IDENTIFICATION OF SILVERLEAF NIGHTSHADE USING MICROSATELLITE MARKERS AND MICROSTRUCTURE

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ABSTRACT

Silverleaf nightshade (*Solanum elaeagnifolium* Cav.) originated in America and is a serious summer-growing perennial weed in Australia. It is often confused with the native Solanaceae species quena (*S. esuriale* Lindl.). Both belong to the "*Leptostemonum*" subclass in the Solanaceae and are remarkably similar in their morphological traits. Correct identification is critical for the successful management of *S. elaeagnifolium*, as different biotypes could vary significantly in their response to control measures, such as herbicides and biocontrol agents. In order to improve the identification of *S. elaeagnifolium*, DNA polymorphism and microstructure of *S. elaeagnifolium* and *S. esuriale* were compared. Thirteen cross-species simple sequence repeat (SSR) primer pairs were utilized to investigate the polymorphism between *S. elaeagnifolium* and *S. esuriale*. SSR markers clearly separated the two species. Three unique SSR alleles were present in *S. esuriale* but not in *S. elaeagnifolium*, which could be used to distinguish the two species. Scanning electron microscope (SEM) examination of the microstructure of the leaf surface of these two species showed that the complex stellate trichomes on the upper leaf surface of *S. elaeagnifolium* had a deep "root" structure penetrating into the palisade mesophyll, while this structure was not found in *S. esuriale*. Combination of molecular phylogeny and SEM will considerably assist in the correct identification of *S. elaeagnifolium*.

Key Words: silverleaf nightshade, quena, perennial weed, SSR, SEM, trichome

INTRODUCTION

Silverleaf nightshade (*Solanum elaeagnifolium* Cav.) is a deep-rooted, summer-growing perennial weed which originated in America (Heiser and Whitaker 1948; Stanton *et al.* 2009). This invasive weed infests at least 350,000 ha in Australia and with the potential to infest 398 million ha (Kwong 2006; Feuerherdt 2009).

Correct identification of silverleaf nightshade is required for selection of herbicides and biocontrol agents (Nissen *et al.* 1995; Lopez-Martinez *et al.* 1999). However, silverleaf nightshade often confused with an Australian native Solanaceae species, quena (*S. esuriale* Lindl.). The misidentification resulted in delays to control of silverleaf nightshade in South Australia (Hosking *et al.* 2000). Currently differentiation between the two species is based on morphological characteristic such as stamens length, spine density or fruit shape (Kidston *et al.* 2006). However, these morphological traits vary considerably in

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Australian silverleaf nightshade populations (Stanton*, et al.* 2009). Identification based solely on morphological traits is unreliable.

Molecular markers have been widely used in *Solanum* species to delineate species and cultivars (Chimote *et al.* 2004) . For example, Chimote*, et al.* (2004) used simple sequence repeat (SSR) markers to differentiate 32 Indian cultivars of potato (*S. tuberosum*). In addition, previous studies have used molecular markers to identify the main clades and phylogenetic relationships within *Solanum* (Bohs 2005; Weese and Bohs 2007). The Australian native species quena was not included in these previous studies.

Micro-morphological parameters, such as leaf trichomes were considered as some of the most distinguishing features in *Solanum* (Roe 1971). Bean (2004) noted that silverleaf nightshade was so similar in morphology to quena that microscopic examination was usually required for identification.

In this study, micro-morphological traits and SSR markers were used to differentiate silverleaf nightshade from the Australian native *Solanum* species quena.

MATERIAL AND METHODS

Molecular Analysis

Silverleaf nightshade leaf samples were collected from Jarklin, Shepparton and Lake Boga (Victoria), and Corowa, Morven and Gulgong (New South Wales). Quena samples were collected from Jarklin (Victoria) and Ungarie, Wellington and Wagga Wagga (New South Wales). Genomic DNA was extracted from leaf material using the standard phenol/ chloroform method (Sambrook *et al.* 1989). The DNA samples of each species from the same location were bulked for PCR amplification. Thirteen SSR primer-pairs from other *Solanum* species were used in this study. Primer details were those mentioned in previous study of Zhu *et al.* (2011). The 5' end of the forward primer of each SSR primer-pair was tailed with M13 sequence and PCR amplification and detection of the amplification products were carried out as described by Raman *et al*. (2005). The alleles were scored in a binary form as the presence or absence (1 or 0) of bands of each SSR primer-pairs for each population and data were analysed using PAST (Hammer *et al.* 2001).

Micro-morphological Analysis

Seven populations of silverleaf nightshade from Jarklin, Shepparton, Lake Boga, Corowa, Morven, Gulgong and Wagga Wagga and four quena populations from Jarklin, Ungarie, Wellington and Wagga Wagga were observed in this study. The 4th leaf from the shoot apex was collected from each individual. Only the adaxial surface of each leaf was examined in this study. Trichomes were removed with forceps from fresh leaves and placed on a 12 mm carbon tab (ProSciTech, Australia). A total of 195 and 74 trichomes were observed for silverleaf nightshade and quena, respectively. Images of these trichomes were obtained using a scanning electron microscope (SEM) (JEOL JCM 5000 NeoScope, Japan). The length of the "root" structure of each trichome was measured using software ImageJ (Ferreira and Rasband 2010). Data were analyzed using unpaired two sample t-test of GenStat 13.0 (Buysse *et al.* 2004).

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RESULTS

Quena populations were clearly differentiated from silverleaf nightshade populations by the unweighted pair group method with arithmetic mean (UPGMA) dendrogram based on the Jaccard's similarity indicating the genetic variability between related species (Figure 1). In addition, three unique alleles were amplified in quena: fragment 85 bp with primer-pair EM 117, 222 bp with EM 135 and 249 bp with ESM 3. These unique alleles could be utilized to distinguish quena from silverleaf nightshade.

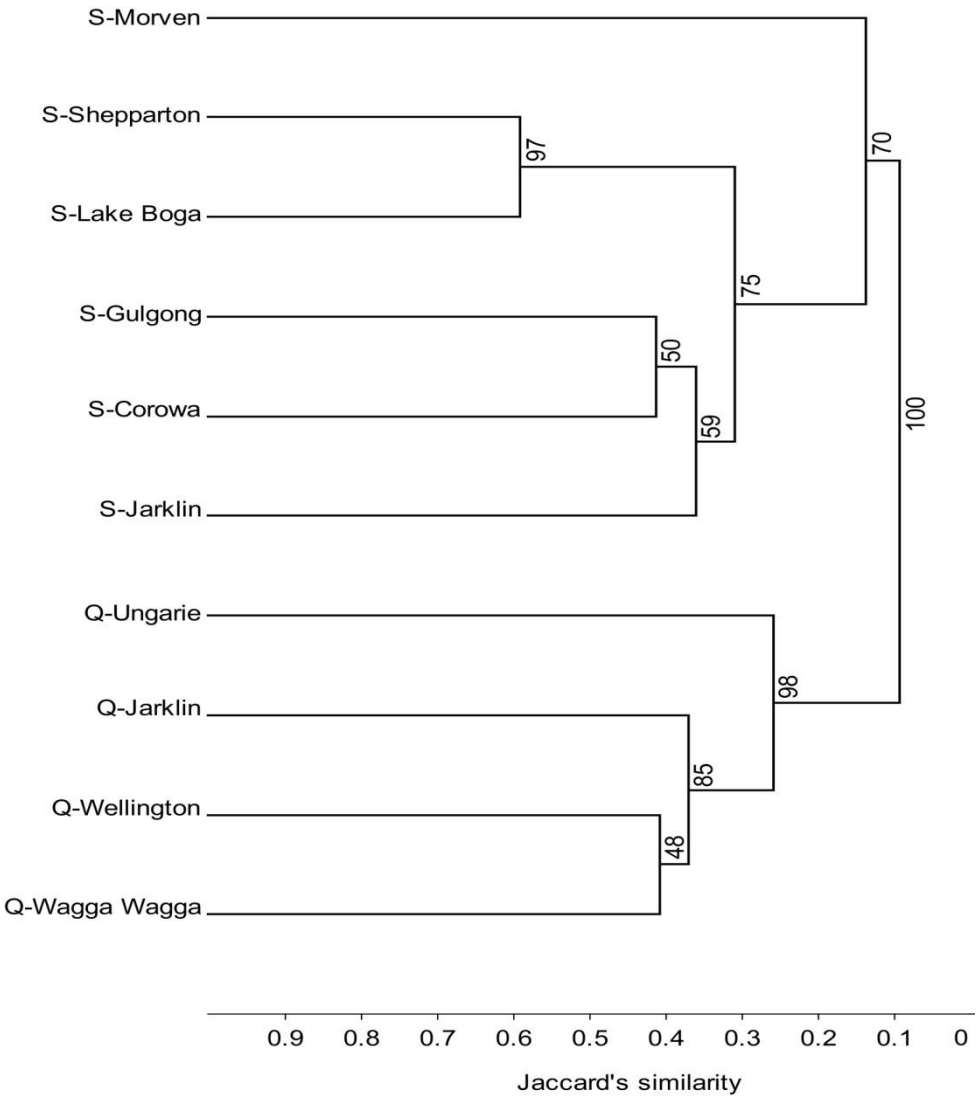

Figure 1. UPGMA dendrogram clearly separated quena (Q) and silverleaf nightshade (S) populations.

Significant difference on the length of trichome "root" structure ($P < 0.001$) was found between these two species (Figure 2). The average lengths of trichomes of silverleaf nightshade and quena were 134 and 33 μm, respectively.

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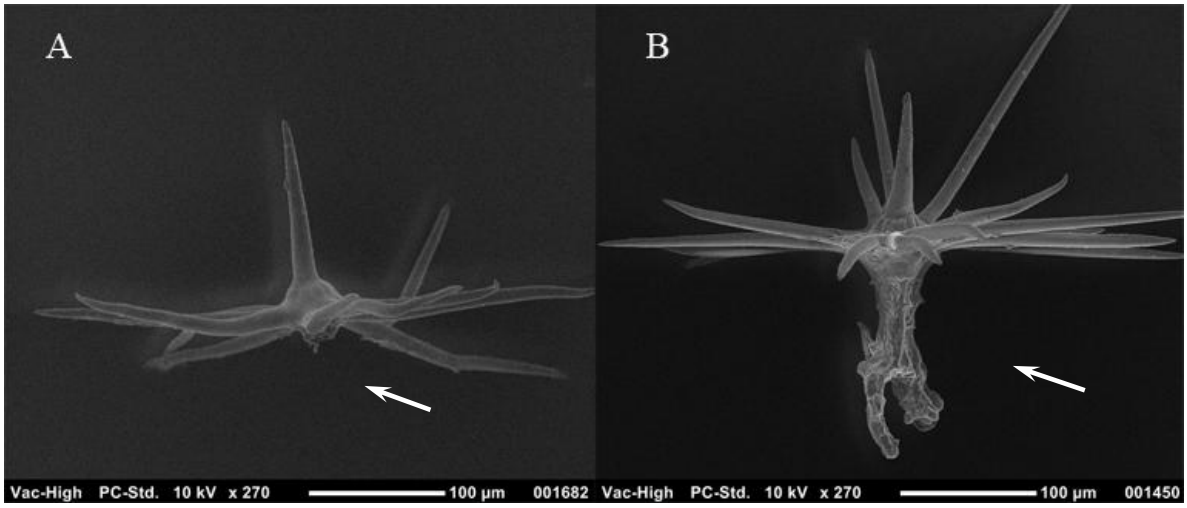

Figure 2. A comparison of trichome structure from adaxial leaf surface between quena and silverleaf nightshade. A: quena; B: silverleaf nightshade. Arrows indicate the root structure under epidermal level.

DISCUSSION

Silverleaf nightshade and quena were separated at a level of about 10% similarity (Figure 1), indicating the great genetic divergence between these two species. Furthermore, the unique alleles found in quena will provide a reliable method to distinguish these two species.

This is the first report on the examined morphological characteristics of quena. Silverleaf nightshade had a much longer “root” structure of the trichome than quena, which has been highlighted by previous studies (Bruno *et al.* 1999; Christodoulakis *et al.* 2009). This “root” deeply penetrates into the palisade mesophyll making it very difficult to pull off from the leaf. The significant difference on trichome structures between quena and silverleaf nightshade found in this study can be considered diagnostic in order to discriminate these two species. This possibility of the impacts of this structure on herbicide uptake needs to be further tested.

Correct identification of silverleaf nightshade will improve the weed management. Generally, quena is easier to manage than silverleaf nightshade (Johnson *et al.* 2006), therefore correct identification will help the herbicide selection and management strategies. In addition, reliable identification is also required for biocontrol, as agent/ weed compatibility has a significant influence on agent success (Nissen*, et al.* 1995).

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