consisted of one cycle of 94ºC for 3 min, 57ºC for 2 min and 72ºC for 1.5 min, followed by 30 cycles at 94ºC for 1 min, 57ºC for 2 min and 72ºC for 1.5 min. The fourth profile consisted of 35 cycles at 96ºC for 1 min, 60ºC for 1 min and 72ºC for 2 min. This profile ended with a 10 min extension at 72ºC. All PCR reactions were run in a Genius thermal cycler (Techne). The PCR products were mixed with 2 μl of loading dye then loaded onto either 2% or 3% agarose gels. The gels were run in 1x TBE buffer in a Pharmacia LKB electrophoresis apparatus (Fisher Biotech) at 80 V for approximately 45 min. Gels were stained with 10 μg/μl ethidium bromide to visualize the PCR products.

Results

Analysis of barley microsatellite markers

Twenty-five barley primer pairs were tested for their ability to amplify across species using four accessions of *Elymus trachycaulus* complex and two accessions of *Pseudoroegneria spicata*. In cases where either no PCR product or weak banding was observed, PCR optimization was carried out by decreasing and/or increasing the annealing temperatures, and switching to "touchdown" PCR conditions. Many primer pairs that failed to amplify under standard conditions successfully amplified product when used with a touchdown protocol.

Eleven primer pairs (44%) gave reproducible amplification products from all four accessions of *Elymus* species and from accessions of *P. spicata*. Two primer pairs (HVM15 and HVM22) were successful in amplifying product from only some of the accessions used in this study. HVM15 failed to amplify product from *E. trachycaulus* ssp. *subsecundus*, but successfully amplified products from the other three subspecies. HVM22 failed to amplify product from both *E. trachycaulus* spp. *subsecundus* and *E. trachycaulus* spp. *violaceus,* but successfully amplified product from *E. trachycaulus*, *E. trachycaulus* spp. *trachycaulus* and *P. spicata.* The primer pairs HVM77 amplified product from *P. spicata*, but failed to amplify product from any of the *E. trachycaulus* species. In total, 13 barley primer pairs (52%) generated reproducible amplification products in both *Elymus* and *Pseudoroegneria* species. One primer (4%) only amplified PCR product from *P. spicata*. The remaining 11 primer pairs (44%) failed to amplify product from both *E. trachycaulus* complex species and *P. spicata.* The results of the cross-species amplification are summarized in Table 3.

Analysis of wheat microsatellite markers

Three wheat primer pairs were tested. Two (WHS37 and WHS65) (67%) gave reproducible amplification products from all four accessions of *E. trachycaulus* complex and from at least one accession of *P. spicata*. The primer pair WHS165 failed to amplify product from any of the accessions tested in this study (Table 3).

Microsatellite polymorphisms

Microsatellite polymorphisms, presumably resulting from variation in the number of nucleotide repeats, were detected by 4 of the 28 primer pairs utilized in this study: HVM5, HVM7, HVM 40 and HVM 44. The number of bands/alleles detected by each primer pair is listed in Table 4. Figure 1 shows an example of the polymor-

Table 3 Results of cross-species amplification of microsatellites from *Elymus trachycaulus* complex and *Pseudoroegneria spicata* species. + indicates successful amplification of PCR product, - indicates non-successful amplification of PCR product, ? indicates primer pairs that did not give reproducible results

Primer pairs	*E. trachycaulus*	*E. trachycaulus* ssp. *subsecundus*	*E. trachycaulus* ssp. *trachycaulus*	*E. trachycaulus* ssp. *violaceus*	*P. spicata*
HVM 2	+	+	+	+	+
HVM 3	+	+	+	+	+
HVM 4	+	+	+	+	+
HVM 5	+	+	+	+	+
HVM 7	+	+	+	+	+
HVM 11	+	+	+	+	?
HVM 15	+	–	+	+	+
HVM 20	+	+	+	+	+
HVM 22	+	–	+	–	+
HVM 23	–	–	–	–	–
HVM 26	–	–	–	–	–
HVM 31	–	–	–	–	–
HVM 33	–	–	–	–	–
HVM 40	+	+	+	+	+
HVM 44	+	+	+	+	+
HVM 51	+	+	+	+	+
HVM 54	–	–	–	–	–
HVM 60	+	+	+	+	+
HVM 63	–	–	–	–	–
HVM 64	–	–	–	–	–
HVM 65	–	–	–	–	–
HVM 67	–	–	–	–	–
HVM 68	–	–	–	–	–
HVM 70	–	–	–	–	–
HVM 77	–	–	–	–	+
WHS 37	+	+	+	+	+
WHS 65	+	+	+	+	+
WHS 165	–	–	–	–	–

Table 4 Number of visible bands produced by polymorphic microsatellite primer pairs

Species	Accession no.	Number of bands produced			
		HVM5	HVM7	HVM40	HVM44
E. trachycaulus	531690	1	1	3	2
E. trachycaulus ssp. *subsecundus*	236685	3	2	1	2
E. trachycaulus ssp. *trachycaulus*	537323	4	1	1	1
E. trachycaulus ssp. *violaceus*	2767712	2	1	1	2
P. spicata	232140	2	1	3	2
P. spicata	286198	2	1	3	2

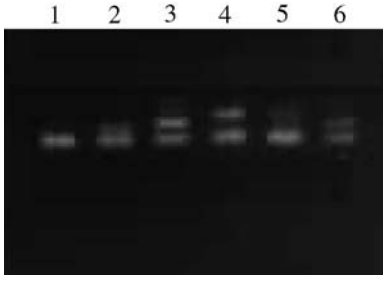

Fig. 1 Polymorphisms detected by the barley microsatellite marker HVM5. Lane 1: *E. trachycaulus*; Lane 2: *E. trachycaulus* ssp. *subsecundus*; Lane 3: *E. trachycaulus* ssp. *trachycaulus*; Lane 4: *E. trachycaulus* ssp. *violaceus*; Lane 5: *P. spicata*; Lane 6: *P. spicata*

phisms detected by the barley microsatellite marker HVM5.

Three of the four polymorphic microsatellite markers (HVM5, HVM40 and HVM44) displayed polymorphisms in both *Pseudoroegneria* and *Elymus* species. The number of alleles produced by each marker in *P. spicata* was consistent in both accessions of the species, but with different alleles, while the number of alleles produced in the four different accessions of *E. trachycaulus* varied.

Discussion

It is possible to detect microsatellite loci by using primer sequences originally designed from the flanking DNA of related species (van Treuren et al. 1997). This process depends on the conservation of priming sites within the flanking sequences to enable amplification, and on the maintenance of repeat arrays long enough to promote polymorphism (Fitzsimmons et al. 1995). Primer sequence conservation has been tested in a variety of plant species, with most of the research focusing on cultivated species (van Treuren et al. 1997). Peakall et al. (1998) tested the transferability of soybean microsatellites within the genus and within other legume genera. They found that 65% of the soybean microsatellite primers amplified microsatellites within the genus and only 13% outside the genus. Van Treuren et al. (1997) carried out a study of *Arabis* species using primers designed from *Arabidopsis thaliana,* and found 42% of the primers successfully amplified DNA. A study utilizing cross-species microsatellite markers for elucidating the population genetic structure of *A. thaliana* showed that of 42 primer pairs with the ability to amplify microsatellite loci from several closely related populations, 79% could amplify microsatellites in more distantly related species (Clauss et al. 2002). Within Triticeae species, Sun et al. (1997) found that 15 out of 18 (83%) wheat microsatellite primer pairs amplified DNA from *Elymus* species. Röder et al. (1995) reported that 1 of 15 (6.7%) wheat microsatellite primer pairs amplified DNA from barley and rye. Hernández et al. (2002) found that more than 50% of wheat and barley primer pairs amplified DNA from wild barley, *Hordeum chilense* Roem et Schult.

The present study has shown that after optimization of the PCR conditions for each primer pair, 52% of barley and 68% of wheat microsatellite primer pairs generated reproducible amplification product from *E. trachycaulus* complex and *P. spicata* species. The amplification success rates of the barley and wheat microsatellite markers used in this study are very similar to those of Hernández et al. (2002), who were successful in amplifying 53% of the wheat markers and 54% of barley markers tested in *H. chilense* and tritordeums. The high levels of microsatellite conservation found by Sun et al. (1997) and Hernández et al. (2002) and in this study are not surprising, considering that cereal genomes may have developed from rearrangements and duplications of one ancestral cereal chromosome (Moore 1995).

Although not enough information is available to enable scientists to predict the transferability of microsatellite markers, the large numbers of these markers being developed in wheat and barley provides a valuable resource (Hernández et al. 2002). In this study over 50% of the barley and wheat microsatellite primer pairs reproducibly amplified products in *E. trachycaulus*. Some of the primer pairs that successfully amplified product in *E. trachycaulus* failed to amplify product from some of the subspecies utilized in this study. This could be due to divergence in the microsatellite flanking sequences, creating a null allele (Sun et al. 1998a). However, 44% of the barley microsatellite markers and 67% of the wheat microsatellite markers that were effective in amplifying DNA products from *E. trachycaulus* complex could be used for genetic analysis of the *E. trachycaulus* complex. These results clearly show that wheat and barley microsatellite markers are both valuable and cost-effective molecular markers for studying the population structure of *E. trachycaulus*.

E. trachycaulus is a tetraploid that combines the genomes (SH) of a *Pseudoroegneria* species (S) and a

wild *Hordeum* species (H) (Dewey 1968, 1975). The barley microsatellite primer pairs were developed from *H. vulgare* containing the I genome (Wang et al. 1994), which is closely related to the H genome in wild *Hordeum* species. *P. spicata* was included to determine whether the successful primer pairs were amplifying microsatellites from the S or the H genome in *E. trachycaulus*. All 15 primer pairs that produced reproducible amplification products from *E. trachycaulus* also amplified products from *P. spicata* (Table 3), suggesting that all successful primer pairs amplified microsatellites from both the S and the H genomes, and were not specific to the H genome alone. This finding is surprising, as previous studies (Clauss et al. 2002; Peakall et al. 1998) have shown that primer success decreases as species become more distantly related.

Four of the 28 microsatellite primer pairs tested (14%) produced visible polymorphisms in *E. trachycaulus* and *P. spicata* species. It can be presumed that these polymorphisms are a result of the variation in the number of nucleotide repeats. In a study by Peakall et al. (1998), 85% of the loci amplified by microsatellite primer pairs designed for a related species were found to be polymorphic. Sun et al. (1998a) characterized 18 accessions of *Elymus* species with microsatellites and found 39% of the loci to be polymorphic. The minute occurrence of polymorphisms found in this study, as compared to other related studies, could be the result of the type of gel used during electrophoresis and/or the small sample size. Agarose was used in this study because it is inexpensive, easily available and user friendly. It was suitable for this study because the main objective was to screen the primers for their ability to amplify non-source DNA. If the main objective had been to detect polymorphisms, a sequencing gel would have been more suitable, and a larger sample size would be included.

In conclusion, we have demonstrated a high successful rate of cross-species amplification of barley and wheat microsatellite markers in *E. trachycaulus* and *P. spicata*. Our results show that wheat and barley microsatellite markers are a valuable resource for the genetic characterization of related species, and are a possible source of polymorphic markers for analyzing the relatively unknown *E. trachycaulus* gene pool.

Acknowledgements The authors thank the Natural Sciences and Engineering Research Council of Canada (NSERC) for funding this work. Support from a Saint Mary's University Senate grant is gratefully acknowledged. The seeds used in this study were kindly provided by the United States Department of Agriculture (USDA).

References

Aung T (1991) Intergeneric hybrids between *Hordeum vulgare* and *Elymus trachycaulus* resistant to Russian wheat aphid. Genome 34:954–960

Barkworth ME (1994) The *Elymus trachycaulus* complex in North America: more questions than answers. In: Wang RRC, Jensen KB, Jaussi C (eds) Proc 2nd Int Triticeae Symp, Logan, Utah, USA, pp189–198

Brown RW, Johnston RS, Johnson DA (1978) Rehabilitation of alpine tundra disturbances. J Soil Water Conserv 33:154–160

Clauss MJ, Cobban H, Mitchell-olds T (2002) Cross-species microsatellite markers for elucidating population genetic structure in *Arabidopsis* and *Arabis (Brassicaeae)*. Mol Ecol 11:591–601

Dewey DR (1968) Synthetic *Agropyron-Elymus* hybrids. III. *Elymus canadensis* x *Agropyron caninum*, *A. trachycaulum*, and *A. striatum*. Am J Bot 55:1133–1139

Dewey DR (1975) Introgression between *A. dasystachyum* and *A. trachycaulum*. Bot Gaz 136:122–128

Dewey DR (1982) Genomic and phylogenetic relationships among North American perennial Triticeae. In: Estes JE (ed) Grasses and grassland. University of Oklahoma Press, Oklahoma, pp 51–88

Fitzsimmons NN, Moritz C, Moore SS (1995) Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution. Mol Biol Evol 12:432–440

Hernández P, Laurie DA, Martin A, Snape JW (2002) Utility of barley and wheat simple sequence repeat (SSR) markers for genetic analysis of *Hordeum chilense* and tritordeum. Theor Appl Genet 104:735–739

Hitchcock AS (1951) Manual of grasses of the United States, 2nd edn. USDA Misc Publ 200

Junghans H, Metzlaff M (1990) A simple and rapid method for the preparation of total plant DNA. BioTechniques 8:176

Liu ZW, Biyashez RM, Saghai Maroof MA (1996) Development of simple sequence repeat DNA makers and their integration into barley linkage map. Theor Appl Genet 93:869–876

Moore G (1995) Cereal genome evolution: pastoral pursuits with "Lego" genomes. Curr Opin Genet Dev 5:717–724

Peakall R, Gilmore S, Keys W, Morgante M, Rafalski A (1998) Cross-species amplification of soybean (*Glycine max*) simple sequence repeats (SSR) within the genus and other legume genera: implications for the transferability of SSRs in plants. Mol Biol Evol 15:1275–1287

Röder MS, Plaschke J, Konig SU, Borner A, Sorrells ME, Tanksley SD, Ganal MW (1995) Abundance, variability and chromosomal location of microsatellites in wheat. Mol Gen Genet 246:327–333

Sun GL, Salomon B, Bothmer RV (1997) Analysis of tetraploid *Elymus* species using wheat microsatellite markers and RAPD makers. Genome 40:806–814

Sun GL, Salomon B, Bothmer RV (1998a) Characterization and analysis of microsatellite loci in *Elymus caninus* (*Triticeae: Poaceae*). Theor Appl Genet 96:676–682

Sun GL, Salomon B, Bothmer von R (1998b) Characterization of microsatellite loci from *Elymus alaskanus* and length polymorphism in several *Elymus* species (*Triticeae: Poaceae*). Genome 41:455–463

Sun GL, Diaz O, Salomon B, Bothmer RV (1998c) Microsatellite variation and its comparison with allozyme and RAPD variation in *Elymus fibrosus* (Schrenk) Tzvel. *(Poaceae)*. Hereditas 129:275–282

Sun GL, Díaz O, Salomon B, Bothmer RV (1999) Genetic diversity in *Elymus caninus* as revealed by isozyme, RAPD and microsatellite markers. Genome 42, 420–431

Sun GL, Díaz O, Salomon B, Bothmer RV (2001) Genetic diversity and structure in a natural *Elymus caninus* population from Denmark based on microsatellite and isozyme analysis. Plant Syst Evol 227:235–244

Sun GL, Salomon B, Bothmer RV (2002) Microsatellite polymorphism and genetic differentiation in three Norwegian populations of *Elymus alaskanus* (Poaceae). Plant Syst Evol 234:101–110

Treuren R van, Kuittinen H, Karkkainen K, Baena-Gonzalez E, Savolainen O (1997) Evolution of microsatellites in *Arabis petraea* and *Arabis lyrata*, outcrossing relatives of *Arabidopsis thaliana.* Mol Biol Evol 14:220–229

Wang RRC, Bothmer RV, Dvorak J, Fedak G, Linde-Laursen I, Muramatsu M (1994) Genome symbols in the Triticeae (Poaceae). In: Wang RRC, Jensen KB, Jaussi C (eds) Proc 2nd Int Triticeae Symp, Logan Utah, USA, pp 29–34