Screening of gene regions for genetic diversity in global parthenium weed (*Parthenium hysterophorus* L.) populations

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Summary Parthenium weed (*Parthenium hysterophorus* L.) is an invasive species in more than 40 countries and a Weed of National Significance in Australia. It causes substantial losses to crop and pasture production and health problems in humans and livestock. The high invasive potential of this species is often attributed to its unique morphological features, competitive ability, and allelopathic potential; however, the level of genetic diversity in this species and how diversity contributes to its success is not currently clear. This study aimed to identify informative gene regions and sequences for the study of parthenium weed population genetics using samples collected from its introduced range in Australia (including two distinct biotypes) and the native ranges in Costa Rica, Mexico and the United States of America. Five gene regions were investigated including one nuclear region (ITS) and four chloroplast regions (*trn*H-*psb*A, *trn*L intron, *trn*L-*trn*F spacer and *matK*). Limited genetic diversity was found both in Australian and native populations, and our findings suggest that these gene regions are potentially not optimal for evaluation of population genetic diversity investigation in parthenium weed due to lack of polymorphism or that such genetic diversity is extremely limited. Our findings agree with previous reports of limited genetic variation observed in Australian, Chinese and Pakistan parthenium weed populations. Therefore, for future studies, we suggest the use of next generation sequencing approach such as targeted sequencing, RADseq (Restriction site Associated DNA Sequencing) or MassARRAY® to develop informative genetic markers for this species in an attempt to evaluate population diversity.

Keywords Parthenium weed, invasive alien species, DNA sequencing.

INTRODUCTION

Parthenium weed is a highly invasive species, but the actual mechanism of its invasion is not well understood (Bajwa *et al.* 2016). Several factors could potentially contribute to its spread including aggressive growth habit, high seed production, absence of natural enemies in introduced range, and abiotic stress tolerance ability (Bajwa *et al.* 2016, Nguyen *et al.* 2017). However, only a few studies have been conducted to: 1) evaluate the role of genetic diversity in parthenium weed invasion; and 2) to screen the gene regions or markers suitable to study the genetic diversity in this species.

In Australia, parthenium weed has two distinct biotypes, Toogoolawah and Clermont, both named from collection sites across Australia, but introduced on separate occasions from the United States of America (USA). The first introduction (Toogoolawah biotype) has remained uniquely non-invasive. However, the second introduction (Clermont biotype) of parthenium weed has spread across a large area in Queensland (Adkins and Shabbir 2014). The Clermont biotype is not only more invasive but also has higher germination and growth rates, reproductive output and competitive ability, as compared to the Toogoolawah biotype (Hanif 2014, Bajwa *et al.* 2017, 2018). Hanif (2014) reported that the Toogoolawah and Clermont biotypes were introduced independently in Australia, possibly from the northern and southern Texas races, respectively. However, limited genetic diversity was observed between both biotypes. Earlier studies have evaluated random amplified polymorphic DNA (*RAPD*), inter-simple sequence repeats (*ISSR*), and microsatellite (*SSR*) markers to assess genetic diversity in parthenium weed and reported very limited genetic diversity among various populations (Graham and Lange 1998, Tang *et al.* 2009, Qian *et al.* 2012, Jabeen *et al.* 2015).

Studying genetic lineages can help in identifying the point of origin of a specific invasive species, which might be useful for its management, particularly when

considering biological control. In addition, a high level of genetic diversity is generally considered as key mechanism associated with successful invasion (Prentis *et al.* 2008). Therefore, it is important to evaluate genetic diversity among different populations of parthenium weed, and to do that, the screening of both chloroplast and nuclear gene regions is a crucial preliminary step. This study therefore aimed to select reliable chloroplast and nuclear gene regions for study of the population genetics of parthenium weed using both native and Australian populations.

MATERIALS AND METHODS

Parthenium weed leaf tissue was sampled from the field both in Australia and its native range in Costa Rica, Mexico and the USA. The sampled locations in each native country covered 2–5 geographically distant locations (Table 1). The Australian samples represented two local biotypes, Clermont and Toogoolawah. Five individuals were sampled in each population. Leaf tissue was stored in silica gel until DNA extraction.

The DNA extraction, PCR, sequencing, alignment and sequence analysis were performed as described by Zhu *et al.* (2017). Based on previous plant-based studies, five gene sequences were tested for their suitability as a potential candidate for population genetics, including one nuclear region (ITS) and four chloroplastic regions (*matK*, *trn*H-*psb*A spacer, *trn*L intron and *trn*L-*trn*F spacer).

RESULTS AND DISCUSSION

PCR and sequencing were nearly 100% successful for all gene regions. All sequences obtained from this study were deposited in the GenBank with accession numbers from MH017879–MH17987. As previously reported, very limited genetic diversity was found among samples, even within the native populations. Only one haplotype was found using the *trn*L-*trn*F spacer, and two haplotypes were found using each of *trn*L intron, *trn*H-*psb*A spacer and *matK* regions, while three were observed using ITS. There was no correlation between haplotypes and biotypes, or between haplotypes and geographical distribution.

This is the first study that evaluated multiple native populations covering vast geographical distances. The low levels of diversity apparent across the native and Australian populations may indicate the examined loci are insufficiently polymorphic to track the population genetics and invasion history of this species. In contrast, Tang *et al.* (2009) reported that the morphologically similar parthenium weed biotypes in China had contrasting genetic makeup and, thus, were attributed to independent introductions.

The results of this study are in agreement with those of previous studies of the species which also found very limited genetic diversity using genetic analysis (Hanif 2014, Jabeen *et al.* 2015). Jabeen *et al.* (2015) studied the genetic structure of 11 populations (95 individuals) from across Pakistan including the two populations (10 individuals) from Australia using *ISSR* fingerprinting. It was found that 18% genetic diversity existed among the populations, while 82% existed within the populations. Genetic diversity was highest among the populations from Pakistan; however, gene flow was limited. An earlier study using the ITS markers reported the Australian biotypes to be more closely related to Mexican biotypes, but significantly different from Indian biotypes of parthenium weed (Graham and Lange 1998).

In conclusion, the gene regions tested in this study showed limited genetic diversity in parthenium weed populations from native and Australian ranges. Future research aiming to study the population genetics of this species may potentially benefit from the use of polymorphic markers including *SSR* or *AFLP*. Next Generation Sequencing (NGS) may also be applied to sequence the chloroplast genome of parthenium weed to search gene regions exhibiting greater polymorphism. In addition, for large population genetic studies, NGS approaches including RADseq or MassARRAY® could be considered to try to obtain highly variable SNPs in the parthenium weed genome.

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Table 1. Parthenium weed samples, their locations and the GenBank accession number of each gene region.

Sample ID	Source country	Latitude (N)	Longitude (E)	GenBank accession number				
				ITS	*matK*	*trn*L intron	*trn*L-*trn*F	*trn*H-*psb*A
PH0001	Costa Rica	9.960	−84.112	MH017936	MH017961	MH017921	MH017894	MH017879
PH0002	Costa Rica	9.960	−84.112	MH017937	MH017962	MH017922	MH017895	MH017880
PH0013	Costa Rica	10.460	−84.292		MH017963	MH017923	MH017896	MH017881
PH0014	Costa Rica	10.460	−84.292	MH017938	MH017964	MH017924	MH017897	MH017882
PH0015	Costa Rica	10.460	−84.292	MH017939	MH017965	MH017925	MH017898	MH017883
PH0016	Australia	−22.876	147.775	MH017940	MH017976		MH017899	
PH0017	Australia	−22.876	147.775	MH017941	MH017977		MH017900	
PH0018	Australia	−22.876	147.775	MH017942	MH017978		MH017901	
PH0019	Australia	−22.876	147.775	MH017943	MH017979		MH017902	
PH0020	Australia	−22.876	147.775	MH017944	MH017980		MH017903	
PH0022	Australia	−22.876	147.775	MH017945	MH017981		MH017904	
PH0027	Australia	−27.139	152.377	MH017946	MH017982		MH017905	
PH0028	Australia	−27.139	152.377	MH017947	MH017983		MH017906	
PH0029	Australia	−27.139	152.377	MH017948	MH017984		MH017907	
PH0030	Australia	−27.139	152.377	MH017949	MH017985		MH017908	
PH0031	Australia	−27.139	152.377	MH017950	MH017986		MH017909	
PH0035	Australia	−27.139	152.377	MH017951	MH017987		MH017910	
PH0161	Mexico	18.006	−93.301	MH017952	MH017966	MH017926	MH017911	MH017884
PH0162	Mexico	18.006	−93.301	MH017953	MH017967	MH017927	MH017912	MH017885
PH0168	Mexico	18.017	−93.315		MH017968	MH017928	MH017913	MH017886
PH0180	Mexico	17.972	−93.381	MH017954	MH017969	MH017929	MH017914	MH017887
PH0186	Mexico	17.855	−93.394	MH017955	MH017970	MH017930	MH017915	MH017888
PH0333	USA	28.180	−97.767	MH017956	MH017971	MH017931	MH017916	MH017889
PH0348	USA	27.838	−97.720	MH017957	MH017972	MH017932	MH017917	MH017890
PH0373	USA	28.550	−96.645	MH017958	MH017973	MH017933	MH017918	MH017891
PH0383	USA	29.313	−96.275	MH017959	MH017974	MH017934	MH017919	MH017892
PH0388	USA	30.538	−96.421	MH017960	MH017975	MH017935	MH017920	MH017893

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