Genetic variation in *Solanum elaeagnifolium* in Australia using SSR markers

Xiaocheng Zhu[A,B], Hanwen Wu[A,C], Harsh Raman[A,C], Deirdre Lemerle[A,B], Rex Stanton[A,B] and Geoffrey E. Burrows[A,B]

[A]Graham Centre for Agricultural Innovation
[B]School of Agricultural and Wine Sciences, Charles Sturt University, Locked Bag 588,
[C]Wagga Wagga Agricultural Institute, PMB,
Wagga Wagga, New South Wales 2678, Australia.

Summary
Silverleaf nightshade (*Solanum elaeagnifolium* Cav.) is a problematic summer-growing perennial weed in Australia. The genetic diversity of silverleaf nightshade is poorly understood. Nine silverleaf nightshade specific and 10 cross-species simple sequence repeat (SSR) primer pairs were utilised to investigate the genetic variations among 94 silverleaf nightshade populations collected in Australia. High genetic diversity was found within silverleaf nightshade populations, with an average genetic similarity of 0.43. The Unweighted Pair Group Method with Arithmetic mean based dendrogram indicated the presence of genetically diverse silverleaf nightshade populations in Australia. However, no well supported genetic structure was found. The Mantel test indicated that there is no significant correlation between genetic variation and geographic distance. These results suggested a lack of geographic structure in genetic diversity, which is probably due to the long distance spread of seeds of silverleaf nightshade. The high genetic diversity of silverleaf nightshade could contribute to the inconsistency in control efficacy between populations.

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

Introduction

Silverleaf nightshade (*Solanum elaeagnifolium* Cav.) is a summer-growing perennial weed and is a Weed of National Significance in Australia (Australian Weeds Committee 2012). It is widely distributed in the cereal cropping zone in southern Australia, with potential to infest 398 million hectares (Kwong *et al.* 2006). It has been reported that silverleaf nightshade can cause up to 77% yield loss in cereals (Heap *et al*. 1997).

To date, classical biological control has not been implemented in Australia despite the species being declared a target for biological control in 1985 (Kwong 2006). In addition, the high regenerative ability of the root system has limited the efficacy of mechanical managements (Stanton *et al*. 2011), thus chemical control is the only useful option for silverleaf nightshade management in Australia. However, herbicide efficacy can be influenced by many factors including plant genetic variation (Marshall and Moss 2008). Therefore, effective management of silverleaf nightshade requires a comprehensive assessment of genetic diversity (Dekker 1997, Holt and Hochberg 1997).

Genetic diversity studies have been conducted in many weed species, such as bitter vine (*Mikania micrantha* (L.) Kunth.) (Wang *et al*. 2012) and false helleborine (*Veratrum album* L.) (Treier

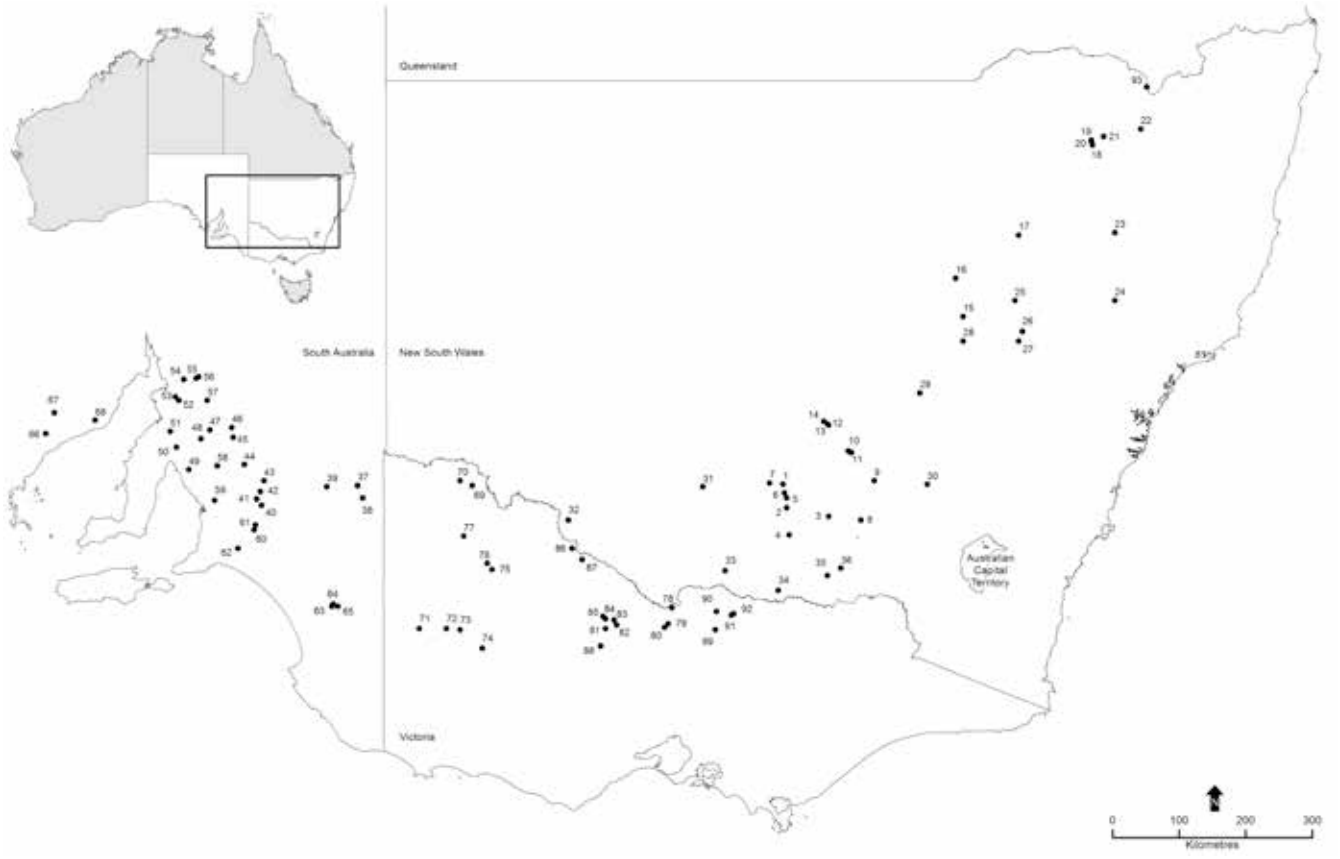

Figure 1. Sampling locations of silverleaf nightshade in New South Wales, South Australia, Victoria and Queensland (the Katanning, Western Australian population is not shown).

and Muller-Scharer 2011). These studies contribute to greater understanding of weed genetic diversity, evolution and invasion. Silverleaf nightshade was found to be genetically diverse in South Australia by random amplification of polymorphic DNA (RAPD) analyses (Hawker *et al*. 2006). However, the genetic diversity of silverleaf nightshade populations across Australia is largely unknown. Genetic diversity studies using genetic markers will contribute to the management of silverleaf nightshade (Dekker 1997).

Simple sequence repeat (SSR) markers are usually co-dominant, and more informative than dominant markers such as RAPDs because they are capable of differentiating homozygous individuals from heterozygous plants (Peakall 1997, McGregor *et al*. 2000). Furthermore SSRs are more reproducible, easily scored and analysed on high throughput genotyping platforms. Thirty six SSR markers have recently been developed for silverleaf nightshade (Zhu *et al*. 2012, 2013). In this study, a subset of 19 high polymorphic primer-pairs was applied to study genetic diversity of 94 populations of silverleaf nightshade collected across Australia.

Materials and methods

Plant material

A total of 670 silverleaf nightshade individuals were collected from 94 locations (populations) in New South Wales, South Australia, Victoria, Queensland and Katanning in Western Australia (Figure 1). One to ten individuals were collected from each location, depending on the level of infestation. Sampled individuals were at least 50 m apart to reduce the probability of collecting clonal plants. In addition, five field samples of quena (*S. esuriale* Lindl., a native *Solanum* species), and five commercial samples of eggplant (*S. melongena* L.; Hortico, Australia), were included for comparison. About 1 g of fresh, undamaged leaf material was collected from each individual plant, placed in a 1.5 mL Eppendorf tube, and then stored at -80ºC in the laboratory until DNA isolation.

Table 1. Simple sequence repeat (SSR) primers used to investigate the genetic diversity between three *Solanum* species, including the number of bands detected by each primer-pair and the corresponding allele sizes, including 19 base pairs (bp) of M13-tailed sequence.[1]

Primer ID	Band number	Estimate allele size (bp)		
		S. elaeagnifolium	*S. esuriale*	*S. melongena*
SLNZ5	4	184 - 203	Fail[2]	188
SLNZ 6	4	256 - 279	Fail	Fail
SLNZ 7	7	226 - 255	Fail	248
SLNZ 8	2	196 - 202	160 - 196	160 - 197
SLNZ 15	4	174 - 187	174 - 187	183
SLNZ 17	2	162 - 164	174	164
SLNZ 20	2	236 - 238	218 - 220	241 - 249
SLNZ 22	14	174 - 246	242	242 - 248
SLNZ26	3	123 -147	160	166
CA158	32	217 - 264	277 - 239	249 - 255
ESM3	22	Null - 355	249 - 258	264 - 268
EM117	30	110 - 178	85 - 178	96 - 116
EM127	9	Null - 222	168 - 268	Fail
EM135	16	Null - 267	222 - 288	283
EM140	6	Null - 231	216 - 235	230 - 233
EM155	7	Null - 334	144 - 171	126 - 295
SSR111	7	Null - 180	174 - 176	183
STI001	4	205 - 217	205	213
STG0010	7	177 - 271	178	178

[1]Detailed sequence information is described previously (Zhu *et al*. 2012, 2013); [2]Fail: No amplification detected.

DNA isolation

Genomic DNA was isolated individually and the quality and concentration was checked as described previously (Zhu *et al*. 2013). The individual DNA concentration was then adjusted to 20 ng μL^{-1}. Equal amounts of DNA from individuals representing the same population were bulked for PCR amplification, as a cost-efficient method of population analysis (Arunyawat *et al*. 2007, Eschholz *et al*. 2008).

PCR reaction and SSR analysis

Nineteen SSR primer-pairs (Table 1) were selected to investigate genetic diversity between populations, on the basis of their high expected heterozygosity value (H_E). The details of these primer-pairs have been described previously (Zhu *et al*. 2012, 2013). The 5′ end of the forward primer of each SSR primer-pair was tailed with a M13 sequence to perform high throughput fragment analysis (Raman *et al*. 2005). PCR amplification and detection of the amplification products were carried out as described elsewhere (Zhu *et al*. 2013).

Data analysis

Binary data, as the presence or absence (1 or 0) of bands of each locus for each population, were scored to construct a similarity matrix by Jaccard's coefficient (Jaccard 1908) using NTSYS-pc 2.1 (Rohlf 2000). The Unweighted Pair Group Method with Arithmetic mean (UPGMA) was calculated using the Sequential, Agglomerative, Hierarchical, and Nested clustering (SAHN) methods of the same software to construct a dendrogram of population genetic relationships. Non-parametric bootstrapping (n=1 000 replicates) was used to estimate statistical support at detected clades, using the Paleontological Statistics Software Package (PAST) (Hammer *et al*. 2001). Correlations between genetic and geographical distance among all pair-wise population comparisons was tested by a Mantel test using NTSYS, with 1 000 random permutations.

Results and discussion

The genetic diversity in 94 populations of silverleaf nightshade was assessed according to allele frequency. The SSR analysis illustrated a high level of genetic variation between silverleaf nightshade populations, with a total of 182 polymorphic bands (alleles) detected. The number of polymorphic bands varied from two with primer pairs SLNZ 8, SLNZ 17 and SLNZ 20 to 32 with the primer pair CA158, with an average of 9.6 polymorphic bands per locus (Table 1). The mean Jaccard's genetic similarity between populations was 0.43, varying from 0.21 to 0.76.

Bulk DNA analysis was used in this study. The reliability of bulk DNA analysis has been checked using a subset of individuals from nine locations (Zhu *et al*. 2013), which achieved similar results (average Jaccard similarity: 0.73 and 0.79 for the bulk and individual analysis, respectively). This method can lead to the loss of the co-dominant feature of SSR analysis and does not allow estimates of the heterozygosity within a population. However, individual genotype information was not essential for estimating between population genetic diversity (Dubreuil *et al*. 1999). The DNA bulking method is highly repeatable and reliable for population genetic studies, such as in maize (*Zea mays* L.) (Eschholz *et al*. 2008) and wild tomatoes (*Solanum peruvianum* L. and *S. chilense* (Dunal) Reiche) (Arunyawat *et al*. 2007).

The 19 SSR markers were successfully used to assess the genetic variation among 94 populations of silverleaf nightshade collected from different states of Australia. The present study detected a high level of genetic polymorphism among silverleaf nightshade populations within Australia, with a mean genetic similarly of 0.43. The high level of genetic variation in Australia might be attributable to multiple introductions (Cuthbertson *et al*. 1976), the heterogeneous nature of the initial introduction(s) and/or the self-incompatibility in silverleaf nightshade. Obligate outcrossing species usually have a higher level of genetic diversity than clonally or self pollinated species (Ward and Jasieniuk 2009).

The Unweighted Pair Group Method with Arithmetic mean (UPGMA) dendrogram based on the Jaccard's coefficient clearly separated quena and eggplant from silverleaf nightshade populations and was supported by a high bootstrap value, suggesting the genetic variability among related species (Figure 2). The 94 populations of silverleaf nightshade were clustered into two main groups with low bootstrap support (<70%), which indicated no well supported structure was found in Australia (Figure 2). In addition, no significant correlation was found between genetic and geographical distance among populations (r = -0.03, t = -091 and p = 0.17). The UPGMA dendrogram and the Mantel test suggested that there is no geographical structure of genetic variation in Australian silverleaf nightshade populations. Similar results were found in other invasive species such as *Flaveria bidentis* Juss. (Ma *et al*. 2011) and *Parthenium hysterophorus* L. (Tang *et al*. 2009).

Long distance distribution of silverleaf nightshade is aided by the spread of fruits (seeds). Transportation of the contaminated livestock or fodder contributes to the dispersal of silverleaf nightshade populations. Such long-distance dispersal events may explain the lack of geographic structure of silverleaf nightshade in Australia. Seeds can generate new plants and hybridise with other genotypes, leading to gene flow, and this may have contributed to the high genetic diversity.

Weeds with high genetic diversity are more likely to develop new phenotypes in response to natural selection pressures, which allow better adaption to the environment or management practices (Dekker 1997). The high genetic diversity in silverleaf nightshade may have

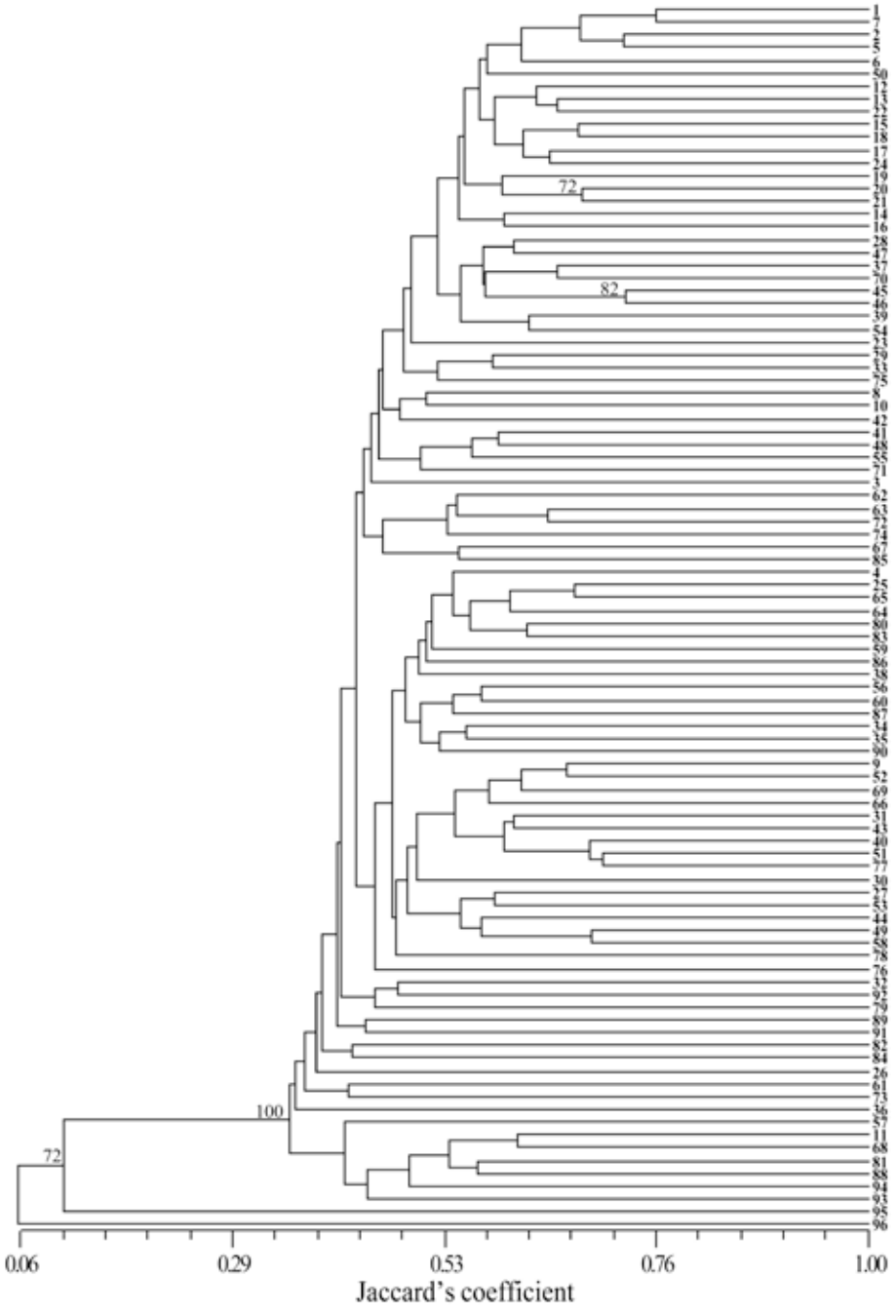

Figure 2. Unweighted Pair Group Method with Arithmetic mean (UPGMA) dendrogram of Jaccard's coefficient from dominant scored alleles of 94 silverleaf nightshade accessions, quena (95) and eggplant (96) from Australia. Only bootstrap values >70% are indicated.

resulted in inconsistent management of this weed in Australia. Similarly, this study also suggests the important role of seed spread in silverleaf nightshade infestations. Attention should therefore be paid to stopping seed set and minimising the movement of agricultural products, livestock and machinery.

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References

Arunyawat, U., Stephan, W. and Stadler, T. (2007). Using multilocus sequence data to assess population structure, natural selection, and linkage disequilibrium in wild tomatoes. *Molecular Biology and Evolution* 24(10), 2310-22.

Australian Weeds Committee (2012). Weeds of National Significance: silverleaf nightshade (*Solanum elaeagnifolium*) strategic plan. Australian Weeds Committee, Canberra. http://www.weeds.org.au/WoNS/silverleafnightshade/docs/SLN_Strategic_Plan_030613.pdf. (accessed 8 August 2013).

Cuthbertson, E.G., Leys, A.R. and McMaster, G. (1976). Silverleaf nightshade - a potential threat to agriculture. *Agricultural Gazette of New South Wales* 87(6), 11-3.

Dekker, J. (1997). Weed diversity and weed management. *Weed Science* 45(3), 357-63.

Dubreuil, P., Rebourg, C., Merlino, M. and Charcosset, A. (1999). Evaluation of a DNA pooled-sampling strategy for estimating the RFLP diversity of maize populations. *Plant Molecular Biology Reporter* 17(2), 123-38.

Eschholz, T.W., Peter, R., Stamp, P. and Hund, A. (2008). Genetic diversity of Swiss maize (*Zea mays* L. ssp. *mays*) assessed with individuals and bulks on agarose gels. *Genetic Resources and Crop Evolution* 55(7), 971-83.

Hammer, Ø., Harper, D.A.T. and Ryan, P.D. (2001). PAST: Paleontological Statistics Software Package for education and data analysis. *Palaeontologia Electronica* 4(1), 1-9.

Hawker, V., Preston, C. and Baker, J. (2006). Genetic variation within and among silverleaf nightshade (*Solanum elaeagnifolium* Cav.) populations in South Australia. Proceedings of the 15th Australian Weeds Conferences, eds C. Preston, J.H. Watts and N.D. Crossman, p. 176. (Weed Management Society of South Australia, Adelaide).

Heap, J.W., Smith, E. and Honan, I. (1997). Silverleaf nightshade: A technical handbook for Animal and Plant Control Boards in South Australia. (Primary Industries South Australia, Animal and Plant Control Commission, Naracoote, South Australia). 42 pp.

Holt, R.D. and Hochberg, M.E. (1997). When is biological control evolutionarily stable (or is it)? *Ecology* 78(6), 1673-83.

Jaccard, P. (1908). Nouvelles recherches sur la distribution florale. *Bulletin de la Societe Vaudoise des Sciences Naturelles* 44, 223-70.

Kwong, R.M., Sagliocco, J.L., Weiss, J., Hunt, T., Morfe, D. and Kularatne, D. (2006). Feasibility of biological control of Solanaceous weeds of temperate Australia: silverleaf nightshade, *Solanum elaeagnifolium* Cav. and prairie ground cherry, *Physalis viscosa* L. A report to the Meat and Livestock Australia Limited, 150 pp.

Ma, J.W., Geng, S.L., Wang, S.B., Zhang, G.L., Fu, W.D. and Shu, B. (2011). Genetic diversity of the newly invasive weed *Flaveria bidentis* (Asteraceae) reveals consequences of its rapid range expansion in northern China. *Weed Research* 51(4), 363-72.

Marshall, R. and Moss, S.R. (2008). Characterisation and molecular basis of ALS inhibitor resistance in the grass weed *Alopecurus myosuroides*. *Weed Research* 48(5), 439-47.

McGregor, C.E., Lambert, C.A., Greyling, M.M., Louw, J.H. and Warnich, L. (2000). A comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosum* L.) germplasm. *Euphytica* 113(2), 135-44.

Peakall, R. (1997). PCR-based genetic markers and their applications to turfgrass breeding. *International Turfgrass Society Research Journal* 8, 243-59.

Raman, R., Raman, H., Johnstone, K., Lisle, C., Smith, A., Martin, P. and Allen, H. (2005). Genetic and in silico comparative mapping of the polyphenol oxidase gene in bread wheat (*Triticum aestivum* L.). *Functional and Integrative Genomics* 5(4), 185-200.

Rohlf, F.J. (2000). NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System. Version 2.1 Exeter Publications. (New York, USA).

Stanton, R., Wu, H. and Lemerle, D. (2011). Root regenerative ability of silverleaf nightshade (*Solanum elaeagnifolium* Cav.) in the glasshouse. *Plant Protection Quarterly*, 26(2), 54-6.

Tang, S.Q., Wei, F., Zeng, L.Y., Li, X.K., Tang, S.C., Zhong, Y. and Geng, Y-P. (2009). Multiple introductions are responsible for the disjunct distributions of invasive *Parthenium hysterophorus* in China: evidence from nuclear and chloroplast DNA. *Weed Research* 49(4), 373-80.

Treier, U.A. and Muller-Scharer, H. (2011). Differential effects of historical migration, glaciations and human impact on the genetic structure and diversity of the mountain pasture weed *Veratrum album* L. *Journal of Biogeography* 38(9), 1776-91.

Wang, T., Chen, G.P., Zan, Q.J., Wang, C.B. and Su, Y.J. (2012). AFLP genome scan to detect genetic structure and candidate loci under selection for local adaptation of the invasive weed *Mikania micrantha*. (*Public Library of Science*) *Plos One* 7(7), e41310. doi:10.1371/journal.pone.0041310

Ward, S.M. and Jasieniuk, M. (2009). Review: sampling weedy and invasive plant populations for genetic diversity analysis. *Weed Science* 57(6), 593-602.

Zhu, X., Raman, H., Wu, H., Lemerle, D., Burrows, G. and Stanton, R. (2013). Development of SSR markers for genetic analysis of silverleaf nightshade (*Solanum elaeagnifolium*) and related species. *Plant Molecular Biology Reporter* 31(1), 248-54.

Zhu, X.C., Wu, H.W., Raman, H., Lemerle, D., Stanton, R. and Burrows, G.E. (2012). Evaluation of simple sequence repeat (SSR) markers from *Solanum* crop species for *Solanum elaeagnifolium*. *Weed Research* 52(3), 217-23.