BioOne COMPLETE

Cross-Species Transferability of Solanum spp. DNA Markers and Their Application in Assessing Genetic Variation in Silverleaf Nightshade (Solanum elaeagnifolium) Populations from Texas, USA

Authors: Singleton, Joshua James, Mangat, Puneet Kaur, Shim, Junghyun, Vavra, Cody, Coldren, Cade, et al.

Source: Weed Science, 68(4) : 396-404

Published By: Weed Science Society of America

URL: https://doi.org/10.1017/wsc.2020.25

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://complete.bioone.org/journals/Weed-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

Weed Science

www.cambridge.org/wsc

Cross-species transferability of *Solanum* spp. DNA markers and their application in assessing genetic variation in silverleaf nightshade (*Solanum elaeagnifolium*) populations from Texas, USA

Joshua James Singleton[1], Puneet Kaur Mangat[1], Junghyun Shim[2], Cody Vavra[1], Cade Coldren[3] and Rosalyn B. Angeles-Shim[3]

[1]Graduate Student, Department of Plant and Soil Science, College of Agricultural Sciences and Natural Resources, Texas Tech University, Lubbock, TX, USA; [2]Research Associate, Department of Plant and Soil Science, College of Agricultural Sciences and Natural Resources, Texas Tech University, Lubbock, TX, USA and [3]Assistant Professor, Department of Plant and Soil Science, College of Agricultural Sciences and Natural Resources, Texas Tech University Lubbock, TX, USA

Research Article

Cite this article: Singleton JJ, Mangat PK, Shim J, Vavra C, Coldren C, Angeles-Shim RB (2020) Cross-species transferability of *Solanum* spp. DNA markers and their application in assessing genetic variation in silverleaf nightshade (*Solanum elaeagnifolium*) populations from Texas, USA. Weed Sci. **68**: 396–404. doi: 10.1017/wsc.2020.25

Received: 23 February 2020
Revised: 27 March 2020
Accepted: 1 April 2020
First published online: 13 April 2020

Associate Editor:
Sara Martin, Agriculture and Agri-Food Canada

Keywords:
Genetic variation; phenotypic plasticity; simple sequence repeats; *Solanum lycopersicoides*

Author for correspondence:
Rosalyn B. Angeles-Shim, Department of Plant and Soil Science, College of Agricultural Sciences and Natural Resources, Texas Tech University, Lubbock, TX 79409.
Email: rosalyn.shim@ttu.edu

Abstract

Silverleaf nightshade (*Solanum elaeagnifolium* Cav.) is an invasive species that has successfully spread outside its native range to become a noxious weed in 21 states in the United States and 42 countries worldwide. The successful establishment of *S. elaeagnifolium* outside its native habitat indicates its innate ability to adapt to a multitude of environments. Phenotypic plasticity and/or genetic adaptation have been identified as key mechanisms underlying the adaptive success of invasive species. Whereas phenotypic plasticity allows a species to buffer changes in the environment by altering its phenotypic attributes within the short term, genetic adaptation is responsible for the longer-term adaptability of plants to heterogeneous environments and is dependent on the amount of genetic variation present in the species. In this study, we screened DNA markers that are specific to tomato (*Solanum lycopersicum* L.) and *Solanum lycopersicoides* Dunal for their interspecific transferability to *S. elaeagnifolium* and determined the applicability of the transferable DNA markers in assessing the extent of genetic variation in populations from Lubbock, Littlefield, and Blackwell, TX. Of the 187 markers screened, 78 successfully amplified targets in *S. elaeagnifolium*, indicating the evolutionary conservation of marker loci across *S. lycopersicum*, *S. lycopersicoides*, and *S. elaeagnifolium*, despite their genetic divergence millions of years ago. Genotyping of *S. elaeagnifolium* populations using 50 DNA markers that consistently amplified clear bands in more than 60% of the plants identified nine polymorphic markers with 0.014 to 0.621 polymorphism information content. Genetic diversity analysis by DNA marker profiling established genetic variation among populations and within individuals of different populations. Unweighted paired group method with arithmetic mean analysis grouped the plants into six clusters that are generally defined by selection pressures unique to each collection site. Results of the study indicate the capacity of *S. elaeagnifolium* for genetic differentiation in response to variable selection pressures within the same geographic region.

Introduction

Silverleaf nightshade (*Solanum elaeagnifolium* Cav.) is an aggressive, highly persistent, invasive species that adversely impacts agricultural and rangeland productivity by competing with crops for nutrients and water, exuding allelopathic compounds that inhibit crop growth, and hosting destructive phytophagous pests and pathogens (Boyd et al. 1984; EPPO 2007). The invasiveness of the species is attributed to its ability to reproduce sexually via insect pollination and asexually through regenerative buds in the roots and stem fragments. Various cytotypes including diploids ($2n = 24$), tetraploids ($2n = 48$), and hexaploids ($2n = 72$) have been reported in Argentina, although *S. elaeagnifolium* specimens in the United States have been identified as diploids (Heap and Carter 1999; Scaldaferro et al. 2012). It is native to northeastern Mexico and the southwestern United States but has since spread across 21 states in the United States and 42 countries throughout Asia, South America, and Australia (Mekki 2007; Roche 1991). In the United States, sizable yield losses in cereal, fiber, vegetable, and forage crops have been attributed to *S. elaeagnifolium* infestation (Boyd et al 1984; Brandon 2005).

Anthropogenic activities have greatly contributed to the widespread distribution of *S. elaeagnifolium*, although the successful establishment of the species outside its native range suggests an inherent mechanism underlying its adaptability to heterogeneous environments

© Weed Science Society of America, 2020. This is an Open Access article, distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives licence (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is unaltered and is properly cited. The written permission of Cambridge University Press must be obtained for commercial re-use or in order to create a derivative work.

Downloaded From: https://complete.bioone.org/journals/Weed-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

(Boyd and Murray 1982; EPPO 2007; Stanton et al. 2009). A key facet to the adaptive success of invasive species in response to environmental alterations is their fitness flexibility as conferred by phenotypic plasticity and genetic adaptation. Phenotypic plasticity is an adaptive strategy that allows a species to buffer rapid changes in the environment by altering its phenotypic attributes, including morphology, growth, survival, and fertility (Davidson et al. 2011; Richards et al. 2006). In the short term, phenotypic plasticity has the ability to enhance the survival of small populations but, in so doing, it reduces the effectiveness of natural selection in promoting genetic adaptation. In the long term, phenotypic plasticity may not be sufficient for a species to avoid extinction (Moritz and Agudo 2013; Nunney 2016). Conversely, genetic adaptation is the rapid selection of adaptive phenotypes and is facilitated by high levels of genetic variation (Barret 2015). This mechanism usually requires strong, divergent selection pressures such as temperature, precipitation, and latitudinal clines (Kollman and Bañuelos 2004; Rice and Mack 1991).

From a management perspective, both phenotypic plasticity and genetic adaptation contribute to the successful establishment and survival of an invasive species outside its native range. Studies that considered the relative importance of both mechanisms in the adaptive success of invasive species indicate that they are not mutually exclusive (Geng et al. 2016; Si et al. 2014). Indeed, ecological and genetic research on introduced *S. elaeagnifolium* populations in Australia and Europe showed a high degree of genetic variation and evidence of morphological plasticity in the species in response to variable water availability (Travlos 2013; Zhu et al. 2013c). Relative to phenotypic plasticity, however, genetic adaptation will have a larger effect on the long-term efficacy of a strategy intended to control invasive species. For example, an *S. elaeagnifolium* population with high genetic variability will have the genetic means to develop tolerance to a particular herbicide in the long term compared with an *S. elaeagnifolium* population with low genetic variability. High genetic variation also translates into genotype variability within a population. When a biological strategy is used to manage *S. elaeagnifolium*, biological control agents may exhibit differential survivability across genotypes, and therefore different efficiencies for control. Understanding the extent of genetic variation that drive the long-term, evolutionary adaptation of *S. elaeagnifolium* will facilitate the design of effective methods to control this weed.

Genetic variation can be quantified based on the amount of genetic polymorphism or heterogeneity present among and within individuals of different populations of a given species (Geng et al. 2016; Sakai et al. 2001). Several molecular marker systems, including amplified fragment-length polymorphism (Vos et al. 1995), random amplified polymorphic DNA (Penner 1996; Welsh and McClelland 1990; Williams et al. 1990), insertions/deletions (indels) (Becerra et al. 2017; Jain et al. 2019; Jamil et al. 2013; Tu et al. 2007), simple-sequence repeats (SSRs) (McCouch et al. 1997; Powell et al. 1996; Taramino and Tingey 1996), and single-nucleotide polymorphisms (Govindaraj et al. 2015), have been successfully used to assess the intra- and interspecific variation in various plant species.

Despite genetic diversity studies on introduced *S. elaeagnifolium* populations in different geographic locations, including Europe, Australia, and South America (Chiarini et al. 2018; Qasem et al. 2019; Zhu et al. 2012, 2013a, 2013b), the available genetic marker resource for the species remains limited. Previous research to evaluate the transferability of SSR markers from related *Solanum* species identified 13 out of 35 SSRs that can amplify targets in *S. elaeagnifolium*, although only 6 were polymorphic. Using these 6 markers, a high degree of genetic variation was established in naturalized populations of *S. elaeagnifolium* in Australia (Zhu et al. 2012). In a separate study, 26 SSR primer pairs were designed based on expressed sequence tags and genomic sequences of *S. elaeagnifolium* (Zhu et al. 2013a). Of the 26 SSR markers, only 9 were polymorphic. These 26 SSRs represent the first set of SSR markers that has been developed specifically for *S. elaeagnifolium* (Zhu et al. 2013a).

To accurately assess the extent of genetic variation that defines the ability of *S. elaeagnifolium* to adapt successfully to different environments, a core set of molecular markers that can capture informative variation across the genome needs to be developed and validated for their applicability in genetic diversity studies. While a few SSR markers have been generated and used to assess the extent of genetic variation in introduced *S. elaeagnifolium* populations in countries such as Australia and Jordan, it is yet undetermined whether these markers are sufficient to capture the genetic variation within the *S. elaeagnifolium* genome, or if they will successfully transfer to *S. elaeagnifolium* populations beyond these two introduced ranges as well as within the species' native range. The goal of this study, therefore, was to expand the genetic marker resource that can be used for genetic diversity studies in *S. elaeagnifolium*. This was accomplished by establishing the transferability to *S. elaeagnifolium* of SSR and indel-based markers from tomato (*Solanum lycopersicum* L.) and *Solanum lycopersicoides* Dunal and determining the applicability of cross-transferable DNA markers in assessing the extent of genetic variation in *S. elaeagnifolium* populations from different localities within the native range of the species in Texas, USA. The ability of SSR markers that have been reported to amplify targets in naturalized populations of *S. elaeagnifolium* in Australia to genotype *S. elaeagnifolium* populations from the United States was also evaluated.

Materials and Methods

Transferability of Solanum spp. DNA Markers to Solanum elaeagnifolium

A total of 187 DNA markers, including 98 genome- and expressed sequence tag (EST)-based SSRs specific to tomato and 54 indel markers specific to *S. lycopersicoides* were screened for their ability to amplify target sequences in the *S. elaeagnifolium* genome. The selected markers mapped in chromosome 1–10 of the tomato genome and did not include markers targeting chromosomes 11 and 12 (Figure 1; Supplementary Table S1). All tomato SSRs were synthesized based on publicly available primer sequences from the Kazusa Marker Database (http://marker.kazusa.or.jp/Tomato), whereas the *S. lycopersicoides*–specific DNA markers were designed following specifications for standard primer design using an in-house analysis of a draft assembly of the *S. lycopersicoides* genome. Twenty-six SSR markers specific to *S. elaeagnifolium*, two specific to potato (*Solanum tuberosum* L.) and seven specific to eggplant (*Solanum melongena* L.) and that have been identified to amplify targets in *S. elaeagnifolium* populations in Australia (Zhu et al. 2012, 2013a) were also screened for their applicability to genotype populations of *S. elaeagnifolium* from the United States (Supplementary Table S1). Synthesis of all DNA markers were outsourced to Sigma (Woodlands, TX, USA).

Genomic DNA was isolated using a modified CTAB method (Murray and Thompson 1980) from six individual *S. elaeagnifolium* plants that were randomly collected from the Horticultural Gardens

Downloaded From: https://complete.bioone.org/journals/Weed-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

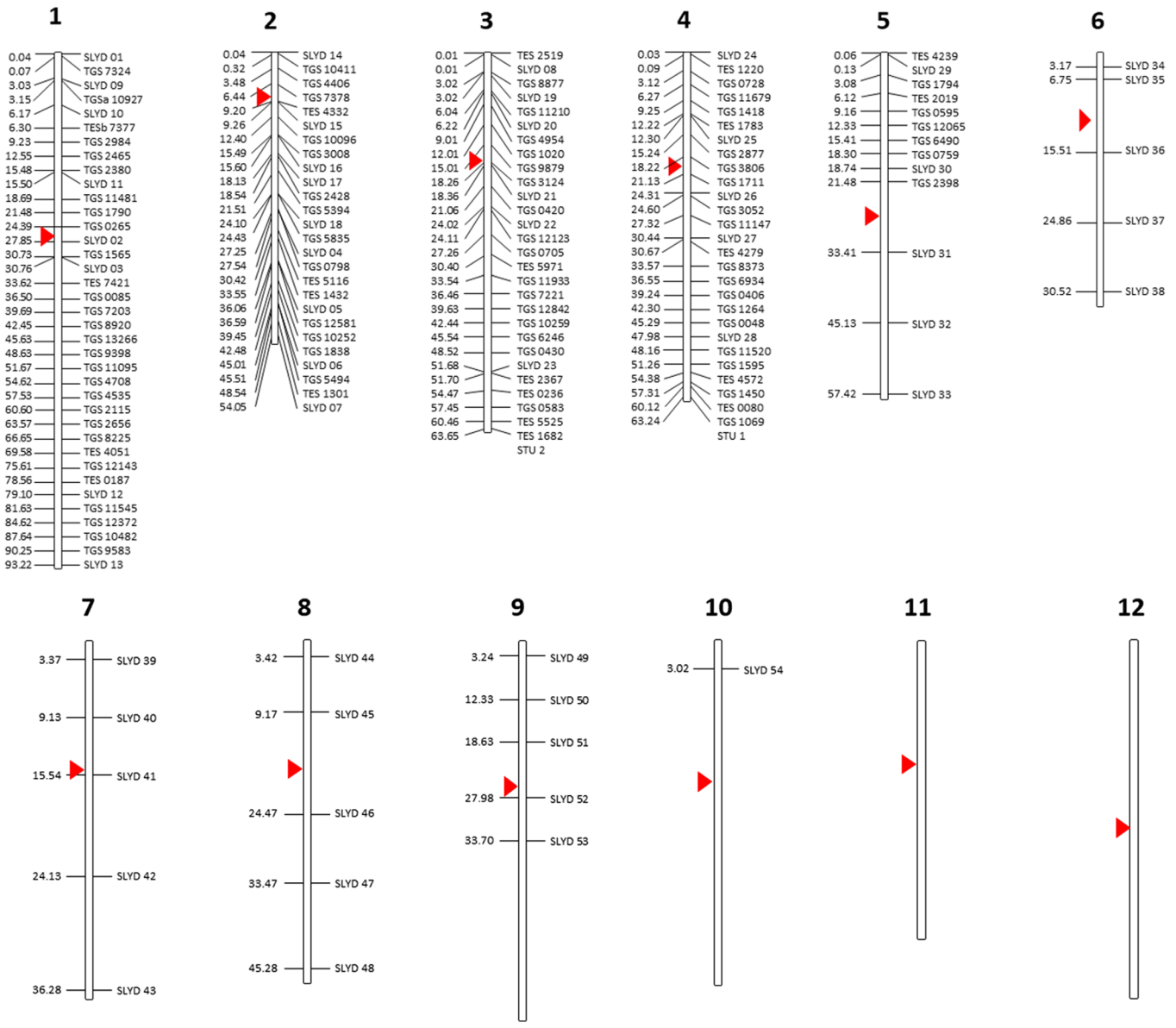


Figure 1. *Solanum*-based DNA markers that were screened for their transferability to *Solanum elaeagnifolium*. All markers were mapped against the tomato chromosome as a point of reference. Numbers on the left indicate the estimated physical distance (Mb) of each marker along the length of each chromosome. Red triangle indicates the centromere.

of the Department of Plant and Soil Science of Texas Tech University (TTU), Lubbock, TX. SSR and indel targets were amplified from the extracted DNA samples following a standard polymerase chain reaction (PCR) protocol (Shim et al. 2015). PCR amplicons were resolved in 3% agarose gel in 1X Tris-borate-EDTA buffer.

Solanum elaeagnifolium Populations

Mature berries from natural populations of *S. elaeagnifolium* were harvested in January 2019 from 100 m by 100 m quadrats in Lubbock (33.58°N, 101.85°W), Littlefield (33.92°N, 102.3°W), and Blackwell, TX (32.09°N, 100.32°W) (Figure 2) and used as sources of seeds. The collection sites in Lubbock included an experimental farm planted mostly with cotton and a native rangeland maintained by TTU. The collection site in Littlefield is a rangeland maintained under a Conservation Reserve Program, and the site in Blackwell is a hunting reserve with interspersed wheat plantings.

Seeds were collected from berries that were harvested from each site and air-dried for 72 h in petri dishes lined with paper towel under greenhouse conditions (ambient temperature of 28 C). The seeds were then directly sown and germinated in plastic flats containing conventional potting media (containing 45% to 50% of composted pine bark, vermiculite, Canadian sphagnum, peat moss, perlite, and dolomitic limestone) or a mixture of sand and potting media (1:1 ratio) without any fertilizer input. The plastic flats were lined with 2-mm polypropylene plastic sheets with 1-cm holes to allow proper drainage after watering every 3 d. All experiments were carried out in a greenhouse of the Horticultural Gardens of TTU.

At the two-true-leaf stage of the seedlings, leaf tissues were sampled for DNA extraction using a Tris/HCl-potassium chloride-EDTA buffer (Angeles-Shim et al. 2020). A total of 147 individual plants from the Lubbock experimental farm (33) and rangeland (52), Blackwell hunting reserve (44), and Littlefield rangeland (18) were genotyped for genetic diversity assessment.

Downloaded From: https://complete.bioone.org/journals/Weed-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

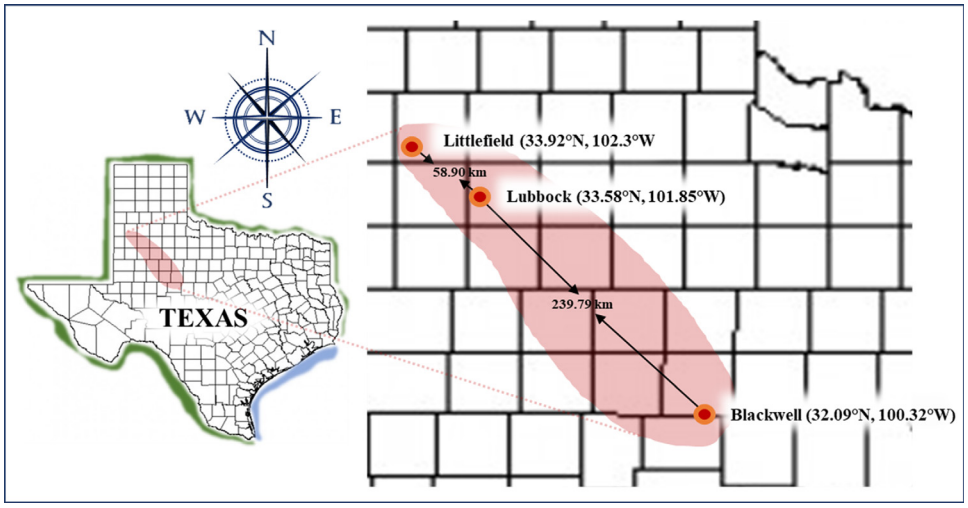


Figure 2. Geographic distribution of *Solanum elaeagnifolium* populations used in the genetic diversity analysis. Seeds of *S. elaeagnifolium* were collected from mature plants from Lubbock, Blackwell, and Littlefield, TX.

Genetic Diversity Analysis

DNA markers identified to amplify targets in the *S. elaeagnifolium* genome (Supplementary Table S2) were used to assess the genetic diversity between and within populations of *S. elaeagnifolium* from Texas, USA. PCR and resolution of amplicons in agarose gel were carried out as described earlier. PCR amplicons were scored codominantly based on molecular weight differences.

Descriptive statistics for the single-locus DNA markers, including the number of different alleles (N_a), number of effective alleles (N_e), and expected heterozygosity (H_e), were generated using GenAlEx v. 6.5b3 (Peakall and Smouse 2012). Polymorphism information content (PIC) of each individual SSR allele was calculated using the formula PIC = $\Sigma\, p_i^2$, where p_i is the frequency of the *i*th allele in the genotypes tested (Weir 1990). A genetic distance matrix based on the DNA marker profiles was generated using GenAlEx v. 6.5b3 and used to calculate both fixation (*F*-statistics) and similarity indices. *F*-statistics, including the average, pairwise genetic differentiation estimates between populations (F_{ST}), as well as inbreeding coefficients (F_{IS} and F_{IT}), were assessed by analysis of molecular variance (AMOVA) based on 9,999 permutations in GenAlEx v. 6.5b3. Similarity indices were calculated based on Jaccard's coefficient. Genetic divergence among the experimental materials was determined by clustering analysis using the unweighted pair group method with arithmetic mean (UPGMA) subroutine in the Molecular Evolutionary Genetics Analysis software based on 1,000 bootstraps (Kumar et al. 1994).

Results and Discussion

Transferability of Solanum lycopersicum– and Solanum lycopersicoides–specific DNA Markers to Solanum elaeagnifolium

Of the 98 tomato-based SSRs screened for cross-species transferability, 28.57% amplified targets in the *S. elaeagnifolium* genome (Table 1). Previous reports on the interspecific transferability of SSRs have been unpredictable at best, with the ability of SSR markers to amplify targets in genomes of species within the same genus ranging from 16.5% to more than 50%, depending on the plant species (Angeles-Shim et al. 2014; Hernández et al. 2002; Mullan et al. 2005). SSRs are tandemly arranged, repetitive sequences that make up a significant portion of the eukaryotic genome. They are hypervariable and have undergone extensive expansion or contraction throughout the course of evolution while simultaneously effecting changes in the nucleotide sequences flanking them (Temnykh et al. 2001). In tomato, these genomic events, combined with mutations, transposon amplifications, chromosomal rearrangements, and gene duplications that also led to the domestication of the species would have generated genomic regions that are highly diverged from those of *S. elaeagnifolium*. The genomic divergence of tomato from *S. elaeagnifolium* would contribute to the low transferability of tomato SSRs in *S. elaeagnifolium*.

Compared with the genome-based SSRs, a higher number of the EST-based tomato SSRs amplified targets in *S. elaeagnifolium*. EST-based markers are specific to transcribed regions of the genome and are generally more conserved across species (Kuleung et al. 2004; Wang et al. 2007). The higher transferability of the EST-based SSRs compared with the genome-based SSRs supports the evolutionary conservation of transcribed regions in the plant genome, specifically among species within a genus.

Of the 54 *S. lycopersicoides* indel markers that were screened for their cross-species transferability, only 18 amplified targets in *S. elaeagnifolium* (Table 1). SLYD 52, which maps at chromosome 9 of *S. lycopersicoides*, amplified multiple loci in *S. elaeagnifolium*. The fragment size generated by the 18 indel markers ranged from 143 to 380 bp, consistent with the in silico predictions for amplicon size.

Phylogenetic and monophyletic analyses of *Solanum* spp. have grouped *S. lycopersicoides* to the potato clade and *S. elaeagnifolium* to the *Leptostemonum* clade (Stern et al. 2011). The high degree of genetic differentiation that separated the two *Solanum* spp. into different clades may account for the <40% transferability of *S. lycopersicoides* indel markers to *S. elaeagnifolium*. Nevertheless, the results of the study indicate the presence of conserved binding sites for indels between *S. lycopersicoides* and *S. elaeagnifolium*.

Other *Solanum*-based SSR markers that have been previously reported to amplify targets in naturalized populations of *S. elaeagnifolium* from Australia (Zhu et al. 2012, 2013a) were also screened for their ability to genotype *S. elaeagnifolium* populations from the United States. Except for SEA 13 and SEA 26, all *S. elaeagnifolium* markers, along with the two potato SSRs and six out of the seven eggplant SSRs, were able to amplify targets

Downloaded From: https://complete.bioone.org/journals/Weed-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

Table 1. Frequency of *Solanum*-based DNA markers that transferred and amplified polymorphic targets in *Solanum elaeagnifolium* populations from Texas, USA.

Source species	DNA marker type[a]	No. of markers screened for transferability	No. of markers that transferred to *S. elaeagnifolium*	% of transferable markers
Solanum lycopersicum	Genome-based SSRs	76	19	25.00
	EST-based SSRs[b]	22	9	40.90
Solanum lycopersicoides	indels	54	18	33.33
Solanum elaeagnifolium	EST-based SSRs[c]	26	24	92.31
Solanum tuberosum	SSR[d]	2	2	100.00
Solanum melongena	SSR[e]	7	6	85.71

[a]Abbreviations: EST, expressed sequence tag; indels, insertions/deletions; SSRs, simple sequence repeats.
[b]EST-based tomato SSRs.
[c]EST-based, *S. elaeagnifolium*-specific markers designed by Zhu et al. (2013).
[d]Potato SSRs screened for transferability to *S. elaeagnifolium* by Zhu et al. (2012).
[e]Eggplant SSRs screened for transferability to *S. elaeagnifolium* by Zhu et al. (2012).

in *S. elaeagnifolium* from the United States. Markers that required a 58 C or 50 to 60 C annealing temperature to amplify targets in the Australian *S. elaeagnifolium* populations gave clear, consistent bands when amplified at an annealing temperature of 55 C using the samples from Texas. The size of the amplified fragments ranged from 220 to 340 bp, consistent with the range of amplicon size obtained from Australian *S. elaeagnifolium* populations (Zhu et al. 2012). SEA 3 and SEA 16, which did not amplify in Australian *S. elaeagnifolium* populations, amplified multiple alleles in *S. elaeagnifolium* populations from the United States, whereas SEA 7 and SEA 22, which amplified multiple loci in the Australian *S. elaeagnifolium* populations, amplified a single locus in U.S. *S. elaeagnifolium* populations. Differences in the ability of the SEA SSRs to amplify targets in the Australian and U.S. *S. elaeagnifolium* populations, as well as in the number of target loci the markers can amplify, suggest a possible diversification of the Australian population following multiple introductions of the species from 1901 to 1914 (Cuthbertson et al. 1976).

Despite the rapid advances in sequencing and genotyping platforms, SSRs remain a marker of choice for most laboratories because of their cost-efficiency, abundance in the genome, degree of polymorphism, and reproducibility across laboratories (Collard et al. 2005, 2008). In the current study, the observed, overall transferability to *S. elaeagnifolium* of DNA markers that are specific to other *Solanum* spp. was moderate (41.71%). This is expected, given the genetic differentiation of *S. elaeagnifolium* that separates the species from tomato and *S. lycopersicoides*. Despite this, the DNA markers that were identified to amplify targets in *S. elaeagnifolium* constitute valuable additions to the genetic marker resource that can be used for genetic studies in *S. elaeagnifolium*. To obtain a better coverage of the *S. elaeagnifolium* genome, *Solanum* spp.–specific DNA markers with targets in chromosomes 11 and 12 can also be evaluated for cross-species transferability and application in assessing genetic variation in *S. elaeagnifolium*. Additionally, sequencing and assembly of the whole genome of *S. elaeagnifolium* will provide a basis for species-specific primer design that can significantly expand the marker resource for *S. elaeagnifolium*.

Descriptive Statistics of DNA Markers Used in Genetic Diversity Assessment Studies

Of the 78 DNA markers used to genotype the *S. elaeagnifolium* populations, 50 consistently amplified clear bands in more than 60% of the individual plants sampled. Of these 50, only 9 generated polymorphic bands, including SLYD10, SLYD 29, SLYD 30, SEA 5, SEA 6 and SEA 19, STU 1, STU 2, and SME 2. All polymorphic markers generated two alleles each. SEA 6, SLYD 10, and STU 2 amplified nine loci with a frequency of ≤5% in a single plant from the TTU Quaker Farm and two individual plants from Blackwell. This indicates the presence of rare, informative bands that are unique to only three individuals in the total sample populations. The calculated mean number of effective alleles was 1.18 ± 1.049, whereas the average expected heterozygosity was 0.241 ± 0.027 (Table 2).

Table 2. Summary statistics of markers used for the assessment of genetic diversity in *Solanum elaeagnifolium* populations from Texas, USA.

Descriptive statistics[a]	Value
Total no. of markers used for genotyping	78
Total no. of markers that amplified in >50% of the experimental materials	50
Average no. of observed alleles per SSR	2
Average no. of effective alleles (N_e)	1.18±0.049
Average expected heterozygosity (H_e)	0.241±0.027
No. of polymorphic SSR markers	9
PIC range	0.014-0.621
Average calculated PIC	0.245

[a]Abbreviations: H_e, expectedheterozygosity; N_e, number of effective alleles; PIC, polymorphisminformation content; SSR, simple sequence repeat.

The number of alleles that are amplified at a particular marker locus is indicative of the genetic diversity within/between germplasm. The more alleles at a particular marker locus, the higher the level of genetic diversity that can be used to distinguish between related lines (Botstein et al. 1980; Geng et al. 2016). The amount of relative information that can be derived from the utilized markers is dependent on their individual PIC values.

In the current study, PIC values for each of the polymorphic markers ranged from 0.014 (SME 2) to 0.621 (SLYD 29), with an average of 0.245 (Table 3). The calculated mean PIC coincided with the mean H_e (0.241) established for the same set of markers (Table 2). PIC value for STU 1 was higher, whereas that for SME 1 was lowed compared with values obtained from genotyping *S. elaeagnifolium* populations from Australia (Zhu et al. 2012). These results further support the hypothesis of *S. elaeagnifolium* diversification following multiple introductions of the species in Australia. SEA 5 and SLYD 10 recorded PIC values that were ≥0.40, whereas SLYD 29 recorded the highest PIC of 0.621.

Based on the mean and individual PIC values of the DNA markers used, a high degree of genetic diversity was established within individuals of the different populations. This was supported by

Downloaded From: https://complete.bioone.org/journals/Weed-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

Table 3. Polymorphism information content of DNA markers that amplified polymorphic targets in *Solanum elaeagnifolium* populations from Texas, USA.[a]

No.	Primer name	No. of alleles	Total no. of individuals in the population	Allele frequency (p_i)				
				A	H_e	B	$\sum p_i^2$	PIC
1	STU 1	2	147	0.884	0.116	0.000	0.795	0.204
2	STU 2	2	147	0.993	0.006	0.000	0.986	0.014
3	SME 2	2	147	0.925	0.075	0.000	0.862	0.138
4	SEA 05	2	147	0.728	0.265	0.007	0.600	0.400
5	SEA 06	2	147	0.980	0.020	0.000	0.960	0.040
6	SEA 19	2	147	0.905	0.095	0.000	0.828	0.172
7	SLYD 10	2	147	0.701	0.286	0.014	0.573	0.427
8	SLYD 29	2	147	0.388	0.449	0.163	0.379	0.621
9	SLYD 30	2	147	0.899	0.054	0.048	0.812	0.188

[a]H_e, expected heterozygosity; PIC, polymorphism information content.

Table 4. Analysis of molecular variance comparing genetic variation within and among individuals and among populations from Texas, USA.

Source	df	Sum of squares	Mean sum of squares	Estimated variation	Percent variation	*F*-statistics		
						F_{ST}	F_{IS}	F_{IT}
Among populations	3	53.68	17.90	0.24	26%	0.32	−0.29	0.12
Among individuals	143	53.27	0.37	0.00	0%			
Within individuals	147	100.00	0.68	0.68	74%			
Total	293	206.95		0.92	100%			

results of the AMOVA showing that 74% of the total genetic variation observed was due to differences within individuals of the population studied (Table 4).

Solanum elaeagnifolium propagates clonally as well as by seed production through obligate outcrossing (Hardin et al. 1972). Plants that are derived from allogamous seeds are able to maintain a certain degree of heterozygosity due to the reshuffling of alleles and creation of novel genetic recombinations that occur during cross-pollination. To capture the maximum genetic variation present within and between *S. elaeagnifolium* populations, we sampled individual plants that were grown from *S. elaeagnifolium* seeds harvested from mature berries of natural populations of *S. elaeagnifolium*. The high genetic variation within individuals of populations used in the study, as well as the low values of F_{IS} and F_{IT} (Table 4), which measure inbreeding in an individual relative to a subpopulation and total population, respectively, reflects the nature of *S. elaeagnifolium* as obligate cross-pollinators. Incidentally, the results of the study also confirm the maintenance of a sexual mode of reproduction in *S. elaeagnifolium* despite its ability to propagate vegetatively through regenerative stem fragments and buds in the roots.

Genetic Diversity and Population Structure of Solanum elaeagnifolium

Based on Jaccard's coefficient, UPGMA analysis distinctly grouped the *S. elaeagnifolium* individuals into six clusters: I, II, III, IV, V, and IV (Figure 3). Clusters I, II, and III were composed solely of individuals from the TTU rangeland in Lubbock. Cluster IV consisted of individuals collected from a hunting reserve in Blackwell. Cluster V mostly included individuals from the rangeland in Littlefield, with the addition of one plant (individual 87) from the TTU rangeland in Lubbock. Finally, Cluster VI was an admixture consisting primarily of individuals collected from the Lubbock experimental farm and a few individual plants from rangelands in Littlefield and Lubbock. In general, individual plants coming from the same collection site clustered together, suggesting the possible role of selection pressures that are unique to each collection site in driving the genetic differentiation of individuals from each population.

Table 5. Pairwise genetic differentiation estimate (F_{ST}) matrix for *Solanum elaeagnifolium* populations from Texas, USA.

Populations	Blackwell	TTU[a] Quaker	TTU rangeland
Blackwell	0.00	0.00	0.00
TTU Quaker	0.38	0.00	0.00
TTU Rangeland	0.46	0.16	0.00
Littlefield	0.29	0.12	0.17

[a]TTU, Texas Tech University.

Selection pressures at each of the collection sites are driven in part by land use and management practices, and each of these has the potential to produce differential adaptation of *S. elaeagnifolium*. Rangelands are generally uncultivated lands for animal grazing and browsing. They are characterized by limited water and nutrient availability and low annual production (Havstad et al. 2009). Hunting reserves, on the other hand, are fenced-in land areas where hunting is carefully controlled. Rangelands and hunting reserves differ in native vegetation, as well as in the degree to which anthropogenic activities impact the composition of flora. Agricultural farmlands are used for the production of crops and are characterized by a high degree of anthropogenic disturbance. Unlike naturally maintained rangelands and hunting reserves, croplands are exposed to a range of conventional management practices, including tillage, irrigation, and agrochemical use (Andreasen et al. 1996; Green and Stowe 1993; Haughton et al. 1999; Kladivko 2001; Møller 2001; Perkins et al. 2000; Rands 1986). Intensive agricultural practices such as these have direct impacts on the ecology of croplands, including the existing flora and fauna. More generally, such varied management practices across the collection sites present viable sources of selection pressures that can drive the differential adaptation of species within a given time frame.

Downloaded From: https://complete.bioone.org/journals/Weed-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

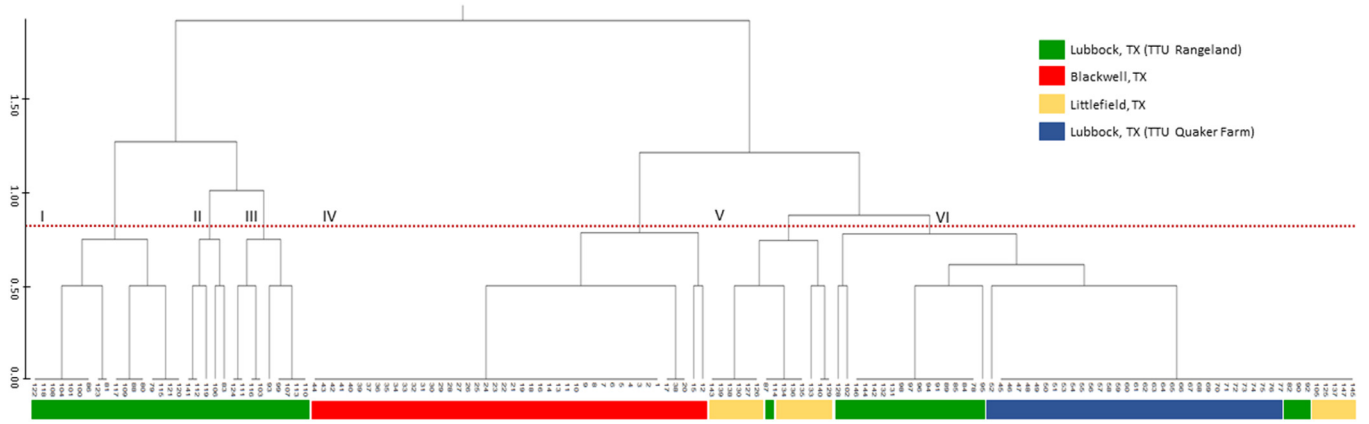


Figure 3. Unweighted pair group method with arithmetic mean clustering of *Solanum elaeagnifolium* populations from three localities in Texas, USA, based on Jaccard's similarity coefficient. Red broken line indicates genetic similarity threshold for the six major clusters identified. Colors indicate the different collection site where each individual plant was sampled.

The multitude of variable environmental stimuli that are unique to a particular ecology have been shown to serve as barriers that constrict gene flow between populations over time, thus increasing genetic differentiation (Linhart and Grant 1996). In the present study, the directional selection pressure from both environmental and/or anthropogenic factors that defines each collection site may have caused allele frequency shifts over time and favored specific genotypes with adaptability to each ecology. This hypothesis is supported by the obtained F_{ST} values that estimate the pairwise genetic differentiation of populations (Wright 1978). The calculated F_{ST} for the populations ranged from 0.17 to 0.46, indicating high levels of genetic divergence. Differentiation was highest between the Lubbock rangeland and Blackwell hunting reserve, where selection pressures are highly variable, and lowest between the Littlefield rangeland and Lubbock agricultural farm (Table 5). Although selection pressures in rangelands and agricultural farms are different, it should be noted that individuals from the Lubbock rangeland also grouped with individuals from the two former populations to create an admixture.

Several individual plants from the Lubbock rangelands clustered with all the plants collected from the experimental farm. The rangeland and experimental farm in Lubbock are located only 1.5 km apart. Given the proximity of the two sites, movement of *S. elaeagnifolium* propagules (i.e., seeds, root and stem fragments) from one location to another is probable through natural dispersal or via agricultural practices. This may account for the genetic similarity between individuals from the rangeland and experimental farm.

Intraspecific variations that were observed among individuals of the same cluster are attributed to the nature of *S. elaeagnifolium* as an obligate outcrosser (Hardin et al. 1972). Cross-pollination facilitates allelic reshuffling that results in novel gene recombinations within the genome. This allows naturally cross-pollinated species to maintain an innate degree of variability. Such genetic variation is important in enhancing the ability of small plant populations to remain viable under fluctuating stresses and novel environmental conditions (Wise et al. 2002). In larger populations, genetic variation can favor rapid genetic adaptation without any major loss in genome-wide variation (Menchari et al. 2007).

Genetic adaptation as facilitated by genetic variation is one of the critical mechanisms underlying the adaptive success of invasive species such as *S. elaeagnifolium*. Understanding the extent of genetic variation that drives the successful establishment of *S. elaeagnifolium* under different selection pressures will facilitate the design of an effective strategy for the long-term control of the species. In this study, 78 SSR and indel markers that are transferable to *S. elaeagnifolium* from *S. lycopersicoides* were identified and used to genotype *S. elaeagnifolium* populations from four different localities within the species' native range in Texas, USA. DNA marker profiling established a high degree of genetic variation among individuals within populations, as well as genetic differentiation of each population in response to the selection pressures that are unique to each collection site. Together, these results indicate the great capacity of *S. elaeagnifolium* for genetic differentiation and, therefore, adaptation to variable selection pressures in potential ranges outside its natural habitat.

To gain a more accurate assessment of the extent of genetic variation present in *S. elaeagnifolium* that allowed it to gain widespread geographic distribution, genetic diversity analysis of populations that have successfully adapted and established in environments that are drastically different from that of its native range (i.e., northern U.S. states, areas with less than ideal cold and/or wet conditions) will be necessary. To this end, the development of a core set of *S. elaeagnifolium*–specific markers that can be used in genetic diversity studies in the species remains an important requirement.

Acknowledgments. This research received no specific grant from any funding agency or the commercial or not-for-profit sectors. No conflicts of interest have been declared.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/wsc.2020.25

References

Andreasen C, Stryhn H, Streibig J (1996) Decline of the flora in Danish arable fields. J Appl Ecol 33:619–626

Angeles-Shim RB, Reyes VP, Del Valle MM, Sunohara H, Shim J, Lapis RS, Jena KK, Ashikari M, Doi K (2020) Marker-assisted introgression of the quantitative resistance gene *pi21* confers broad-spectrum resistance of rice to blast. Rice Sci 27:113–123

Angeles-Shim RB, Vinarao RB, Balram M, Jena, KK (2014) Molecular analysis of *Oryza latifolia* Desv.-derived introgression lines and identification of value-added traits for rice (*O. sativa* L.) improvement. Hered 105:676–689

Barret SC (2015) Foundations of invasion genetics: the Baker and Stebbins legacy. Mol Ecol 24:1927–1941

Downloaded From: https://complete.bioone.org/journals/Weed-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

Becerra V, Paredes M, Ferreira ME, Gutiérrez E, Díaz LM (2017) Assessment of the genetic diversity and population structure in temperate japonica rice germplasm used in breeding in Chile, with SSR markers. Chil J Agric Res 77:15–26

Botstein D, White RL, Skalnick MH, Davies RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphism. Am J Hum Genet 32:314–331

Boyd JW, Murray DS (1982) Growth and development of silverleaf nightshade (*Solanum elaeagnifolium*). Weed Sci 30:238–343

Boyd JW, Murray DS, Tyrl RJ (1984) Silverleaf nightshade, *Solanum elaeagnifolium*, origin, distribution and relation to man. Econ Bot 38:210–217

Brandon LJ (2005) Weed Management in Roundup Ready Flex Cotton. MSc thesis. Lubbock: Texas Tech University. 57 p

Chiarini FE, Scaldaferro MA, Bernardello G, Acosta MC (2018) Cryptic genetic diversity in *Solanum elaeagnifolium* (Solanaceae) from South America. Aust J Bot 66:531–540

Collard, BCY, Jahufer MZZ, Brouwer JB, Pang ECK (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. Euphytica 142:169–196

Collard BCY, Vera Cruz CM, McNally KL, Virk PS, Mackill DJ (2008) Rice molecular breeding laboratories in the genomics era: current status and future considerations. Int J Plant Genomics 2008:524847

Cuthbertson EG, Leys AR, McMaster G (1976) Silverleaf nightshade—a potential threat to agriculture. Agric Gaz NSW 87:11–13

Davidson AM, Jennions M, Nictra AB (2011) Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. Ecol Lett 14:419–431

[EPPO] European and Mediterranean Plant Protection Organization (2007) *Solanum elaeagnifolium*. Datasheets on quarantine pests. Bull OEPP 37:236–245

Geng Y, van Klinken RD, Sosa A, Li B, Chen J, Xu CY (2016) The relative importance of genetic diversity and phenotypic plasticity in determining invasion success of a clonal weed in the USA and China. Front Plant Sci 7:213

Govindaraj M, Vetriventhan M, Srinivasan M (2015) Importance of genetic diversity assessment in crop plants and its recent advances: an overview of its analytical perspectives. Genet Res Int 2015:431487

Green RE, Stowe TJ (1993) The decline of the corncrake *Crex crex* in Britain and Ireland in relation to habitat change. J Appl Ecol 31:667–692

Hardin JW, Doerkson G, Herndon D, Hobson M, Thomas F (1972) Pollination and floral biology of four weedy species in southern Oklahoma. Southwest Nat 16:403–412

Haughton A, Bell J, Boatman N, Wilcox A (1999) The effects of different rates of the herbicide glyphosate on spiders in arable field margins. J Arachnol 27:249–254

Havstad K, Peters D, Allen-Diaz B, Bartolome J, Bestelmeyer B, Briske D, Brown J, Brunson M, Herrick J, Huntsinger L, Johnson P, Joyce L, Pieper R, Svejcar T, Jiao J (2009) The western United States rangelands: a major resource. Pages 75–93 *in* Wedin WF, Fales SL, eds. Grassland Quietness and Strength for a New American Agriculture. Madison, WI: ACSESS Digital Library

Heap JW, Carter RJ (1999) The biology of Australian weeds. 35. *Solanum elaeagnifolium* Cav. Plant Prot Q 14:2–12

Hernández P, Laurie DA, Martín A, Snape JW (2002) Utility of barley and wheat simple sequence repeat (SSR) markers for genetic analysis of *Hordeum chilense* and tritordeum. Theor Appl Genet 104:735–739

Jain A, Roorkiwal M, Kale S, Garg V, Yadala R, Varshney RK (2019) InDel markers: an extended marker resource for molecular breeding in chickpea. PLoS ONE 14:e0213999

Jamil M, Rana IA, Ali Z, Awan FS, Shahzad Z, Khan AS (2013) Estimation of genetic diversity in rice (*Oryza sativa* L.) genotypes using simple sequence repeats. Mol Plant Breed 36:285–291

Kladivko EJ (2001) Tillage systems and soil ecology. Soil Till Res 61:61–76

Kollman J, Bañuelos MJ (2004) Latitudinal trends in growth and phenology of the invasive alien plant *Impatiens glandulifera* (Balsaminaceae). Divers Distrib 10:377–385

Kuleung C, Baenziger PS, Dweikat I (2004) Transferability of SSR markers among wheat, rye and triticale. Theor Appl Genet 108:1147–1150

Kumar S, Tamura K, Nei M (1994) MEGA: molecular evolutionary genetics analysis software for microcomputers. Bioinformatics 10:189–191

Linhart YB, Grant MC (1996) Evolutionary significance of local genetic differentiation in plants. Annu Rev Ecol Syst 27:237–277

McCouch SR, Chen X, Panaud O, Temnykh S, Xu Y, Cho Y, Huang N, Ishii T, Blair M (1997) Microsatellite marker development, mapping and applications in rice genetics and breeding. Plant Mol Biol 35:89–99

Mekki M (2007) Biology, distribution and impacts of silverleaf nightshade (*Solanum elaeagnifolium* Cav.) Bull OEPP 37:114–118

Menchari Y, Délye C, le Corre V (2007) Genetic variation and population structure in black-grass (*Alopecurus myosuroides* Huds.), a successful, herbicide-resistant, annual grass weed of winter cereal fields. Mol Ecol 16:3161–3172

Møller AP (2001) The effect of dairy farming on barn swallow *Hirundo rustica* abundance, distribution and reproduction. J Appl Ecol 38:378–389

Moritz C, Agudo R (2013) The future of species under climate change: resilience or decline? Science 341:504–508

Mullan DJ, Platteter A, Teakle NL, Appels R, Colmer TD, Anderson JM, Francki MG (2005) EST-derived SSR markers from defined regions of the wheat genome to identify *Lophopyrum elongatum* specific loci. Genome 48:811–822

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4325

Nunney L (2016) Adapting to a changing environment: modeling the interaction of directional selection and plasticity. J Hered 107:15–24

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537–2539

Penner G (1996) RAPD analysis of plant genomes. Pages 251–268 *in* Jauhar PP, ed. Methods of Genome Analysis in Plants. Boca Raton,FL: CRC Press

Perkins AJ (2000) Habitat characteristics affecting use of lowland agricultural grassland by birds in winter. Biol Conserv 95:279–294

Powell W, Machray G, Provan J (1996) Polymorphism revealed by simple sequence repeats. Trends Plant Sci 1:215–222

Qasem JR, Abdallat AM, Hasan SM (2019). Genetic diversity of silverleaf nightshade (*Solanum elaeagnifolium* Cav.), an invasive problematic weed in Jordan. Weed Res 59:222–234

Rands MRW (1986) The survival of gamebird (Galliformes) chicks in relation to pesticide use in cereal fields. Ibis 128:57–64

Rice KJ, Mack RN (1991) Ecological genetics of *Bromus tectorum*. Oecologia 88:91–101

Richards CL, Bossdorf O, Muth NZ, Gurevitch J, Pigliucci M (2006) Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. Ecol Lett 9:981–993

Roche C (1991) Silverleaf nightshade (*Solanum elaeagnifolium* Cav.). Washington, DC: Pacific Northwest Extension Publication. 2 p

Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thompson JN, Weller SG (2001) The population biology of invasive species. Annu Rev Ecol Syst 32:305–332

Scaldaferro M, Chiarini F, Santiñaque F, Bernardello G, Moscone E (2012) Geographical pattern and ploidy levels of the weed *Solanum elaeagnifolium* (Solanaceae) from Argentina. Genet Resour Crop Evol 59:1833–1847

Shim J, Torollo G, Angeles-Shim RB, Cabunagan RC, Choi IR, Yeo US, Ha WG (2015) Rice tungro spherical virus resistance into photoperiod-insensitive japonica rice by marker-assisted selection. Breed Sci 65:345–351

Si C, Dai Z, Lin Y, Qi S, Huang P, Miao S, Du D (2014). Local adaptation and phenotypic plasticity both occurred in *Wedelia trilobata* invasion across a tropical island. Biol Invasions 16:2323–2337

Stanton RA, Heap JW, Carter RJ, Wu H (2009) *Solanum elaeagnifolium*. Pages 274–293 *in* Panetta FD, ed. The Biology of Australian Weeds. Volume 3. Melbourne: RG and FJ Richardson

Stern S, Fátima M, Bohs A, Bohs L (2011) Molecular delimitation of clades within New World species of the "spiny solanums" (*Solanum* subg. *Leptostemonum*) Taxon 60:1429–1441

Taramino G, Tingey S (1996) Simple sequence repeats for germplasm analysis and mapping in maize. Genome 39:277–287

Temnykh S, DeClerck G, Lukashova A, Lipovich L, Cartinhour S, McCouch S (2001) Computational and experimental analysis of microsatellites in rice

Downloaded From: https://complete.bioone.org/journals/Weed-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

(*Oryza sativa* L.): frequency, length variation, transposon associations, and genetic marker potential. Genome Res 11:1441–1452

Travlos IS (2013) Responses of invasive silverleaf nightshade (*Solanum elaeagnifolium*) populations to varying soil water availability. Phytoparasitica 41:41–48

Tu M, Lu B-R, Zhu Y, Wang Y (2007) Abundant within varietal genetic diversity in rice germplasm from Yunnan Province of China revealed by SSR fingerprints. Biochem Genet 45:789–801

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hoernes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Wang HY, Wei YM, Yan ZH, Zheng YL (2007) EST-SSR DNA polymorphism in durum wheat (*Triticum durum* L.) collections. J Appl Genet 48:35–42

Weir BS (1990) Genetic Data Analysis. Methods for Discrete Population Genetic Data. Sunderland, MA: Sinauer. 377 p

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18:7213–7218

Williams J, Kubelik A, Livak K, Rafalski J, Tingey S (1990) DNA Polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Wise C, Ranker T, Linhart Y (2002) Modeling problems in conservation genetics with *Brassica rapa*: genetic variation and fitness in plants under mild, stable conditions. Conserv Biol 16:1542–1554

Wright S (1978) Evolution and the Genetics of Populations. Variability within and among 427 Natural Populations. Volume 4. Chicago: University of Chicago Press. 580 p

Zhu XC, Raman H, Wu H, Lemerle D, Burrows GE, Stanton R (2013a) Development of SSR markers for genetic analysis of silverleaf nightshade (*Solanum elaeagnifolium*) and related species. Plant Mol Biol Rep 31: 248–54

Zhu XC, Wu HW, Raman H, Lemerle D, Stanton R, Burrows GE (2012) Evaluation of simple sequence repeat (SSR) markers from *Solanum* crop species for *Solanum elaeagnifolium*. Weed Res 52:217–223

Zhu XC, Wu HW, Raman H, Lemerle D, Stanton R, Burrows GE (2013b) Genetic variation and structure of *Solanum elaeagnifolium* in Australia analysed by amplified fragment length polymorphism markers. Weed Res 53:344–354

Zhu XC, Wu HW, Stanton R, Burrows G, Lemerle D, Raman H (2013c) Time of emergence impacts the growth and reproduction of silverleaf nightshade (*Solanum elaeagnifolium* Cav.). Weed Biol Manag 13:98–103

Downloaded From: https://complete.bioone.org/journals/Weed-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use