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Genotypic diversity among pasture and roadside populations of the invasive buffelgrass (*Pennisetum ciliare* L. Link) in north-western Mexico

R. Gutierrez-Ozuna [a], L.E. Eguiarte [b], F. Molina-Freaner [a,*]

[a] Departamento de Ecología de la Biodiversidad, Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 1354, Hermosillo, Sonora, C. P. 83000, Mexico
[b] Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70-275, México D. F., C. P. 04510, Mexico

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ABSTRACT

Buffelgrass (*Pennisetum ciliare* L. Link) is an African agamospermous grass that has been widely introduced into arid regions of the world to improve rangelands for cattle production and as a result, it has invaded adjacent habitats. In this study, ISSR markers were employed to assess genotypic variation in populations of *P. ciliare* from north-western Mexico. We sampled plants from pasture and from colonizing roadside populations in order to explore if invasion success is associated with greater levels of genotypic diversity. Three ISSRs primers produced 27 reproducible bands that were used to screen 480 plants from sixteen populations. Mean values of the proportion of distinguishable genotypes (G/N) were 0.29, Nei's genotypic diversity was 0.75 and multilocus evenness (E) was 0.50. Sixty-seven multilocus genotypes were detected among 480 plants, with 46 (69%) restricted to single populations and 21 (31%) found in two or more populations. Our results showed no significant differences in genotypic diversity between pasture and roadside populations, suggesting that in *P. ciliare* invasion success is not directly associated with greater levels of genotypic variation. Probably, other factors such as phenotypic plasticity and propagule pressure could be major determinants of the invasion success of buffelgrass in this region of Mexico.

1. Introduction

The spread, naturalization and invasion of exotic plant species into native plant communities have been recognized as a serious ecological problem threatening biological diversity (Wilcove et al., 1998) and affecting ecosystem function (D'Antonio and Vitousek, 1992). However, only a small fraction of all introduced taxa reproduce and spread over large areas; most taxa fail at some stage before reaching such levels of success (Richardson et al., 2000). Because only a small fraction of introduced species become invasive (Williamson, 1996; Richardson et al., 2000), much effort has been focused on understanding what makes some species better invaders than others (Rejmànek and Richardson, 1996; Kolar and Lodge, 2001).

A growing number of studies have focused on genetic traits of invasive species and the role of genetic diversity in invasion success. Genetic and evolutionary processes may be key features in determining whether invasive species establish and spread (Sakai et al., 2001), but general patterns of genetic variation associated with successful colonizers have not been identified (Roy, 1990; Schierenbeck et al., 1995). Invasive species may evolve both during their initial establishment and during subsequent range expansion, especially in response to selection pressures generated by the novel environment (Sakai et al., 2001). Colonization events often involve genetic bottlenecks and consequently drift may lead to reduced genetic variation in the introduced population (Barrett and Husband, 1990). Reduced genetic variation may limit the population ability to evolve and invade the new habitat. However, genetically diverse founding populations and multiple introductions increase the likelihood of successful invasion (Sakai et al., 2001).

Understanding the role of genetic diversity during the invasion process is important to predict the response of invasive species to novel environments (Sakai et al., 2001). Empirical studies dealing with the genetics of plant invasions have found a wide spectrum of patterns regarding the role of genetic variation during invasion. These studies range from cases in which genetic variation plays an important role (Maron et al., 2004; Lavergne and Molofsky, 2007) to cases in which invasion success is not associated with genetic variation (Poulin et al., 2005; Meinberg et al., 2006). Despite the increasing number of studies dealing with genetics of plant invaders, little is known about the evolutionary mechanisms that permit geographic range expansion and evolution of invasiveness in introduced species

* Corresponding author. Tel./fax: +52 662 2139303.
E-mail address: freaner@servidor.unam.mx (F. Molina-Freaner).

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(Lavergne and Molofsky, 2007). Studies exploring the role of genetic variation during the different steps involved in the introduction dynamics could assess whether invasion results from the introduction of pre-adapted genotypes or occurs as a consequence of postimmigration processes (Novak, 2007).

Buffelgrass (*Pennisetum ciliare* L. Link = *Cenchrus ciliaris*) is an African perennial bunch grass that has been widely introduced to arid regions of the world to improve rangelands for cattle production (Cox et al., 1988). Although sexual seed production has been detected in low frequency (Hignight et al., 1991), reproduction is mainly through agamospermy (Bashaw, 1962; Bray, 1978). Buffelgrass was first introduced to north-western Mexico during the 1950s but large-scale conversion of desert and thorn scrub to buffelgrass pastures began in the 1970s (Cox et al., 1988; Castellanos et al., 2002). In this region of Mexico, buffelgrass land conversion shows an exponential growth, covers around 8% of the area and if current rates are maintained, in 10 years is likely to occupy 3 million hectares (Castellanos et al., 2002; Franklin et al., 2006). The introduced material came from Texas as common buffel (T-4464), a selection from samples collected in Kenya (Holt, 1985; Alcalá, 1995). In addition, several varieties were tested in the region and distributed to ranchers (Servín and Ramírez, 1981; Enriquez et al., 1982; Martin-R et al., 1989). However, nothing is known about levels of genetic variation in buffelgrass from pasture populations. One of the unforeseen consequences of the introduction of this species has been the invasion of adjacent, desert habitats (Cox et al., 1988; Burguess et al., 1991; Bowers et al., 2006). In this region, buffelgrass colonizes and spreads into roadside shoulders, city lots, hillsides and unconverted desert habitats (Búrquez et al., 2002), whose ecological consequences are yet to be documented. The spread of pasture plant stands to adjacent non-planted sites by *P. ciliare* has been reported only in some regions of the world (Cox et al., 1988; Ibarra-F et al., 1995). Therefore, the invasion of adjacent habitats from pasture populations in north-western Mexico offers a good system to explore whether genetic and genotypic variation plays an important role in the invasion success.

In this paper, we describe levels of genotypic and genetic diversity within and among populations of *P. ciliare* in north-west Mexico using ISSR (inter-simple sequence repeats) markers. By examining levels of variation in pasture and roadside (invasive) populations, we explore whether genotypic variation plays an important role during the process of invasion. If invasive populations exhibit greater levels of clonal diversity, genetic variation might play an important role during the invasion of buffelgrass. In contrast, if pasture and roadside population exhibit the same level of variation, other processes may explain the invasion success, like phenotypic plasticity or propagule pressure, which is a composite measure of the number of individuals released into a region to which they are not native. This measure includes both the absolute number of individuals involved in any one release event and the number of discrete release events (Allendorf and Lundquist, 2003; Lockwood et al., 2005).

2. Material and methods

2.1. Population sampling

Sixteen populations of *P. ciliare* located in the Mexican states of Sonora (12 populations), Baja California Sur (three populations) and Sinaloa (one population) were sampled during September of 2004 (Fig. 1). Sampling tried to cover the current distribution of buffelgrass (pasture and roadside populations) in north-western Mexico. Leaf tissue was collected from 480 plants, using 30 individuals per population. The sampling scheme tried to combine plants from pastures with plants growing along adjacent roadsides, as often they represent migration corridors of invasive plants (Von der Lippe and Kowarik, 2007). In seven sites, we sampled both groups of plants; in eight sites, we sampled only roadside individuals, whereas in just one locality we collected only pasture plants (Table 1). The objective of this sampling method was to evaluate if clonal diversity of invasive populations is greater than pasture populations and, if invasion success is associated with greater levels of genotypic diversity. To increase the likelihood of picking leaves from different clones, leaf samples were taken from individuals at least 10 m apart. Samples were immediately stored in liquid nitrogen and once in the lab, they were transferred to an ultra cold freezer (−80 °C). In this study, populations were defined as discrete groups of plants covering an area of at least 1 ha.

2.2. DNA extraction

Leaf tissue (0.5 g) was ground into fine powder in liquid nitrogen. Genomic DNA was extracted adding 260 μL of CTAB extraction buffer and 975 μL of STE buffer. The mixture was then agitated and subsequently centrifuged at 12,000 rpm for 8 min. The supernatant was eliminated and the precipitated was resuspended in 250 μL of CTAB buffer and 750 μL of STE buffer. Samples were centrifuged at 12,000 rpm for 8 min, the supernatant was eliminated and the precipitated was resuspended in 600 μL of 2× CTAB buffer. The samples were treated with ribonuclease 7000 u/mL (4 μL per sample) at 37 °C for 20 min to digest RNA. After this treatment, 25 μL of proteinase-K (20 mg/mL) was added and incubated at 65 °C for 30 min. The samples were placed on ice for 15 min. DNA was isolated using chloroform: octanol 24:1 separation (adding 600 μL per sample and homogenising), centrifuged at 9000 rpm for 12 min, and the supernatant was transferred to a fresh centrifuge tube. DNA was precipitated with 600 μL of cold isopropanol (−20 °C) and maintained for 12 h at −20 °C. After that time, samples were centrifuged at 12,500 rpm for 7 min and the supernatant was eliminated. The pellet was washed agitating gently for 5 min with 70% ethanol (−20 °C). The pellet was dried, resuspended in 100 μL of PCR grade water, and stored at 4 °C. DNA concentration of each extract was quantified using an Eppendorf biophotometer.

2.3. ISSR amplification

A total of 22 simple sequence repeats (from the University of British Columbia primer set 9) were screened, and primers that gave promising amplification products were screened again after adjusting their PCR conditions. Eventually, three ISSR primers that produced clear and reproducible bands were selected to screen for genetic variation: 810 [$(GA)_8$ T], 840 [$(GA)_8YT$] and 850 [$(GT)_8YC$]. Single letter abbreviation for mixed base position, $Y = (C, T)$.

All polymerase chain reactions (PCR) were run as single primer reactions in a total volume of 30 μL, consisting of 45 ng of template DNA, 1× PCR buffer, 0.2 mM of each dNTP, 0.5 μM primer, 0.7 units Taq polymerase, 3 mM $MgCl_2$ for primers 810 and 840, 2.5 mM $MgCl_2$ for primer 850, and PCR grade water. The reactions were performed using a Thermo Hybaid PCR Express thermal cycler with hot bonnet with the following PCR profile: Initial denaturation was carried out for 3 min at 95 °C, followed by 35 cycles of 30 s at 94 °C, 45 s at optimal annealing temperatures (53 °C for primer 810, 55 °C for primer 840, and 52 °C for primer 850), 75 s at 72 °C, and a final 5 min extension at 72 °C, followed by a 4 °C soak. A negative control tube was also included in each reaction to check for contamination. Amplification products were stored at 4 °C until electrophoresis on 2% horizontal agarose gel in 1× TAE buffer (Tris–Acetic acid–EDTA) with a constant voltage of 180 V for 2 h 15 min and visualized under ultraviolet light after staining in ethidium bromide. Gels were