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Evaluation of simple sequence repeat (SSR) markers from *Solanum* crop species for *Solanum elaeagnifolium*

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Summary

Molecular markers specific for *Solanum elaeagnifolium* (silverleaf nightshade) are currently not available. A total of 35 simple sequence repeat (SSR) primer pairs from potato (*Solanum tuberosum*), tomato (*S. lycopersicum*) and eggplant (*S. melongena*) were tested for cross-species transferability in *S. elaeagnifolium*. Among them, 13 primer pairs successfully produced alleles (bands). The polymorphism information content ranged from 0 to 0.84. The transferable rate of SSR from potato, tomato and eggplant to *S. elaeagnifolium* was 20%, 40% and 46% respectively. SSR analysis revealed high level of genetic diversity among 40 individuals collected within a paddock. Highly polymorphic and transferable cross-species SSR markers would be useful for determining the extent of genetic diversity in *S. elaeagnifolium* populations.

Keywords: silverleaf nightshade, invasive weed, cross-species SSR, microsatellite, genetic diversity.

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Introduction

Solanum elaeagnifolium Cav. (silverleaf nightshade) is a diploid (2n = 2x = 24), deep-rooted, summer growing perennial that originated in south-western United States and northern México (Stanton *et al.*, 2009). It reproduces both sexually (obligate outcrossing) and vegetatively (Hardin *et al.*, 1972). *Solanum elaeagnifolium* has been introduced around the world (Stanton *et al.*, 2009). It was first reported in Australia in 1901 and gradually spread over New South Wales (NSW), Victoria (VIC) and South Australia (SA) (Stanton *et al.*, 2009). This invasive weed currently infests at least 350 000 hectares in Australia, with the potential to infest 398 million hectares (Feuerherdt, 2009). *Solanum elaeagnifolium* competes with pastures and other crops for soil water and nutrients and causes up to 77% reduction in cereal crop yields (Stanton *et al.*, 2009). Current management strategies are ineffective and unreliable, especially for dense and large infestations (Wassermann *et al.*, 1988). Improved management of this weed would require a better understanding of genetic diversity in *S. elaeagnifolium*, because genetically diverse weed species will affect the choice of appropriate control strategies, such as the selection of biocontrol agents (Dekker, 1997).

Molecular markers such as simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD) have been widely used in the assessment of

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genetic diversity in weeds (O'Hanlon *et al.*, 2000). However, there is limited information available on the level of genetic variation in *S. elaeagnifolium*. Hawker *et al.* (2006) employed RAPD markers to investigate genetic diversity of *S. elaeagnifolium* populations in SA and found high levels of variation. SSR markers have been shown to be much more polymorphic and reproducible between laboratories than RAPD markers (McGregor *et al.*, 2000). Molecular marker resources specific to *S. elaeagnifolium* have not been developed worldwide. However, SSR primers sequences have been published and applied in genetic diversity analyses of some members of *Solanum*: potato (*Solanum tuberosum* L.), tomato (*S. lycopersicum* L.) and eggplant (*S. melongena* L.).

In this study, the transferability of cross-species SSR markers derived from potato, tomato and eggplant to *S. elaeagnifolium* was evaluated, and the suitability of these markers for genetic diversity analysis validated using *S. elaeagnifolium* plants collected from a single paddock in NSW. These SSR markers could be used in the future to investigate the genetic diversity of *S. elaeagnifolium* throughout Australia and elsewhere around the world.

Materials and methods

Plant material

Young leaf samples from 40 individuals were collected following a zigzag pattern (Menchari *et al.*, 2007) from a paddock heavily infested with *S. elaeagnifolium* at 4–5 plants m^{-2} in Ungarie, central west NSW [latitude (N): −35°36′, longitude (E): 146°55′]. Individuals were sampled at least 50 m apart, to reduce the probability of sampling clonal material. Leaves were collected from each plant and placed in 1.5 mL Eppendorf tubes and then stored at −80°C in the laboratory until DNA extraction.

DNA extraction

Genomic DNA was isolated from leaf material using a standard phenol/chloroform method (Sambrook *et al.*, 1989). Quality of DNA was checked by electrophoresis (MI-DEAR, SYS-MD 120, USA) in a 1.0% agarose gel at 200 V for 10 min.

PCR and SSR analysis

Thirty-five SSR primer pairs (synthesised by Sigma Aldrich, Sydney, Australia) were selected on the basis of their high polymorphism information content (PIC) values in the source species (Tables 1 and S1). A nineteen-nucleotide-long M13 sequence (5′-CAC GAC GTT GTA AAA CGA C-3′) was tailed to the 5′ end of the forward primer of each SSR primer pair (Raman *et al.*, 2005).

PCR amplification was carried out in 12 μL of reaction mixture consisting of 50–100 ng of template DNA, 1.2 μL of 10× buffer, 0.24 μL of 25 mM $MgCl_2$, 1.2 μL of 2 mM dNTP's, 0.08 μL of Taq (5 units μL^{-1}) (Promega, Australia), 0.05 μL of forward primer (3 μM μL^{-1}), 0.1 μL of reverse primer (3 μM μL^{-1}), 0.15 μL of M13 (2 μM μL^{-1}) labelled with one of the fluorescent dyes (D2, D3 or D4; Beckman Coulter, Brea, CA, USA). After an initial denaturation of 4 min at 94°C, 30 cycles of 1 min at 94°C, 1 min at 45–58°C (depending on the primers, Tables 1 and S1) and 1 min at 72°C were performed, followed by a final extension of 10 min at 72°C. The amplifications were carried out in a Gene Amp PCR System 2700 (Applied Biosystems, Singapore). PCR products were separated on a CEQ 8000 genetic analysis system (Beckman Coulter Inc.) as described previously (Raman *et al.*, 2005). Individuals with null alleles were confirmed by at least three different PCR amplifications. In addition, null alleles detected for single-locus SSRs were further checked using the software program Micro-Checker, set at the 95% confidence interval (Van Oosterhout *et al.*, 2004).

Data analysis

Scoring of SSRs was based on their amplified fragment size (base pairs). The PIC value of each SSR locus was calculated by PowerMarker V3.0 software (Liu & Muse, 2005), while the observed and expected heterozygosity were calculated using POPGENE version 1.32 (Yeh & Boyle, 1997). For each fragment size, binary scores (1 for present, 0 for absent) were assigned for each allele to calculate genetic similarity matrices.

A similarity matrix for all test samples was calculated by Jaccard's coefficient using Similarity for Qualitative data (SIMQUAL) in NTSYS-pc 2.1 (Rohlf, 2000). A dendrogram based on this matrix was computed to illustrate genetic relationships among individuals. This was performed by the Unweighted Pair Group Method with Arithmetic mean (UPGMA) using the SAHN procedures of the same software. The relationship between the similarity and cophenetic (ultrametric) matrices was determined by Mantel test using the matrix comparison plot based on the product-moment correlation, *r*, and 1000 permutations were used in the Mantel test.

Results

Thirteen of the 35 tested markers (37%) generated bands (Table 1). Primers from eggplant had the highest levels of

Table 1 Cross-species simple sequence repeat (SSR) primers that generated amplicons in *S. elaeagnifolium*, including eight producing results consistent with single-locus SSRs and five consistent with multi-locus SSRs

Locus	Repeat motif	Source species	PIC_{SO}	T_A (°C)	Primer sequence (5′–3′)	Reference
Single-locus amplification						
STI001	$(AAT)_n$	Potato	0.69	58	F: CAGCAAAATCAGAACCCGAT R: GGATCATCAAATTCACCGCT	Ghislain *et al.*, (2009)
CA158	(GA)32	Tomato	0.85	55	F: CATGCACGTACAACCTGTTT R: TAGTTCCCTTGCTGCAGTAA	Martins *et al.*, (2006)
SSR63	(AT)39	Tomato	0.80	50	F: CCACAAACAATTCCATCTCA R: GCTTCCGCCATACTGATACG	Kwon *et al.*, (2009)
SSR111	(TC)6(TCTG)6	Tomato	0.88	50	F: TTCTTCCCTTCCATCAGTTCT R: TTTGCTGCTATACTGCTGACA	Kwon *et al.*, (2009)
TSR2	(AT)15	Tomato	0.81	50	F: TCAAGTGAGTTTATCTGCCCAC R: GCTCATCCTACACATTCATGCTC	Yi *et al.*, (2008)
EM135	(CA)11(GA)20	Eggplant	0.75	58	F: ATCCTGTTGCTGCTCATTTTCCTC R: AGGAGGATCCAAGAGGTTTGTTGA	Nunome *et al.*, (2003)
EM140	(AC)4GC(AC)5T(AC)3ATGC(AC)4AT(AC)6(AT)5G(TA)13	Eggplant	0.52	53–48	F: CCAAAACAATTTCCAGTGACTGTGC R: GACCAGAATGCCCCTCAAATTAAA	Munoz-Falcon *et al.*, (2009)
EM141	(AT)16(GT)19	Eggplant	0.83	50	F: TCTGCATCGAATGTCTACACCAAA R: AAAAGCGCTTGCACTACACTGAAT	Nunome *et al.*, (2003)
Multi-locus amplification						
STG10	$(TG)_n$	Potato	0.69	55–50	F: CGATCTCTGCTTTGCAGGTA R: GTTCATCACTACCGCCGACT	Ghislain *et al.*, (2009)
EM117	(AC)19(AT)11	Eggplant	0.74	55	F: GATCATCACTGGTTTGGGCTACAA R: AGGGGAGAGGAAACTTGATTGGAC	Nunome *et al.*, (2003)
EM127	(AC)13(AT)13	Eggplant	0.60	55–50	F: CAGACACAACTGCTGAGCCAAAAT R: CGGTTTAATCATAGCGGTGACCTT	Munoz-Falcon *et al.*, (2009)
EM155	(CT)38	Eggplant	0.64	50–45	F: CAAAAGATAAAAAGCTGCCGGATG R: CATGCGTGAGTTTTGGAGAGAGAG	Munoz-Falcon *et al.*, (2009)
ESM3	(TA)9(GA)8	Eggplant	0.51	55–50	F: ATTGAAAGTTGCTCTGCTTCAC R: ACATCGTTCCGCCTCTATTG	Munoz-Falcon *et al.*, (2009)

PIC_{SO}, polymorphism information content (PIC) in source species; T_A, annealing temperature used in this study.

transferability (46%, 7/15) to *S. elaeagnifolium*, followed by tomato (40%, 4/10) and potato (20%, 2/10) (Table S1). A total of 88 bands were amplified from these 13 SSR loci, ranging from one for the SSRs TSR2 and EM141 to 21 for the SSR EM117 (Table 2). Eight of these SSR primer pairs produced 1 or 2 discrete bands and were considered as single-locus markers. Among them, two (TSR2 and EM141) were monomorphic. Amplification of five primer pairs (STG10, EM117, EM127, EM155 and ESM3) resulted in the amplification of three or more bands, consistent with multilocus gene expression; therefore, these bands were scored as present or absent. The observed heterozygosity ranged from 0 to 0.85 and the expected heterozygosity from 0 to 0.87. The PIC value, a measure of allelic diversity, varied from 0 for the primers TSR2 and EM141 to 0.84 for the primer CA158.

Jaccard's genetic similarity coefficients among the 40 individuals analysed in this study ranged from 0.15 to 0.79, with the mean value of 0.37 (Table S2), indicating high genetic diversity among the individuals within this paddock.

Both product-moment correlation, r (0.75), and the Mantel test statistic, Z (t = 9.55), were highly significant ($P < 0.01$) for the clustering shown in Fig. 1. According to the UPGMA dendrogram, two main clusters were defined (Fig. 1). Seven individuals were grouped into Custer A and the remaining 33 individuals formed Cluster B.

Discussion

Transferability of SSR markers

This study is the first report evaluating cross-species SSR markers in *S. elaeagnifolium*. A total of 13 cross-species SSR markers amplified DNA fragments in this weed. The rate of SSR transferability to *S. elaeagnifolium* ranged from 20% to 46%, depending on the source *Solanum* species. Higher transferability of SSR markers was observed between *S. elaeagnifolium* and eggplant and tomato, as compared with potato. It is possible that closely related species may have similar primer binding sites (Rossetto, 2001). Both

Table 2 Comparison of information provided and band size of 13 simple sequence repeat (SSR) markers between *S. elaeagnifolium* and source species, including eight producing results consistent with single-locus SSRs and five consistent with multi-locus SSRs

Locus	N_A/N_B	H_O	H_E	PIC_{SE}	FS_{SO}	FS_{SE}	Fluorescent Dye used
Single-locus amplification							
STI001	2	0.23	0.20	0.18	185–208	205–211	D3
CA158	13	0.43	0.87	0.84	198–250	217–249	D3
SSR63	2	0	0.18	0.16	250	Null–183	D2
SSR111	4	0	0.54	0.49	188	Null–179	D3
TSR 2	1	0	0	0	219–301	286	D3
EM135	11	0.85	0.75	0.70	260	233–262	D4
EM140	5	0	0.62	0.54	277–290	Null–223	D4
EM141	1	0	0	0	228	184	D4
Multi-locus amplification							
STG10	5	–	–	–	175–192	177–271	D4
EM117	21	–	–	–	123	120–172	D2
EM127	12	–	–	–	200–210	Null–294	D4
EM155	4	–	–	–	232–264	113–298	D3
ESM3	7	–	–	–	230–243	Null–351	D4
Mean	6.77						

N_A, number of alleles in *S. elaeagnifolium*; N_B, number of bands in *S. elaeagnifolium*; observed (H_O) and expected (H_E) heterozygosity; PIC_{SE}, average PIC of each marker in *S. elaeagnifolium*; FS_{SO}, fragment size of source species; FS_{SE}, fragment size of *S. elaeagnifolium*.

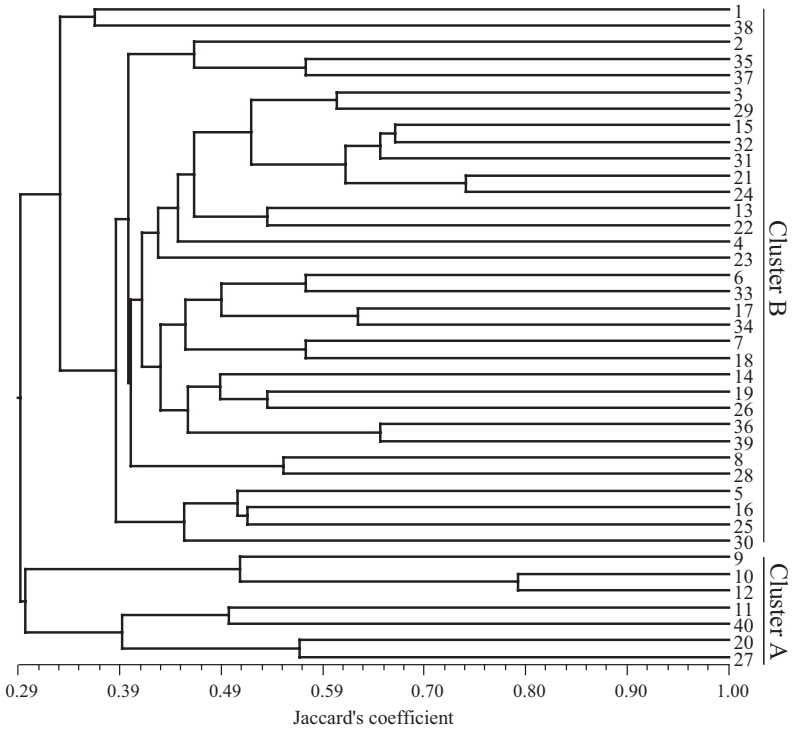

Fig. 1 Unweighted pair group method with arithmetic mean (UPGMA) based dendrogram showing the genetic variation of 40 individual samples of *S. elaeagnifolium* collected from Ungarie, NSW, Australia.

S. elaeagnifolium and eggplant are located in the *Leptostemonum* clade of *Solanum*, while tomato and potato are located in the *Potato* clade, based on *ndhF*, *trnTF* and *waxy* DNA sequence data (Weese & Bohs, 2007). Varying rates of cross-species SSR transferability have been reported previously. For example, Torres *et al.* (2008) found a low (27%) transferability rate of SSR markers from potato to naranjilla (*Solanum quitoense* Lam.). However, the within-subgenus transferable rate of SSR markers in these two *Solanum* studies is much lower than in other species, such as in *Magnolia* (90.9%) and *Vitis* (93.5%) (Rossetto, 2001). *Solanum*, as

one of the largest genera of flowering plants, might have undergone a long evolutionary process or have a much higher speciation rate than other species (Whalen & Caruso, 1983). These factors may cause genetic divergence among *Solanum* species, which may be one of the reasons leading to poor amplification when using cross-species genetic markers (Whalen & Caruso, 1983; Torres *et al*., 2008). The results presented here suggest that eggplant and tomato are reliable sources of cross-species SSRs for *S. elaeagnifolium*.

Information provided by markers

Differences in the level of SSR polymorphism (0–0.84) were observed (Table 2). This may be attributed to gene conservation between source species and *S. elaeagnifolium,* source of the SSRs (genomic and EST-SSR) and/or nature of the SSRs (nucleotide repeat unit, such as di-, tri- and tetra). These results show that the high PIC values of SSR markers in source species (Tables 1 and S1) were not preserved in *S. elaeagnifolium*. For example, the SSR markers of high PIC values in source species, such as STI001, SSR63, TSR 2 and EM141, did not possess similarly high PIC values in *S. elaeagnifolium* (Tables 1 and 2). Similarly, it has also been reported that many SSR markers when transferred from *Cirsium acaule* (L.) Scop. and *Zostera marina* L. to other *Cirsium* and *Zostera* species, respectively, did not produce similar levels of polymorphism (Reusch, 2000; Jump *et al*., 2002). This phenomenon suggests that a highly informative marker from a source species does not necessarily lead to high levels of polymorphism in a test species. In addition, some of these 13 SSR loci did not produce similar sized alleles in this investigation compared with those in the source species (SSR63, EM140, EM141, EM155, EM127 and ESM3) (Table 2). Differences in the size of alleles in the source species and the individuals analysed in this study may be attributed to chromosomal rearrangements during the evolution of the *S. elaeagnifolium* genome, or strand slippage during DNA replication.

We considered five SSRs that generated multiple bands as multilocus markers. Amplification of multiple alleles might be attributed to the divergence and/or duplication of genomic regions (Senthilvel *et al*., 2008), which has been detected in many plants such as pearl millet [*Pennisetum glaucum* (L.) R.Br.] and tea (*Camellia sinensis* L.) (Senthilvel *et al*., 2008; Sharma *et al*., 2009).

Genetic variation among Solanum elaeagnifolium

A high level of genetic variation was identified in the population studied, having an average genetic similarity of 0.37. Two main groups were clustered according to a UPGMA dendrogram (Fig. 1). The predominant 'Cluster B' contains 82.5% (33) of the individuals, distributed across the paddock. This may be a reflection that the spread of *S. elaeagnifolium* within a paddock can be assisted by cultivation practices and grazing animals (Stanton *et al*., 2009). 'Cluster A' consisted seven individuals which were also distributed across the paddock. No specific grouping of individual samples was observed according to GPS location (data not shown). 'Cluster A' may be indicative of the range of genetic diversity among individuals in this paddock. Alternatively, these individuals may have been introduced into the paddock from elsewhere. Dispersal of *S. elaeagnifolium* from one paddock to another may occur through seed-contaminated fodder, agricultural produce and/or farm machinery (Stanton *et al*., 2009). Differentiating between these two alternative scenarios requires further genetic analysis of additional paddocks. The high level of genetic variation identified in this study is consistent with the report of Hawker *et al*. (2006). It is believed that a high level of genetic diversity will contribute to the adaptation of weed species to various environments and contribute to the capacity of weeds to respond to selection pressures, reducing the effectiveness of weed management (Dekker, 1997). For example, differential herbicide responses have been reported in many weed species, such as *Alopecurus myosuroides* Huds. (blackgrass) (Marshall & Moss, 2008) and *Amaranthus retroflexus* L. (redroot pigweed) (Scarabel *et al*., 2007). A genetically diverse weed species could also limit the effectiveness of biocontrol. The biocontrol agent, *Puccinia chondrillina*, showed differential pathogenicity between genotypes of *Chondrilla juncea* L. (Burdon *et al*., 1984). Therefore, the high genetic variation of *S. elaeagnifolium* may represent a challenge to effectively managing this weed using biological control.

In conclusion, this research demonstrates the transferability of 13 cross-species SSR markers for genetic research in *S. elaeagnifolium*. The highly polymorphic SSRs identified in this study can be used for genetic mapping, genetic diversity analysis and molecular evolution studies. These SSR markers are currently being employed to determine the genetic diversity of *S. elaeagnifolium* collected from different regions of Australia. To this end, amplicons from multi-locus SSRs could be sequenced and then the flanking sequence of SSRs could be used to generate single-locus markers.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Cross-species SSR primers not transferable to *S. elaeagnifolium*. PIC_{SO}, polymorphism information content (PIC) in source species; T_A, annealing temperature used in this study.

Table S2 Matrix of genetic similarity between individuals based upon Jaccard's coefficient.

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