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Transferability of Sequence Tagged Microsatellite Site (STMS) Primers across Four Major Pulses

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Abstract. The transferability of genome-specific sequence tagged microsatellite site (STMS) primers from field pea (*P. sativum*) and chickpea (*C. arietinum*) to other major pulses was examined. Overall, field pea STMS primers amplified products in most of the accessions in comparison to that of the chickpea STMS primers, which amplified products in relatively few accessions. The highest level of successful amplifications with a single primer was 89% for field pea and 33% for chickpea primers respectively. The potential transferability of the STMS primers among species, expressed as the total mean percentage of positive amplifications, was 53% for the field pea STMS primers and 9% for the chickpea STMS primers. The individual mean percentage of successful transferability of field pea STMS primers across lentil, vetch and chickpea/*Cicer* sp. accessions was 60%, 39% and 62%, respectively. Whereas, for the chickpea STMS primers successful transferability was 5%, 3% and 18% for lentil, vetch and field pea, respectively. The transferability of these STMS primers indicates a high level of sequence conservation in these regions across species. Together with their locus-specificity, co-dominant nature and potential to amplify multiple alleles, their transferability makes STMS markers a powerful tool for genetic mapping, diversity analysis and genotyping.

Key words: conservation, pulse, STMS, transferability

Introduction

Microsatellites are short sequence head-to-tail repeats of approximately 1 to 5 nucleotides in length that are present in genomes of all higher eukaryotes (Tautz and Renz, 1984; Lagercrantz et al., 1993). Also known as simple sequence repeats (SSRs) or simple tandem repeats (STRs), variation in tandem repeat number at a particular locus often causes the length of the microsatellite to vary (Levinson and Gutman, 1987; Jeffreys et al., 1988; Zischler et al., 1992). For the amplification of the regions between SSRs, inter SSR PCR technique employs 5'- or 3'-anchored nucleotide repeats as single PCR primers. Sequence tagged site (STS) markers are derived usually from known sequences in the genome and have been successfully used for wheat (Talbert et al., 1994), barley (Tragoonrung et al., 1992) and rice (Inoue et al., 1994).

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The regions flanking microsatellite repeats are more conserved and often a valuable source for the design of locus-specific primers to amplify the internal microsatellite regions. Primers designed to these flanking regions amplify sequence tagged microsatellite sites (STMSs). The production of STMS primers involves the following steps: 1) a genomic library is screened for microsatellite repeats, 2) clones containing repetitive sequences are identified, and 3) locus-specific primers which flank the repeats are designed. Their co-dominant mode of inheritance, high level of polymorphism and ubiquitous occurrence throughout the genome therefore ensures that STMS markers will have extensive applications in genetic mapping (Weising et al., 1998). Thus, STMS are becoming the marker of choice, when compared to RAPDs and other methods of oligonucleotide fingerprinting, due to the highly reproducible and reliable identification of alleles.

So far, there are reports on the conservation of primer binding sites among different species of whale (Schlotterer et al., 1991) and primates (Deka et al., 1994). For plants, the regions flanking microsatellites were found to be conserved within brassica genera (Lagercrantz et al., 1993; Kresovich et al., 1995) chickpea (Hüttel, 1996) and citrus (Kijas et al., 1995). Primer sequence conservation of microsatellite markers has been reported among cultivated rice subspecies, and related wild species (Wu and Tanksley, 1993), and grapevine species (Thomas and Scott, 1993). Potential transferability of sequence tagged site (STS) PCR markers between wheat and barley has been demonstrated (Erpelding et al., 1996) and also the transferability of soybean simple sequence repeats (SSRs) loci to other legume genera has been established (Peakall et al., 1998). The conservation of chickpea STMS loci between accessions and species of *Cicer* has been reported (Choumane et al., 2000). Since sequence information of repetitive and flanking regions is not available for many of the major crop species, transferability of STMS loci across species would be highly advantageous. Moreover the laborious cloning, sequencing and screening procedures employed in order to identify potential species-specific STMS markers, would be reduced if markers from related species were transferable.

The aims of this study were to:

1. explore the transferability of field pea STMS primers to lentil, vetch and chickpea/*Cicer* sp;
2. to explore the transferability of chickpea STMS primers to lentil, vetch and field pea; and
3. to analyze the potential of primer sequence conservation between four major pulses in order to evaluate the potential transferability of STMS markers for genetic mapping purposes.

Materials and Methods

Plant material and DNA isolation

Seeds of vetch (*Vicia* sp.), lentil (*Lens culinaris* ssp. *culinaris*), chickpea (*Cicer arietinum*), *Cicer* sp., and field pea (*Pisum sativum*) were obtained from The Victorian Institute for Dryland Agriculture (VIDA), Horsham, Australia (Table 1).

Table 1. List of plant material used in this study.

Pulse	Species	Accession	Sample number
Vetch	<i>Vicia sativa ssp. sativa</i>	Blanchefleu	1
	<i>Vicia sativa ssp. sativa</i>	Languedoc	2
	<i>Vicia villosa ssp. dasycarpa</i>	Capello	3
	<i>Vicia benghalensis</i>	Popany	4
	<i>Vicia villosa ssp. dasycarpa</i>	Namoi	5
	<i>Vicia ssp.</i>	Wild vetch	6
Lentil	<i>Lens culinaris ssp. culinaris</i>	Cobber	1
	<i>Lens culinaris ssp. culinaris</i>	Digger	2
	<i>Lens culinaris ssp. culinaris</i>	Laird B	3
	<i>Lens culinaris ssp. culinaris</i>	Indianhead	4
	<i>Lens culinaris ssp. culinaris</i>	ILL0882	5
	<i>Lens culinaris ssp. culinaris</i>	ILL4532	6
Chickpea	<i>Cicer arietinum</i>	Kaniva	1
	<i>Cicer arietinum</i>	Lasseter	2
	<i>Cicer arietinum</i>	ATC41890	3
Cicer sp.	<i>Cicer echinospermum</i>	ATC42013	4
	<i>Cicer bijugum</i>	ATC42258	5
	<i>Cicer reticulatum</i>	ATC42291	6
Field pea	<i>Pisum sativum</i>	Excell	1
	<i>Pisum sativum</i>	Bohatyr	2
	<i>Pisum sativum</i>	Soupa	3
	<i>Pisum sativum</i>	Paravic	4
	<i>Pisum sativum</i>	Alma	5
	<i>Pisum sativum</i>	Magnet	6

Seedlings were grown in 10 cm diameter pots in the glasshouse. DNA extractions were carried out from young leaves using the adapted CTAB method of Taylor et al. (1995). The quantity of DNA was measured spectrophotometrically at $\lambda=260$ nm and the working concentrations were adjusted to 10 ng/ μ L.

STMS PCR amplifications

For PCR, twenty-two STMS primer-pairs each for field pea and chickpea were chosen based on similarity in melting temperature ($\sim 60^\circ\text{C}$; Table 2). The primer sequences for chickpea STMS primers were developed by Winter et al. (1999); and for field pea the sequences were developed by members of the Agrogene field pea microsatellite consortium (Agrogene, France). PCR was performed against six accessions from each of the pulse groups; field pea, chickpea/*Cicer* sp., lentil and vetch (Table 1). PCR amplification was performed in a 25 μ L volume containing 50 ng of genomic DNA, 0.75 units of *Taq* DNA polymerase (Gibco, BRL Life Technologies, USA), 0.24 mM each of dATP, dCTP, dGTP and dTTP, 1.0 μ M of each primer (Gibco, BRL Life Technologies, USA) and buffer with a final concentration of 2.5 mM MgCl_2 (Gibco, BRL Life Technologies, USA). PCR cycling conditions were an initial denaturation step at 94°C for 3 min, followed by 37 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 2 min with a final extension step at 72°C for 5 min. PCR products were run on a 1.4% agarose gel in TBE

Table 2. Transferability of field pea STMS primers across species. Accessions names are given in Table 1.

Field pea STMS primers	Vetch					Lentil					Chickpea/ <i>Cicer</i> sp.					percent transferability			
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3		4	5	6
PSMPA6 ^a	+						+	+	+	+			+	+	+	+	+	+	66.6
PSMPA7 ^a		+					+	+	+	+	+			+	+	+	+	+	72.2
PSMPA8 ^a	+	+	+	+	+		+	+	+	+	+				+	+	+	+	77.7
PSMPA9 ^a	+	+					+	+	+	+	+					+	+	+	55.5
PSMPB14 ^a		+					+	+	+	+	+	+			+			+	55.5
PSMPB16 ^a	+																		5.5
PSMPD21 ^a	+	+											+		+	+	+	+	38.8
PSMPD24 ^a	+	+	+	+	+		+	+	+	+	+	+	+		+				72.2
PSMPSAA1 ^a	+	+					+	+	+	+				+	+			+	50.0
PSMPSAA5 ^a									+	+						+		+	22.2
PSMPSAA7 ^a			+			+									+			+	16.6
PSMPC20 ^a			+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	83.3
PSMPSAA135 ^b		+	+	+			+	+	+	+	+			+	+	+	+	+	72.2
PSMPA5 ^a		+	+	+			+	+	+	+	+	+	+	+	+	+	+	+	83.3
PSMPSAD118 ^c																			0.0
PSMPSAD123 ^c	+	+		+	+		+	+	+	+		+		+	+		+	+	72.2
PSMPSAD126 ^c			+		+		+	+	+	+	+	+	+	+				+	66.6
PSMPSAD134 ^c									+										5.5
PSMPSAD135 ^c	+	+		+	+		+		+	+	+	+	+	+	+	+	+	+	83.3
PSMPSAD138 ^c	+	+	+	+			+	+	+	+	+	+	+	+	+	+	+	+	88.8
PSMPSAD141 ^c	+													+		+	+	+	27.7
PSMPSAD144 ^c	+	+	+	+			+							+		+	+	+	55.5

Sources:

^aDanisco Seed, Højbygardvej 14, Holeby, Denmark.

^bFrederic Muel, GSP, 12 Av Georges V, 75008 Paris, France.

^cPaul Taylor, University of Melbourne, Parkville 3052, Victoria, 3010 Australia.

buffer, stained with ethidium bromide and visualized with an UV transilluminator. Amplification products were scored as positive (+) only if an intense, reproducible band was observed. Mean scores, based on mean ability to amplify accessions tested within a species, were calculated as a measure of primer transferability.

Results and Discussion

Measures of intra- and inter-species transferability of field pea and chickpea STMS primers to the other three pulse species tested are shown in Tables 2 and 3 respectively. Overall, the *Pisum*-derived STMS primers amplified microsatellite sequences from the majority of the accessions assessed in this study (Figure 1). In comparison, the *Cicer*-derived STMS primers amplified relatively few sequences. Only one field pea STMS primer-pair was unable to amplify across all the 18 accessions compared to the 11 chickpea STMS primers (Tables 2 and 3).

The percentage of successful transferability of field pea and chickpea derived STMS primers are shown in Tables 2 and 3, respectively. Five out of the 22

Table 3. Transferability of Chickpea STMS primers across species. Accessions names are given in Table 2.

Chickpea STMS primers ^d	Vetch					Lentil					Field pea					percent transferability				
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3		4	5	6	
TA8	+	+												+			+			22.2
TA21														+			+			11.1
TAA55							+	+	+	+										27.7
TA64																				0.0
TA96				+																11.1
TA114																				0.0
TA180				+					+								+			16.6
TA200																				0.0
GA16																	+			5.5
GA102														+			+			11.1
TR29																				0.0
TR44																				0.0
TAA137																				0.0
TS45																				0.0
TS53																				0.0
TS57																				0.0
TS72																				0.0
TA1						+		+		+	+	+						+		33.3
TA2						+				+				+	+			+		27.7
TA3																				0.0
TA18			+		+		+													16.6
TA39																+	+			11.1

Source:

^dPrimer sequence obtained from Winter et al., 1999.

field pea STMS primer-pairs were >75% successfully transferable across the 3 genera or 9 species and 18 accessions assessed. Moreover, field pea STMS primer-pair, PSMPSAD138 was most successful, amplifying 16 out of the total 18 accessions (89%). Furthermore, 14 field pea STMS primer-pairs produced >50% positive amplifications and four field pea primer-pairs were between 10 and 40% successful. Chickpea STMS primers however, amplified products from a limited number of accessions with no amplification products produced from the majority of accessions. For chickpea STMSs, the highest level of successful amplification was 33% generated by primer-pair TA1, 10 other primer-pairs amplified at a success rate between 5 and 30%. The potential transferability of STMS primers to amplify across species, expressed as the total mean percentage of successful amplification, was 53% for field pea STMS primers and 9% for chickpea STMS primers. The individual mean percentage of successful transferability of field pea STMS primers across lentil, vetch and chickpea/*Cicer* species was 60%, 39% and 62% respectively. For the chickpea STMS primers it was 3%, 5% and 18% for vetch, lentil and field pea, respectively.

Extensive polymorphism of the flanking regions was observed between species as well as among accessions within species as detected by differences in the size and number of the amplification products obtained. For instance, the number

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 M

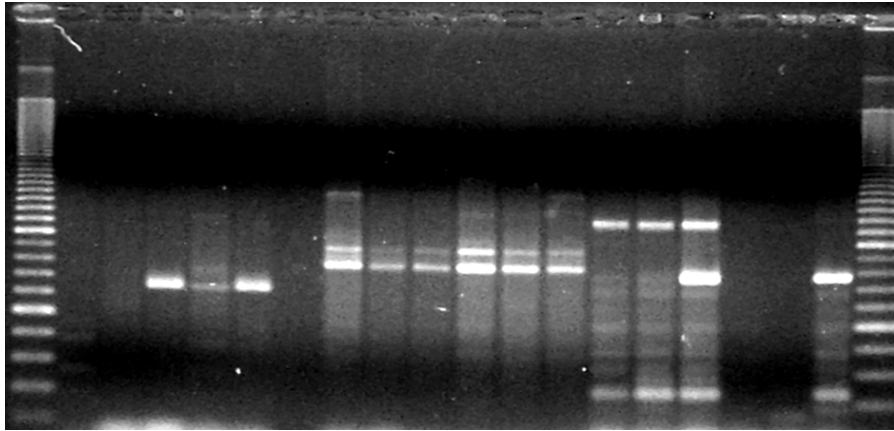


Figure 1. Transferability analysis of field pea STMS primer-pair, PSMPAD126 across major pulse species. Lanes 1-6 = *Vicia* sp; lanes 7-12 = *Lens culinaris* ssp. *culinaris*; lanes 13-15 = *Cicer arietinum*; lanes 16-18 = *Cicer* sp. and M = molecular weight marker.

of amplification products generated by the field pea STMS primer-pair, PSMPAD138 ranged from two for accession kaniva of chickpea and six for accession cobber of lentil. Differences in number of alleles amplified could be caused either by the production or loss of primer binding sites at similar loci. In the case of the field pea primer-pairs PSMPA5 and PSMPC20, a monomorphic 200 kbp fragment was amplified in 15 accessions representing lentil, vetch and chickpea/*Cicer* species. Difference in the sizes of the amplification products was also noticed. Variation in the size of bands from different individuals was likely to be due to differences in the number of tandem repeats present between the primer sites (Choumane et al., 2000). Field pea STMS primer-pair PSMPA9 produced cultivar-specific bands such as a 300 kbp fragment in the vetch accessions blanchefleur and languedoc, a 700 kbp band in five of the lentil accessions and a 900 bp product in three of the chickpea accessions; ATC42013, ATC42258 and ATC42291.

Transferability of field pea and chickpea derived STMS markers, across four major pulses was confirmed in this study. Field pea STMS primers successfully amplified across most accessions used, indicating a very high level of sequence conservation among the flanking regions of these microsatellites regions. This was not surprising, since field pea, vetch and lentil, belong to the same tribe, *Vicieae*. However, the transferability of chickpea STMS primers across field pea, lentil and vetch was low. This indicated that the sequences flanking the microsatellite regions in chickpea were different to that in the other pulses. This may be expected since chickpea is placed in the tribe, *Cicereae* (Davidson and Davidson, 1993). Alternatively, the methodology used to identify the flanking sequence of the microsatellites in chickpea may have been more selective for *Cicer*-specific sequences. Whereas the methods used for field pea sequence identification may have selected sequence regions highly conserved across the pulses. The true

benefits of the transfer of these markers between species lie in the reduced need to undergo expensive and labor intensive methodology to develop species-specific STMS markers.

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