Transferability of simple sequence repeat (ssr) markers

Transferability of simple sequence repeat (SSR) markersdeveloped inred clover (*Trifolium pratense* L.) to some *Trifolium* species

Abstract

Microsatellite markers previously developed for red clover (*Trifolium pratense* L.) were used in cross-amplification tests of eight other *Trifolium* species. Of the 20 SSR loci tested, 9(47. 37%) had positive results and were found to be transferable to all eight species tested. The number of alleles detected at each locus ranged from 4-12, with an average of 7. 2 in *T. tumens* Steven ex M. Bieb., 5. 8 in *T. resupinatum* L., 5. 1 in *T. fragiferum* L., 4. 5 in *T. tomentosum* L., 3. 4 in *T. bullatum* Boiss. & Hausskn. ex Boiss., 3. 2 in *T. clusii* Godr. & Gren., 2. 7 in *T. spumosum* L. and 2. 1 in *T. physodes* Stev. ex M. B. This study has shown that SSR markers developed for red clover effectively amplify DNA from other species and this approach may be applicable for the analysis of intra- and interspecific genetic diversity of target *Trifolium* speciesfor which, until now, no information about their genomic SSR status has been available.

Keywords: Cross-species transferability, Genomic SSR, *Trifolium* , *T. pratense* .

Introduction

Microsatellites, or Simple Sequence Repeats (SSRs), are one of the most useful DNA markers for studying population genetic structure and dynamics (Moreno *et al* ., 2011; Zhang and Hewitt, 2003). However, the main limit on the use of these markers for analysis of genetic diversity in different species

is the high cost for developing specific primers (Miranda *et al* ., 2012). Thus, a more widespread use of SSRs in plants would be facilitated if we were able to transfer SSR loci across species (Manoj *et al* ., 2013; Gutierrez *et al.* , 2005). Many studies on comparative genetics have revealed that gene content and order are highly conserved among closely related plant species(Manoj *et al* ., 2013; Kalia *et al* ., 2011; Varshney *et al* ., 2005; Kuleung *et al* ., 2004). It is assumed successful transferability depends on the extent of sequence conservation in the primer sites flanking the microsatellite loci and the stability of these sequences during evolution (Moreno *et al* ., 2011; Zhang and Hewitt, 2003). Therefore, primer pairs designed based on the sequences obtained from one species could be used to detect microsatellites in related species. Several studies have also demonstrated the successful cross-transferability of SSR markers from one species to other species of the same genus (Miranda *et al* ., 2012; Castillo *et al* ., 2008; Gimenes *et al.* , 2007; Alves *et al* ., 2006; Bravo *et al* ., 2006). The cross-transferability of SSRs is mainly useful in comparative genome mapping and phylogenetic studies. Also, this method of microsatellite detection is especially useful in species where neither sequence information nor the genetic maps are available (Manoj *et al* ., 2013).

The genus *Trifolium* L. is organized into eight sections including 255 species (Gillett and Taylor, 2001; Zohary and Heller, 1984). Many members of this species have agronomic value, including red clover (*T. pretense*), white clover (*T. repens*), strawberry clover (*T. fragiferum*), and Persian clover (*T. resupinatum*). Except for red and white clovers, little information is available on the genomics of other members of the genus. Thus, in the

present study, we studied the transferability of 20 red clover SSR loci to eight new targets species belonging to *Fragifera* (*T. fragiferum, T. tumens, T. physodes, T. resupinatum, T. tomentosum, T. bullatum* , *T. clusii*) and *Mistyllus* (*T. spumosum*) sections.

Materials and Methods

Plant materials and DNA extraction

Eight *Trifolium* species were studied: *T. fragiferum, T. tumens, T. physodes, T. resupinatum, T. tomentosum, T. bullatum* , *T. clusii* and *T. spumosum.* Plants of each species were identified morphologically according to Haerinasab and Rahiminejad (2012).

Leaves from 8 populations of *T. fragiferum* , 12 populations of *T. tumens* , 1 population of *T. physodes* , 11 populations of *T. resupinatum* , 6 populations of *T. bullatum* , 3 populations of *T. clusii* , 14 populations of *T. tomentosum* and 2 populations of *T. spumosum* were sampled to test transferability of primers previously developed in red clover (*T. pratense*). Genomic DNA was extracted according to Ellison *et al* . (2006). DNA concentration was estimated on 0. 8% agarose gels.

Microsatellite markers and PCR analysis

Twenty primer pairs previously developed for *T. pratense* (Sato *et al* ., 2005) (Table 1) were tested in the eight target species.

Table 1 – Primer Sequences and characteristics of 20 tested red clover specific SSR primer pairs.

Alelle frequency	Reverse primer (5′ to 3′)	Forward primer(5′ to 3′)	Calculated size
12	GAAGAGATAGCTTGCCTTGGA	CACGTTACTCAATTTGGATCTTTG	157
9	TGGGGAAGTGAAGGATGTTC	ATTTGAGCACAAGGCCTCAC	206
6	ATCAACTCGATGGGAACACC	TTTTCTGGCGACGAATTAGG	199
14	GATTTCGATCCTCCTCCTCC	AATAACAATATGCGGCTTTGC	162
13	GCAGATTATGAGGAATAACATTG	AAATTATCATTTTGCAAATTTTA	182
12	TTCAATCGGGAGTGTCAGTG	CGATTGCTACAAACACAGCC	139
4	GGTGCTAGCTCCAACCTCAG	CCTGCTCCGTACCATTGTTT	189
8	GGTGGTGTTGCTGATTACGA	CCTCAGCAGAATCTTCACCC	208
9	CCCCCAAAATACAAAACCCT	GAGAAAAGAAAGAAGTCTCTGAAGGA	220

6	CCTTTCAGAACAGATGGCGT	TACCCTCTTGAGCACCCATT	243
11	CGGCAGACGAAGTGACAAAT	GCCGATATTGCTAGGTTGGA	110
8	CTCGCTGAAGGAGGAAACAG	TGCAAACTCCGCTTTATGC	200
10	AGCTCAAGCTCAACGGACAT	GGCACGAGGCACACTACTTC	107
6	CGAAGCAGGTTGGAAAACAT	GCACGAGGCACACACTACTT	188
7	TTGGCATCTCAAAGCTGAAA	GCCAAGCCCACCAATACATA	231
15	TCTGTTTCTTGTCTCGGCCT	CATGGCTGCCTGAGGTTAAT	212
11	CACTAATTCAGACCACCAGCA	TCGGTGAGCTGTGACTAACG	217
8	AAACAAACCAAGCAGCACCT	ACGGTGGAATTATGGGATGA	244

11	TTCAACATGCAGGCTAAGAAAA	CGCAATCTTTCTTCTCATTTCA	199
8	ATGAGCACCTTCACCAATCC	CATGTCAGCATATCCATTTTCC	280

* Microsatellite loci that generated amplification products in this study.

PCR amplifications were carried out in a volume of 25 µl, containing 1× buffer, 0. 2 mM of each dNTP, 250 nM of each SSR primer (forward, reverse), 1. 5 mM of MgCl2, 50-100 ng template genomic DNA and 1. 2 units of Taq DNA Polymerase.

In order to improve the PCR results, the maximum and minimum optimal annealing temperatures of each pair of primers to be tested were determined (See Table 2.). The PCR thermal profile for all species was as follows: denaturation period of 4 min at 94°C, followed by twenty cycles of 1 min at 94°C, 1 min at the maximum optimal temperature for each primer pair, and 1 min at 72°C, followed by 15 cycles of 1 min at 94°C, 1 min at theminimum optimal temperature for each primer pair, 1 min at 72°C, and a final extension for 10 min at 72°C. PCR products were separated on a 10% non-denaturing polyacrylamide gel using 1× TBE buffer and visualized by the silver staining method of Tixier *et al* . (1997), with the exception of using NaOH instead of $NaCO_3$. All SSR fragments for the 57 populations were scored manually.

Data analysis

The results for the 20 loci were summarized for each species in a vector of 0 (no amplification) and 1 (successful amplification). The number of total alleles detected in all eight species was determined for each SSR locus. The polymorphic information content (PIC) of each SSR marker was calculated using the formula: PIC = 1 – ∑(P *i*) ^{2} (Botstein *et al.* , 1980) where P *i* is the frequency of the *i* th allele calculated for each SSR marker.

Results and discussion

Twenty SSR primer pairs developed for *T. pratense* were tested for their ability to amplify across eight other species of *Trifolium* (*T. fragiferum, T. tumens, T. physodes, T. resupinatum, T. tomentosum, T. bullatum* , *T. clusii* and *T. spumosum*). Transferability was successful for 9 (47. 37%) SSRs in all species tested. From these microsatellites, all of the 9 primers were polymorphic in the target species.

Our results are in accordance with many previous studies that have shown interspecific transferability of SSRs, for example across species of Araucariaceae (31. 8 to 77. 3%; Moreno *et al* ., 2011), in the genus *Glycine* (65%; Peakall *, et al* ., 1998), from *Hordeum vulgare* to *H. bulbosum* (51. 61%; Khodayari *et al.* , 2011) and to *H. chilense* (26%; Castillo *et al* ., 2008), from *Arachis hypogaea* to other *Arachis* species (63. 1%; Gimenes *et al.* , 2007), from *Theobroma cacao* to *Theobroma grandiflorum* (60. 4%; Alves *et al* ., 2006), and across species of the genus *Arachis* (78%; Bravo *et al* ., 2006). Usually, closely related species share similar SSR priming sites, resulting in a high frequency of cross-species amplification. Our findings provide clear evidence for the potential transferability of SSRs across

Trifolium species and demonstrate that these priming site are located in conserved regions of the genome.

In the present study, the number of alleles detected at each locus ranged from 4 to 12, with an average of 7. 2 in *T. tumens* , 5. 8 in *T. resupinatum* , 5. 1 in *T. fragiferum* , 4. 5 in *T. tomentosum* , 3. 4 in *T. bullatum* , 3. 2 in *T. clusii* , 2. 7 in *T. spumosum* and 2. 1 in *T. physodes.* A maximum of 65 alleles were detected for *T. tumens* , while only 19 alleles were detected for *T. physodes* . The number of alleles detected by all transferable SSR loci is listed in Table 2. The number of alleles for each primer determined in this study is different from what has been reported for red clover by Sato *et al* . (2005), except for the RCS1920 and RCS2667 loci (See Table 1 and 2.). According to Wang *et al* . (2009), this may have occurred because of the different methodologies used, namely capillary electrophoresis in the genotyping studies where these markers were originally described and polyacrylamide gel electrophoresis in the present study. Another possible explanation is that these differences are the result of variations in the number of tandem repeat polymorphisms on the tested loci. We also found differences in reproducibility of amplifications, with variable fragment sizes, compared with what has been reported for red clover by Sato *et al* . (2005). Bravo *et al* . (2006) argue that there may be considerable variation both in the number of repetitions as well in the levels of polymorphism between the species for which SSR markers were previously developed and the species that showed a cross reaction.

The PIC values for 9 SSR primers varied from 0. 45 for RCS3666 to 0. 65 for RCS0883 (Table 2).

Table 2. The results of cross-amplification of red clover SSR primers across other *Trifolium* species.

PIC	N0. of alleles	Fragment size (bp)	Annealing temp. range (°C)	SSR ID			
T. spumosum	*T. tomentosum*	*T. clusii*	*T. bullatum*	*T. resupinatum*	*T. tumens*	*T. physodes*	*T. fragiferum*
0. 65	9	9	10	9	12	10	4
0. 59	1	2	2	2	3	4	1
0. 64	3	7	1	4	8	10	2
0. 61	1	7	5	3	9	5	1
0. 51	3	4	1	4	4	5	4
0. 59	1	2	2	2	4	8	1

0. 45	1	1	1	1	1	4	1
0. 59	4	8	6	5	5	9	4
0. 51	1	1	1	1	6	10	1
0. 57	2. 6	4. 5	3. 2	3. 4	5. 7	7. 2	2. 1

In conclusion, the cross-species transferability observed in this study demonstrates the utility of red clover SSRs for the analysis of intra- and interspecific genetic diversity and evolutionary studies of other *Trifolium* species, for which no information on genomic SSRs is available. Our findings provide encouragement for the establishment of effective strategies for conservation of pastureland genetic resources, along the lines proposed byBarbará *et al* . (2007). As highlighted by Noor & Feder (2006), the possibility of cross amplification of genetic markers allows comparative studies among closely related taxa to be carried out, as well as providing some insight into the genetic mechanisms of speciation and population divergence.

References

Alves, R. M., Sebbenn, A. M., Artero, A. S. & Figueira, A. (2006). Microsatellite loci transferability from *Theobroma cacao* to *Theobroma grandiflorum* . Molecular Ecology Notes, 6, 1219-1221.

Barbará, T., Palma-Silva, C., Paggi, G. M., Bered, F., Fay, M. F. & Lexer, C. (2007). Cross-species transfer of nuclear microsatellite markers: potential and limitations. Molecular Ecology, 16, 3759-3767.

Botstein, D., White, R. L., Skolnick, M. & Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. The American Journal of Human Genetics, 32(3), 314–331

Bravo, J. P., Akemi, H. A., Lara, C. M., Angelici, C. D., Lopes, C. R. & Gimenes, M. A. (2006). Transferability and use of microsatellite markers for the genetic analysis of the germplasm of some *Arachis* section species of the genus *Arachis* . Genetics and Molecular Biology, 29(3), 516-524.

Castillo, A., Budak, H., Varshney, R. K., Dorado, G., Graner, A. & Hernandez, P. (2008). Transferability and polymorphism of barley EST-SSR markers used for phylogenetic analysis in *Hordeum chilense* . BMC Plant Biology, 8, 97.

Ellison, N. W., Liston, A., Steiner, J. Williams, M. & Taylor, N. (2006). Molecular phylogenesis of the Clover genus (Trifolium – Leguminnosae). Molecular phylogenetics and evolution, 39, 688-705.

Gillett, J. M. & Taylor, N. L. (2001). The World of Clovers. Iowa State University Press, Ames, Iowa, USA.

Gimenes, M. A., Hoshino, A. A., Barbosa, A. V. G., Palmieri, D. A. & Lopes, C. R. (2007). Characterization and transferability of microsatellite markers of the cultivated peanut (*Arachis hypogaea*). BMC Plant. Biology, 7, 1-13.

Gutierrez, M. V., Vaz Patto, M. C., Huguet, T., Cubero, J. I., Moreno, M. T. & Torres, A. M. (2005). Cross-species ampliï¬ cation of *Medicago truncatula* microsatellites across three major pulse crops. Theoretical and Applied Genetics, 110, 1210–1217.

Haerinasab, M. & Rahiminejad, M. R. (2012). A taxonomic revision of the genus *Trifolium* L. sect. *Fragifera* Koch (Fabaceae) in Iran. Iranian Journal of Botany, 18 (1), 22-30.

Kalia, R. K., Rai, M. K., Kalia, S., Singh, R. & Dhawan, A. K. (2011). Microsatellites markers: an overview of the recent progress in plants. Euphytica, 177, 309–334.

Khodayari, H., Saeidi, H., Rahiminejad, M. R. & Komatsuda, T. (2011). Transferability and polymorphism of barley microsatellite Markers across H-genome containing species in the genus *Hordeum* (*H. vulgare* and *H. bulbosum*). Iranian Journal of Botany, 17 (2), 200-211.

Kuleung, C, Baenziger, P. S. & Dweikat, I. (2004). Transferability of SSR markers among wheat, rye, and triticale. Theoretical and Applied Genetics, 108, 1147–1150.

Miranda, F. D., Gontijo, A. B. P. L., Santiliano, F. C., Favoreto, F. C. & Soares, T. C. B. (2012). Transferability and Characterization of Microsatellite Markers

in five Bromeliaceae species belonging to the subfamilies Pitcairnioideae and Bromelioideae. Biota Neotropica, 12(3), 319-323.

Rai, M. K., Phulwaria, M. & Shekhawat, N. S. (2013). Transferability of simple sequence repeat (SSR) markers developed in guava (*Psidium guajava* L.) to four Myrtaceae species. Molecular Biology Reports, 40(8), 5067-5071.

Moreno, A. C., Marchelli, P., Vendramin, G. G. & Gallo, L. A. (2011). Cross transferability of SSRs to five species of Araucariaceae: a useful tool for population genetic studies in *Araucaria araucana* . Forest Systems, 20(2), 303-314.

Noor, M. A. F. & Feder, J. L. (2006). Speciation genetics: evolving approaches. Nature Reviews Genetics, 7, 851-861.

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

Sato, S., Isobe, S., Asamizu, E., Ohmido, N., Nakamura, Y., Kaneko, T., Sakurai, N., Okumura, K., Klimenko, I., Sasamoto, S., Wada, T., Watanabe, A., Kohara, M., Fujishiro, T. & Tabata, S. (2005). Comprehensive structural analysis of the genome of red clover (*Trifolium pratense* L.). DNA Research, 12, 301-364.

Tixier, M. H., Sourdille, P., Röder, M., Leroy, P. & Bernard, M. (1997). Detection of wheat microsatellites using a non-radioactive silver-nitrate staining method. Journal of Genetics and Breeding, 51, 175-177.

Varshney, R. K., Graner, A. & Sorrells, M. E. (2005). Genic microsatellite markers in plants: features and applications. Trends in Biotechnology, 23, 48–55.

Zhang, D. X. & Hewitt, G. M. (2003). Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. Molecular Ecology, 12, 563-584.

Zohary, M. & Heller, D. (1984). The genus *Trifolium* . The Israel Academy of Science and Humanities. Jerusalem, Israel.