

# [Transferability of simple sequence repeat (ssr) markers](https://assignbuster.com/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(bp)  | SSR motif  | SSR ID  | No  |
| 12  | GAAGAGATAGCTTGCCTTGGA  | CACGTTACTCAATTTGGATCTTTG  | 157  | AAG  | RCS0883\*  | 1  |
| 9  | TGGGGAAGTGAAGGATGTTC  | ATTTGAGCACAAGGCCTCAC  | 206  | AAC  | RCS0907  | 2  |
| 6  | ATCAACTCGATGGGAACACC  | TTTTCTGGCGACGAATTAGG  | 199  | AG  | RCS1479  | 3  |
| 14  | GATTTCGATCCTCCTCCTCC  | AATAACAATATGCGGCTTTGC  | 162  | AAG  | RCS2773  | 4  |
| 13  | GCAGATTATGAGGAATAACATTG  | AAATTATCATTTTGCAAATTTTA  | 182  | AAT  | RCS0033  | 5  |
| 12  | TTCAATCGGGAGTGTCAGTG  | CGATTGCTACAAACACAGCC  | 139  | AC  | RCS2343  | 6  |
| 4  | GGTGCTAGCTCCAACCTCAG  | CCTGCTCCGTACCATTGTTT  | 189  | AAC  | RCS1735\*  | 7  |
| 8  | GGTGGTGTTGCTGATTACGA  | CCTCAGCAGAATCTTCACCC  | 208  | AAG  | RCS2667\*  | 8  |
| 9  | CCCCCAAAATACAAAACCCT  | GAGAAAAGAAAGAAGTCTCTGAAGGA  | 220  | AAG  | RCS1920\*  | 9  |
| 6  | CCTTTCAGAACAGATGGCGT  | TACCCTCTTGAGCACCCATT  | 243  | AGC  | RCS1928\*  | 10  |
| 11  | CGGCAGACGAAGTGACAAAT  | GCCGATATTGCTAGGTTGGA  | 110  | AC  | RCS2202\*  | 11  |
| 8  | CTCGCTGAAGGAGGAAACAG  | TGCAAACTCCGCTTTATGC  | 200  | ATC  | RCS1225\*  | 12  |
| 10  | AGCTCAAGCTCAACGGACAT  | GGCACGAGGCACACTACTTC  | 107  | ATC  | RCS1737  | 13  |
| 6  | CGAAGCAGGTTGGAAAACAT  | GCACGAGGCACACACTACTT  | 188  | ATC  | RCS1518  | 14  |
| 7  | TTGGCATCTCAAAGCTGAAA  | GCCAAGCCCACCAATACATA  | 231  | GGAT  | RCS0843  | 15  |
| 15  | TCTGTTTCTTGTCTCGGCCT  | CATGGCTGCCTGAGGTTAAT  | 212  | AC  | RCS3666\*  | 16  |
| 11  | CACTAATTCAGACCACCAGCA  | TCGGTGAGCTGTGACTAACG  | 217  | AAC  | RCS3052  | 17  |
| 8  | AAACAAACCAAGCAGCACCT  | ACGGTGGAATTATGGGATGA  | 244  | ATC  | RCS1327  | 18  |
| 11  | TTCAACATGCAGGCTAAGAAAA  | CGCAATCTTTCTTCTCATTTCA  | 199  | AAG  | RCS0793  | 19  |
| 8  | ATGAGCACCTTCACCAATCC  | CATGTCAGCATATCCATTTTCC  | 280  | AAAG  | RCS1897\*  | 20  |

\* 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  | 11  | 150-230  | 47-55  | RCS0883  |
| 0. 59  | 1  | 2  | 2  | 2  | 3  | 4  | 1  | 5  | 190-210  | 48-52  | RCS1225  |
| 0. 64  | 3  | 7  | 1  | 4  | 8  | 10  | 2  | 11  | 160-310  | 46-52  | RCS1735  |
| 0. 61  | 1  | 7  | 5  | 3  | 9  | 5  | 1  | 4  | 220-320  | 46-52  | RCS1920  |
| 0. 51  | 3  | 4  | 1  | 4  | 4  | 5  | 4  | 4  | 205-250  | 50-58  | RCS1928  |
| 0. 59  | 1  | 2  | 2  | 2  | 4  | 8  | 1  | 3  | 190-250  | 48-52  | RCS2667  |
| 0. 45  | 1  | 1  | 1  | 1  | 1  | 4  | 1  | 2  | 190-235  | 48-52  | RCS3666  |
| 0. 59  | 4  | 8  | 6  | 5  | 5  | 9  | 4  | 4  | 75-125  | 48-51  | RCS2202  |
| 0. 51  | 1  | 1  | 1  | 1  | 6  | 10  | 1  | 2  | 250-320  | 48-52  | RCS1897  |
| 0. 57  | 2. 6  | 4. 5  | 3. 2  | 3. 4  | 5. 7  | 7. 2  | 2. 1  | 5. 1  |  |  | Mean  |

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.

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