

The polyploid nature of *Cenchrus ciliaris* L. (*Poaceae*) has been overlooked: new insights for the conservation and invasion biology of this species – a review

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Abstract. Climate change, associated with increased aridity, and high grazing pressure by livestock results in the scarcity and loss of perennial *Poaceae* in arid ecosystems. The species threatened by this include *Cenchrus ciliaris* L., a native perennial grass of the tropical and sub-tropical arid rangelands of Africa and Western Asia and now introduced in Central and South America, and Australia. This species reproduces predominantly through aposporous apomixis although sexual individuals have been occasionally identified. *Cenchrus ciliaris* is characterised by a significant, heritable, phenotypic polymorphism and three ploidy levels including tetraploids ($2n=4x=36$), pentaploids ($2n=5x=45$) and hexaploids ($2n=6x=54$). Under water-deficit conditions, *C. ciliaris* shows plasticity in growth characteristics and aboveground biomass. This phenotypic plasticity has led to the identification of genotypic-associated responses conferring more productivity. This underlines the importance of conserving the genetic diversity of *C. ciliaris* in order to ensure the persistence of the vegetation cover in the arid ecosystems in which it occurs. Observations from cytogenetic and molecular data converge to underline the possibility of sexual reproduction, recombination and gene flow within and between populations of *C. ciliaris*. Genetic mechanisms, such as polyploidy, hybridisation between ploidy levels and apomixes, are generating and then maintaining the diversity of *C. ciliaris*. This review emphasises the role of polyploidy in the evolutionary development of *C. ciliaris* and how it may be a crucial factor for its conservation in some countries and its weedy nature in others.

Additional keywords: buffelgrass, facultative apomixis, genetic diversity, phenotypic variation, water stress.

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Introduction

Climatic change, associated with increasing aridity, leads to a high loss of vegetation cover in arid and semiarid ecosystems (Reynolds *et al.* 2007; Gornall *et al.* 2010; Hoffmann and Sgrò 2011; Sowers *et al.* 2011). Because perennial herbaceous plants result in higher levels of ground cover and persist for longer than annual species, they are the most intensively grazed by cattle (Chaieb *et al.* 1996). They are the species most affected by dryland degradation and constitute one factor contributing to desertification (Whitford 2002; Jauffret and Visser 2003).

Cenchrus ciliaris L. [*Poaceae* syn. *Pennisetum ciliare* (L.) Link, buffelgrass] is a native perennial grass of tropical and sub-tropical arid rangelands of Africa and Western Asia. Because *C. ciliaris* is highly valued as a pastoral species (Arshadullah *et al.* 2011) and suited to a range of harsh conditions (Mansoor *et al.* 2002), it has been widely introduced as a pasture species and for erosion control in Australia, Mexico and South America (Marshall *et al.* 2012). Its establishment in Australia has been associated with a loss of native species (Clarke *et al.* 2005; Jackson 2005; Eyre *et al.* 2009; Smyth *et al.* 2009) and alteration of fire regimes (Miller *et al.* 2010).

Adaptive characteristics

This C_4 perennial is the predominant grass in many arid lands (Marshall *et al.* 2012). Despite its wide distribution, the expansion of overgrazing associated with the severity of climatic factors in arid ecosystems has led to a continuous thinning of stands and the threat of extinction of this species throughout its natural area of occurrence (Chaieb *et al.* 1996; Whitford 2002). Considering the ecological interest offered by *C. ciliaris*: drought tolerance (Bhatt *et al.* 2007; De La Barrera and Castellanos 2007; Kharrat-Souissi *et al.* 2012a), high fodder production and nutritive value to livestock (Kharrat-Souissi *et al.* 2010; Arshadullah *et al.* 2011), rapid regeneration in response to rainfall (Bose and Balakarishnan 2001), tolerance of heavy grazing (M'seddi *et al.* 2004a) and soil stabilisation abilities because of a deep root system (Chaieb *et al.* 1996; Mnif and Chaieb 2009), the persistence of this species in its native range in arid ecosystems is a crucial factor for restoration ecology of degraded arid rangelands. For these reasons, *C. ciliaris* has been the subject of numerous applied studies, which have focussed on its phenotypic polymorphism, its ecological significance (M'seddi *et al.* 2002; Arshad *et al.* 2007; Jorge *et al.* 2008; Visser *et al.* 2008; Kharrat-Souissi *et al.* 2010; Arshadullah *et al.* 2011), resistance to drought (Mansoor *et al.* 2002; Mnif and Chaieb 2010; Kharrat-Souissi *et al.* 2012a), biological invasion capacity (Friedel *et al.* 1996; Franklin *et al.* 2006; Gutierrez-Ozuna *et al.* 2009; Marshall *et al.* 2012) and its genotypic diversity (Nisar *et al.* 2010; Al-Soqeer 2011; Kharrat-Souissi *et al.* 2011).

For effective conservation and management of *C. ciliaris*, it is important to understand intra-specific genetic diversity and its relationship to other traits. The association between biological and ecological characteristics, such as mode of reproduction (apomixis or sexuality), genetic characterisation (polyploidy and genotypic diversity), ability to cope with environmental disturbance and the capacity for colonising new habitats, will contribute considerably. One option for increasing availability of this species in its native range is through the development of an optimal seed collection strategy. The basic tenet of native seed production is to provide seed of local origin and of the widest adaptive genetic diversity possible. This review, based on an analysis of published data, focuses on the conservation, invasion biology and use of *C. ciliaris* in restoration of degraded arid and semiarid ecosystems. The specific objectives of this paper are, therefore, to:

- (1) Document the facultative nature of apomixis in *C. ciliaris*;
- (2) Underline neglected aspects concerning the invasion biology of *C. ciliaris*;
- (3) Understand the intra-specific phenotypic variation;
- (4) Emphasise the polyploid nature of *C. ciliaris* and its ecological implications;
- (5) Underline the adaptive response of the species to water deficit in arid environments, and
- (6) Consider the implications of molecular genotypic data for conservation and management of *C. ciliaris*.

Facultative nature of apomixis in *Cenchrus ciliaris*

Cenchrus ciliaris is one of few pastoral species that are apomictic, producing clones from seeds. This asexual reproduction provides the advantage of rapid multiplication allowing the development

of a significant aboveground yield and consequently the formation of an extremely dense ground cover by *C. ciliaris* (Ozias-Akins *et al.* 2003; Akiyama *et al.* 2005; Miles 2007; Ozias-Akins and Van Dik 2007; Singh *et al.* 2007). Asexual reproduction may have benefits in stable environments, while sexual reproduction offers a net advantage by allowing more rapid generation of genetic diversity allowing adaptation to a changing environment. *Cenchrus ciliaris* was considered for many years as an obligate apomict, with apospory followed by pseudogamy (Savidan 2000; Visser *et al.* 2000). Apospory involves the formation of an unreduced embryo sac and the development of the unreduced egg cell without fertilisation. The discovery of an obligatory sexual plant in south Texas in 1958 (Bashaw 1962) provided the possibility to breed new genotypes since it has been used as a female parent and was pollinated with pollen from apomictic accessions in order to produce a wide range of hybrids possessing many different characters (Taliaferro and Bashaw 1966). After the discovery of this sexual plant, other authors determined that some other genotypes of *C. ciliaris* were also facultative apomicts (Bray 1978; Sherwood *et al.* 1980; Visser *et al.* 2000; Gupta *et al.* 2001). The use of sexual female parents of *C. ciliaris* in hybridisation programs may be advantageous for breeders by producing new genotypes from local germplasm (Bashaw 1980). This breeding approach has been used successfully to produce high-yielding, apomictic hybrids (Bashaw 1980).

Agamospermy is frequently associated with polyploidy (Roche *et al.* 2001) and almost all apomictic plants are polyploid (Asker and Jerling 1992). The *C. ciliaris* complex contains three ploidy levels including tetraploids ($2n=4x=36$), pentaploids ($2n=5x=45$) and hexaploids ($2n=6x=54$) (Fisher *et al.* 1954; Bashaw and Hignight 1990; Kharrat-Souissi *et al.* 2013). The high phenotypic polymorphism (Arshad *et al.* 2007; Jorge *et al.* 2008; Kharrat-Souissi *et al.* 2011) in the presence of polyploidy suggests that apomixis in *C. ciliaris* may be facultative. The flexibility in reproductive behaviour that is frequently associated with an increase in ploidy might be highly relevant for colonising species by increasing the ability to reproduce under suboptimal conditions and allowing for asexual reproduction in the absence of suitable mates (Bessa-Gomes *et al.* 2003). The mode of reproduction of each ploidy level of *C. ciliaris* was investigated using Flow Cytometric Seed Screening (FCSS) (Kharrat-Souissi *et al.* 2013). The same profiles of nuclear DNA histograms were found for all three cytotypes, with a dominant peak of 2C somatic embryonic nuclei and a smaller 3C peak corresponding to the endosperm (Kharrat-Souissi *et al.* 2013). Although many reports have shown the utility of FCSS for assessing modes of reproduction (Matzk *et al.* 2000; Hörandl *et al.* 2008; Hörandl and Tensch 2009; Krahulcová and Rotreklová 2010), these results obtained for native populations of *C. ciliaris* (Kharrat-Souissi *et al.* 2013) did not clarify the reproductive mode, because the endosperm cells of both the aposporous and sexual plants yield 3C values. Likewise, Matzk *et al.* (2000) concluded that for facultative apomictic species like *Panicum maximum* and *C. ciliaris*, FCSS could not differentiate sexual from apomictic genotypes. Despite these profiles with 2C-, 3C- and 4C-seed nuclei (Kharrat-Souissi *et al.* 2013), it is presumed that the pentaploid populations of *C. ciliaris* are maintained by apomixis. It would be more appropriate to detect aposporous apomixis in the

case of *C. ciliaris* using the traditional cytological technique of dissecting immature ovaries. In this case the aposporous plants of the *Panicum* type, which produce a 4-nucleate sac(s) per ovule, can be easily differentiated from sexually reproducing plants which display a solitary 8-nucleate sac (Savidan 1982; Koltunow 1993; Visser *et al.* 2000).

In *C. ciliaris*, apospory inherits as a dominant trait under genetic control of a single locus and transmits with an apospory-specific genomic region (ASGR) located on a single chromosome (Roche *et al.* 1999; Jessup *et al.* 2002; Ozias-Akins and Van Dik 2007). Cytogenetic investigations of tetraploid *C. ciliaris* have demonstrated that the ASGR is located near the centromere in a genomic region that is hemizygous and heterochromatic (condensed part of chromatin genetically inactive) (Goel *et al.* 2003, 2006; Ozias-Akins *et al.* 2003; Akiyama *et al.* 2005). The ASGR-carrier chromosome has a unique morphological characteristic when compared with other chromosomes in the genome (Akiyama *et al.* 2005) and is ~20 Mb larger than its presumed homologous chromosomes (Goel *et al.* 2003; Akiyama *et al.* 2005). Goel *et al.* (2003) suggest that chromosome structure, proximity to centromere and hemizygoty (the state of having only one copy of a gene in the diploid cell instead of the usual two copies) may all play a role in the low recombination rates in the ASGR.

Neglected aspects concerning invasion biology of *Cenchrus ciliaris*

Invasive species constitute a major threat to natural ecosystems, and conservation strategies generally involve elimination of these species. Whereas *C. ciliaris* is endangered in its native habitat, e.g. drylands in Tunisia, it has invaded extensive areas in Australia, Mexico and South America (Jackson 2005; Gutierrez-Ozuna *et al.* 2009; Miller *et al.* 2010), constituting a threat to natural and managed ecosystems (Marshall *et al.* 2012). Factors that affect the capacity of plants to be rapid or efficient colonisers include wide environmental tolerance, high relative growth rate, effective dispersal, high competitive ability and high levels of phenotypic plasticity (Levin 2000). In addition, the ploidy level of the invasive alien species constitutes an important determinant of invasiveness in plants (te Beest *et al.* 2012). Genome duplication has played a major role in the evolutionary history of plants and can drastically alter a plant's genetic make-up, morphology, physiology and ecology within only one or a few generations (te Beest *et al.* 2012). Polyploidy results in increased leaf and flower size, cell size and chloroplast count (Dhawan and Lavania 1996). These phenomena are collectively referred to as the gigas effect (Acquaah 2007). This allows increasing growth rate, photosynthesis, survival and, therefore, conferring a competitive advantage of polyploids to succeed in strongly fluctuating environments. Polyploidy could give introduced species an important advantage in negotiating novel habitats during the colonisation process (Richardson *et al.* 2000). In this context, Pandit *et al.* (2011) and te Beest *et al.* (2012) suggest that the success of invasive plants can be enhanced by polyploidisation (whole genome duplication), where polyploids have a higher survival rate and fitness than diploids. Until now, the polyploid nature of *C. ciliaris* has been neglected in the programs of conservation and restoration in the native range of the species

and also in the context of biological invasion. Because polyploidy is a decisive factor of genome evolution and genotypic variation of plants (Ainouche and Jenczewski 2010), the conservation strategies for a polyploid complex should consider the frequency of each ploidy level, origin and size of their distribution area and their functional role in diversity. Accordingly, with respect to the *C. ciliaris* complex, further assessment of ploidy levels should be undertaken in order to understand the ecology, distribution, and the biodiversity impact of this invasive species.

Intra-specific phenotypic variation

Although several studies of the morphological variability of *C. ciliaris* have been conducted (M'seddi *et al.* 2002, 2004b; Mnif *et al.* 2003, 2005a; Arshad *et al.* 2007; Jorge *et al.* 2008; Visser *et al.* 2008; Arshadullah *et al.* 2011), few comparisons between populations and studies of the heritable nature of important traits have been undertaken. In one rare study, the progeny from different populations was tested. Here, variability among populations, within populations and among individuals of the same offspring was examined (Kharrat-Souissi *et al.* 2011). Multiple morphometric variables mainly linked to productivity (tiller number, spike number and aboveground biomass) were examined at the vegetative and reproductive stages. These measurements were quantitative, while previous studies concerning the phenotypic diversity of *C. ciliaris* were merely semiquantitative, using a visual scoring method (M'seddi *et al.* 2002; Visser *et al.* 2008).

In these studies the phenotypic variables measured showed considerable differences among populations of *C. ciliaris* and among maternal parent plants within the same population (Kharrat-Souissi *et al.* 2011). This was a novel result compared with previous studies on the morphological variability of *C. ciliaris*. High similarity among individuals belonging to the same offspring was found, indicating that the measured attributes were highly heritable. This no doubt results from the apomictic reproductive mode of *C. ciliaris* that leads to the formation of seeds genetically identical to the mother plant (Ozias-Akins *et al.* 2003; Akiyama *et al.* 2005). As stated above, apomixis is a heritable trait (Grimanelli *et al.* 2001; Jessup *et al.* 2002; Curtis and Grossniklaus 2007; Ozias-Akins and Van Dik 2007; Yadav *et al.* 2012), but is rarely obligatory, and such is the case for *C. ciliaris*. In mixed breeding systems (sexual and apomictic), the genetic factors controlling apomixis can be potentially transferred from an apomictic individual to the offspring of a sexual plant via pollen. In turn, the pollen of the sexual individuals does not fertilise an apomictic plant and will not result in the inheritance of the sexual traits because the egg cell develops parthenogenetically (Hörandl and Tensch 2009). Because of this unidirectional hybridisation, apomicts have a superior colonising ability. The faster moving apomicts will build up a barrier against the slower moving sexual populations because the latter will always be pollinated mainly by the more abundant apomictic individuals. Consequently, sexuality would be replaced finally by apomixis (Mogie *et al.* 2007) and is thought to be a causal factor for the wide geographical distribution of apomictic complexes (Hörandl and Tensch 2009). This model is based on a simple heritability mechanism of apomixis via a single genetic factor and all progeny expressing the trait.

Kharrat-Souissi *et al.* (2011) showed that heritable phenotypic traits mainly associated with productivity (e.g. aboveground biomass) were highly correlated: number of tillers, number of spikes, plant height and tuft diameter. This has implications in breeding programs as selection for one trait will have an effect on another. In addition, selection could be practiced on a highly heritable trait which correlates with a more complex trait such as yield (Majidi *et al.* 2009). However, according to our results, the aboveground biomass is an efficient discriminating variable for identifying the most productive genotypes (Kharrat-Souissi *et al.* 2010, 2011). M'seddi *et al.* (2004b) has established that tuft diameter and tuft height are also good indicators which can be used to increase the productivity of *C. ciliaris*. Generally, increase in plant size has been proposed as an explanation for invasion success, inducing increased competitiveness and vigour (Jakobs *et al.* 2004; Stastny *et al.* 2005). Indeed, the variability in productivity in *C. ciliaris* could be an important factor in the invasion biology of this species, because the population growth rate is known to affect the speed of invasion (Elliott and Cornell 2012). These last authors showed that the presence of phenotypic polymorphism within a species can result in faster range expansions than if only a single phenotype is present in the landscape. This has implications for predicting the speed of biological invasions, suggesting that speeds cannot just be predicted from looking at a single phenotype and that the full community of phenotypes needs to be taken into consideration.

The phenotypic polymorphism, measured in *C. ciliaris*, did not show any clear relationship with the eco-geographical features of the source population (Kharrat-Souissi *et al.* 2010, 2011). These results accord with those of M'seddi *et al.* (2002, 2004b) and Jorge *et al.* (2008) demonstrating a generally poor correlation between environmental data and agro-morphological data of *C. ciliaris*. This lack of correlation can be explained by the colonising ability by its seeds, which are light and surrounded by stiff bristles, making them widely dispersed by wind over long distances (Pitt 2004). This frequent gene flow via seed dispersal contributes to a certain level of diversity among populations of *C. ciliaris*. These findings are in line with data from Gutierrez-Ozuna *et al.* (2009) showing that seeds were widely dispersed between *C. ciliaris* populations introduced into north-western Mexico. The invasiveness of *C. ciliaris* in arid and semiarid areas of Australia (Friedel *et al.* 2006) has also been attributed predominantly to propagation/dispersal mechanisms (Eyre *et al.* 2009). The speed of a species' invasion depends upon its dispersal ability (Elliott and Cornell 2012).

Polyploidy of *Cenchrus ciliaris*

Before the discovery of the sexual individual in *C. ciliaris*, the knowledge of ploidy was not important, because the breeders were looking to select the most productive apomictic ecotypes. But once the sexual individuals were used in breeding programs and crosses between sexual and apomictic plants were made, knowledge of the ploidy level of the plants used in hybridisation became more important, because breeding success depends on using meiotically stable parental lines with compatible chromosome numbers (Burson *et al.* 2012).

Flow cytometry for ploidy level determination

The use of flow cytometry to determine nuclear DNA content and hence to screen for ploidy level in *C. ciliaris* permitted the prediction of the chromosome number for each population. The 2C DNA values were assessed for 28 natural populations collected from northern to southern Tunisia (Kharrat-Souissi *et al.* 2013). Genome size ranged from 2C = 3.03–4.61 pg, revealing three ploidy levels corresponding to 4x (tetraploid 2n = 36), 5x (pentaploid) and 6x (hexaploid), and mean 2C DNA amounts were of 3.03, 3.70 and 4.48 pg, respectively (Kharrat-Souissi *et al.* 2013). A very low variation in nuclear DNA content between plants of the same ploidy level (Kharrat-Souissi *et al.* 2013) supports a theory of stable genome size. The 1Cx values (monoploid genome size for n = 9) were similar for tetraploid, pentaploid and hexaploid cytotypes (0.757, 0.746 and 0.745 pg, respectively), (733 M bp). This stability is in contrast with the frequent occurrence of genome downsizing (decrease in monoploid genome size, 1Cx, according to Greilhuber (2005), in high polyploids. This intra-cytotype stability in nuclear DNA amount in *C. ciliaris* indicates that there was very little change during the life history of these cytotypes, which suggests a recent origin of pentaploids and hexaploids.

In a recent study, Burson *et al.* (2012) determined the DNA content of many accessions of *C. ciliaris* from the USDA National Plant Germplasm System by flow cytometry. This germplasm collection is dominated by tetraploids (54%), with some pentaploids (25%) and a few hexaploids (4%). This contrasts with the relative frequencies found in natural Tunisian populations: 4, 11, and 82%, respectively (Kharrat-Souissi *et al.* 2013). According to several authors (Hignight *et al.* 1991; Visser *et al.* 1998, 2000; Burson *et al.* 2012), the most common chromosome number reported for *C. ciliaris* corresponds to the tetraploid cytotype (2n = 4x = 36). However, at least for Tunisia, representing a small portion of the native range of *C. ciliaris*, the hexaploid is the most frequent cytotype (Kharrat-Souissi *et al.* 2013). In Tunisia, populations were characterised by assessing 5–14 individuals per population. A stable genome size within and between populations of each ploidy level was found, falling clearly into distinct classes corresponding to the three different cytotypes (Kharrat-Souissi *et al.* 2013). Unfortunately, Burson *et al.* (2012) tested only one individual plant of each accession and failed to test if different ploidy levels were associated with certain populations or with mixed populations.

In addition to the existence of different ploidy levels in *C. ciliaris*, several authors (Visser *et al.* 1998; Mnif *et al.* 2005a; Burson *et al.* 2012) reported frequent aneuploidy [gains (hyper-aneuploidy) or loss (hypo-aneuploidy) of chromosome, in one chromosome set] resulting from unequal segregation of chromosomes. Aneuploidy has a greater effect on phenotype than polyploidy and causes developmental abnormalities, which can reduce organismal fitness in species (Birchler and Veitia 2007). One natural population in arid southern Tunisia was uniformly hyper-aneuploid (2n = 2x = 55), probably apomictic (Kharrat-Souissi *et al.* 2013). Agamospermy must play an important role in formation and preservation of chromosomal abnormalities in *C. ciliaris*. As stated above, *C. ciliaris* reproduces mainly by apospory and the unreduced egg cell develops into an embryo parthenogenetically, requiring pollination/fertilisation

for endosperm development via pseudogamy. Because early pollination tends to increase the frequency of $2n+n$ fertilisation in apomictic grasses (Martinez *et al.* 1994), the chances for early pollination and $2n+n$ fertilisation are increased in *C. ciliaris* as a protogynous species (i.e. shedding of pollen occurs after the stigma has stopped being receptive) (Burson *et al.* 2002).

Chromosome number confirmation of ploidy level

Although flow cytometry indicated ploidy levels in *C. ciliaris*, determination of chromosome number at the mitotic stage was required to confirm these putative ploidy levels. *Cenchrus ciliaris* has small chromosomes that make them difficult to count using traditional cytological methods. However, a protoplast dropping technique (Geber and Schweizer 1987) was appropriate for counting on complete mitotic cells with satisfactory spreading of chromosomes (Kharrat-Souissi *et al.* 2013). Thus, the three inferred cytotypes were confirmed by chromosome counting to be tetraploids ($2n=4x=36$), pentaploids ($2n=5x=45$) and hexaploids ($2n=6x=54$) (Kharrat-Souissi *et al.* 2013). The hexaploid chromosome number has already been reported for Tunisian populations of *C. ciliaris* (Mnif *et al.* 2005b) but tetraploid and pentaploid are new for this area (Kharrat-Souissi *et al.* 2013). These chromosome counts corroborate other researchers' findings (Fisher *et al.* 1954; Hignight *et al.* 1991; Visser *et al.* 1998, 2000). Burson *et al.* (2012) even reported the existence of two septaploids ($2n=7x=63$) in *C. ciliaris*.

The existence of pentaploid cytotypes in *C. ciliaris* suggests ability for hybridisation between tetraploids and hexaploids (Kharrat-Souissi *et al.* 2013). This is yet one more argument that this species exhibits a mixed breeding system (apomixes and sexual) and that sexuality offers a potential mechanism for hybridisation and creation of intermediate cytotypes. However, polyploidisation may play an important role by restoring sexual reproduction after hybridisation. Hybridisation and polyploidisation events should increase adaptation to a variable climate due to a larger genetic diversity and may also assist the 'evolution of invasiveness'.

Molecular cytogenetic evidence of polyploidy and ecological implications

In order to characterise the three ploidy levels of *C. ciliaris*, a physical mapping of chromosomes was carried out using fluorescence *in situ* hybridisation (Kharrat-Souissi *et al.* 2012b). Using double fluorescence *in situ* hybridisation, it was shown that the two rDNA gene families, 5S and 18S-5.8S-26S (18S), displayed intra-specific variation in the number of loci, following the different ploidy levels of *C. ciliaris* (Kharrat-Souissi *et al.* 2012b). The number of 5S and 18S rDNA sites corresponded to the ploidy level displaying four signals for tetraploid, five for pentaploids and six for hexaploids (Kharrat-Souissi *et al.* 2012b), showing a proportional increase of ribosomal loci number during polyploidisation (Kharrat-Souissi *et al.* 2012b). The tetraploid cytotype has been previously studied by Akiyama *et al.* (2005) showing four signals for both 5S and 18S. The positions of rRNA gene loci were consistent and their numbers were proportional in the three ploidy levels, observations which further support a recent origin for pentaploid and hexaploid cytotypes (Kharrat-Souissi *et al.* 2012b).

Although different ploidy levels for *C. ciliaris* have been recorded by several authors (Fisher *et al.* 1954; Hignight *et al.* 1991; Visser *et al.* 2000; Burson *et al.* 2012), the geographical distribution of these ploidy levels has not been studied. The distribution of *C. ciliaris* cytotypes in Tunisia (Fig. 1) seems to follow a north-south bioclimatic gradient (Kharrat-Souissi *et al.* 2013). A single tetraploid population was found in the semiarid north, the three pentaploid populations appeared in the centre of the country, particularly in a region where rainfall is between 100 and 200 mm, while the 24 hexaploid populations occupied the semiarid to the Saharan region where annual average rainfall is below 100 mm (Fig. 1). The wider distribution of hexaploids may suggest that these populations are better adapted to a broader range of climatic conditions (Kharrat-Souissi *et al.* 2013).

Several cytogeographical studies have showed that diploids and polyploids often occupy different parts of the landscape and that polyploids replace their diploid relatives along ecological gradients. Gradients in rainfall have shown that polyploids generally occupy drier habitats than their diploid relatives (Chen 2007; Hegarty and Hiscock 2008; Maherali *et al.* 2009; Treier *et al.* 2009; Paun *et al.* 2011). This clear distinction between cytotypes was not shown by Waters *et al.* (2010), who found a coexistence of different cytotypes of some Australian Danthonieae. Polyploids are also often less likely to become extinct than related diploids in disturbed habitats (Stebbins 1985; Otto and Whitton 2000; Fawcett *et al.* 2009). These authors concluded that polyploids have a greater tolerance of extreme ecological conditions and are often better adapted to dry conditions than their diploid counterparts. A further argument comes from the observation that increasing environmental stress (temperature variation and drought) increases the production of unreduced gametes and thus polyploidy (Fawcett *et al.* 2009; Parisod *et al.* 2010).

Molecular diversity

Genetic resource management of *C. ciliaris* and its use in programs of rehabilitation of degraded rangelands requires documentation of both levels and structure of its genetic diversity to optimise the sourcing of seeds with respect to potential existence of local adaptations. Due to the polyploidy nature of *C. ciliaris* and their relatively low cost, AFLP markers were developed to investigate the structure of variation at several loci among and within 11 Tunisian populations and among the three ploidy levels of *C. ciliaris* (Kharrat-Souissi *et al.* 2011). In other regions of the world (north-western Mexico, Pakistan, central Saudi Arabia), genotypic diversity of *C. ciliaris* has been studied (Gutierrez-Ozuna *et al.* 2009; Nisar *et al.* 2010; Al-Soqeer 2011) unfortunately without taking into account possible different ploidy levels. Although these authors sampled numerous individuals per population, they did not examine individuals descending from the same mother plants, and thus failed to investigate the frequency of sexual reproduction within *C. ciliaris*.

In the study on Tunisian populations (Kharrat-Souissi *et al.* 2011), AFLP markers revealed *C. ciliaris* to have genotype diversity within populations and between individuals descending from the same mother plant (average polymorphism within offspring = 1%, max. = 5%). This variability for a predominantly

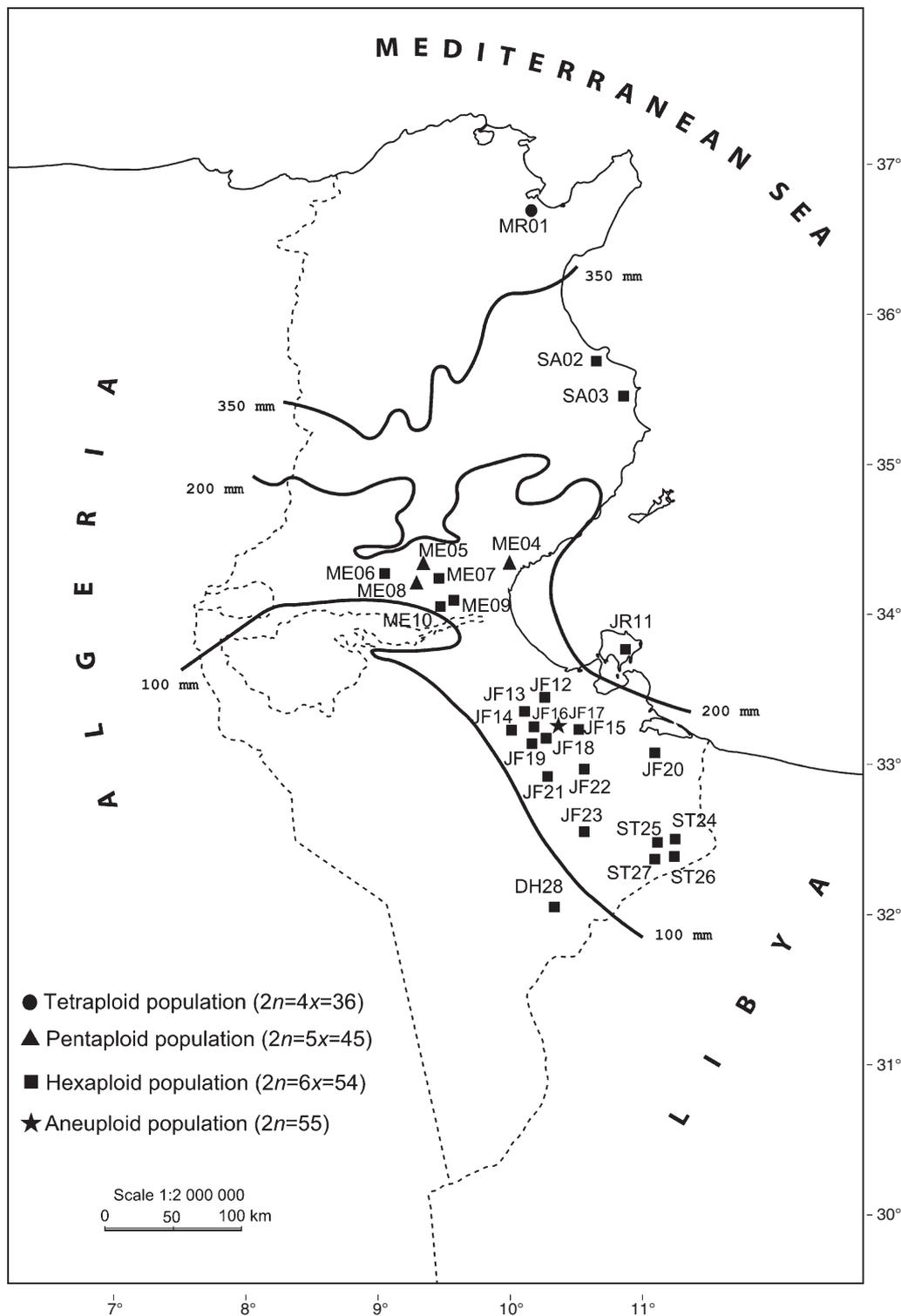


Fig. 1. Geographical distribution of different cytotypes of *Cenchrus ciliaris* in Tunisia (Kharrat-Souissi et al. 2013).

agamosperous species shows that sexual reproduction may occur in some *C. ciliaris* populations (Kharrat-Souissi et al. 2011). This result supports the finding of Hignight et al. (1991) that *C. ciliaris* can reproduce via a process of facultative

apomixes. In Tunisia, *C. ciliaris* has more genetic diversity than in populations from the Al-Qassim region of Saudi Arabia (Al-Soqeer 2011). This latter author surmised that the low level of genetic variation of Saudi (Al-Qassim) *C. ciliaris* populations

was not surprising given the presumed predominantly apomictic reproductive system of this species. The mean G/N value (the proportion of distinct genotypes in a population) observed for *C. ciliaris* in Tunisia (G/N=0.55; Kharrat-Souissi *et al.* 2011) was also higher than that observed for invasive Mexican populations (G/N=0.29, Gutierrez-Ozuna *et al.* 2009).

Each of the Tunisian populations studied for their genotypic diversity was characterised by the presence of both unique genotypes and genotypes, which were common to several individuals (clones) (Kharrat-Souissi *et al.* 2011). The fact that the AFLP diversity (polymorphism and G/N values) varied across populations suggests that the rates of occurrence of the two reproduction modes (apospory and sexual) are not homogeneous in Tunisia. Combined clonal/sexual reproduction processes enable species to disperse to new territories and to form relatively long-lived populations by replicating the best-adapted individuals (Grant 1971; Bayer 1983, 1990; Novak and Mack 2000).

A neighbour-joining tree, based on AFLP markers, performed for the Tunisian *C. ciliaris* populations revealed three major groups of genotypes: tetraploid, pentaploid and a mix of pentaploid and hexaploids (Kharrat-Souissi *et al.* 2011). The existence of pentaploids in two groups with different genotypes suggests that this cytotype may have two origins. This result highlights the evolutionary potential in the *C. ciliaris* complex and supports the statement that gene exchanges between different ploidy levels occur (Chapman and Abbott 2010). The groups of genotypes from the neighbour-joining tree do not correspond completely to the geographical origin of samples (Kharrat-Souissi *et al.* 2011). Gutierrez-Ozuna *et al.* (2009) also showed, in north-western Mexican populations of *C. ciliaris*, that genetic differentiation had no geographic pattern and apparently was not associated with environmental gradients. The lack of geographical structure of genotypic diversity of *C. ciliaris* could be attributed to the dispersal capacity of seeds by wind over a long distance (Pitt 2004). Gutierrez-Ozuna *et al.* (2009) concluded that invasion success of *C. ciliaris* in north-western Mexico is not directly related to genotypic variation and other factors, such as phenotypic plasticity and propagule pressure, could be major determinants of the invasion success of this species.

There are no previous studies examining the relationship between morphological and genotypic variability for *C. ciliaris*. In this context, there was a lack of correlation between genotypic distances from AFLP and phenotypic distances from the morphological study (Kharrat-Souissi *et al.* 2011). This result suggests that neutral markers (AFLP) and complex heritable traits (morphological traits) do not involve the same processes. The significant morphological differences found among populations of *C. ciliaris* may result from the high selection pressure rather than genetic drift, indicating the local adaptation of certain genotypes. In similar studies, species (*Cichorium intybus* L. and *Cichorium spinosum* L.), separated on the basis of morphological characters, could not be distinguished using molecular tools (Gemeinholzer and Bachmann 2005). Estimation of genetic diversity based on morphological characters is very difficult and is influenced by environmental effects (Afghan *et al.* 2005). By contrast, molecular markers offer an efficient measure of genetic relationship without any influence of environmental factors (Ubi *et al.* 2006).

To summarise the importance of cytogenetic and molecular data of *C. ciliaris*, we note that different observations support the notion that apomixis is not an absolute reproductive mode for this species. First, the pentaploids most probably arose through an event of sexual reproduction, being hybrids from the fusion of 2x and 3x gametes. Second, the existence of pentaploids in two groups with different genotypes suggests that two hybridisations or introgression events may have occurred. Finally, the diversity of AFLP genotypes shows that recombination is more frequent than expected for a predominantly agamosperous species.

Adaptive response to water deficit in arid environments

The contribution of the phenotypic polymorphism of *C. ciliaris* in response to a water deficit in the ecological context of dry lands was studied in southern Tunisia (Kharrat-Souissi *et al.* 2012a). Although *C. ciliaris* is well known for its predominantly apomictic nature, previous studies on its drought resistance (Mansoor *et al.* 2002; Mnif and Chaieb 2010) did not compare numerous offspring from the same population nor maternal siblings, and thus failed to examine the heritable part of the response to drought stress. Recently, highly heritable variation in the responses to applied water deficit for several measured attributes (e.g. productivity, leaf area, leaf hairiness and stomata density) has been observed for *C. ciliaris* (Kharrat-Souissi *et al.* 2012a). Different groups of genotypes responded in different ways to the applied water deficit. Although all individuals of *C. ciliaris* grew more slowly and produced less aboveground biomass under water deficit, some genotypes were more strongly affected than others and their aboveground biomass showed the largest decrease (Kharrat-Souissi *et al.* 2012a). These same genotypes maintained their leaf area under water stress, which allowed them to maintain their photosynthetic tissue. In contrast, other genotypes reduced their leaf surface area. This latter strategy may be advantageous under hot conditions and unpredictable rainfall, such as in arid environments, because reducing the leaf surface limits water loss through transpiration (Blum 2005; Taiz and Zeiger 2006). The capacity of *C. ciliaris* genotypes to express different phenotypes adapted to water stress show the important degree of phenotypic plasticity of this species in arid climates.

Several studies have shown that drought-tolerant species reduce water loss by reduction of leaf area simultaneously with a reduction in biomass production (Lazaridou and Noitsakis 2003; Lazaridou and Koutroubas 2004). For *C. ciliaris*, the strong decrease in leaf area was correlated with only a small decrease in aboveground biomass (Kharrat-Souissi *et al.* 2012a). *Cenchrus ciliaris* belongs to the C₄ group of grasses which adapt well to dry environments (Maroco *et al.* 2000). This species is one of many grasses that can produce forage for livestock with limited precipitation, which help it to underpin its invasive weedy potential in areas where it has been introduced (Cox *et al.* 1988; Ward *et al.* 2006). *Cenchrus ciliaris* can out-compete native vegetation for soil nutrients and moisture and has the potential to displace native plant communities in countries where it has been introduced. Accordingly, Eyre *et al.* (2009) has shown that this species can colonise successfully in south-west Queensland at the expense of native species. Indeed, most studies in Australia have reported a negative relationship between *C. ciliaris* occurrence and native species' biomass, species richness and/or individual

native plant species cover (Fairfax and Fensham 2000; Franks 2002; McIvor 2003; Clarke *et al.* 2005; Jackson 2005; Smyth *et al.* 2009).

Genotypes that have increased their leaf hair density under water deficit have also been found in *C. ciliaris* (Mansoor *et al.* 2002; Kharrat-Souissi *et al.* 2012a). This increase of leaf hair density appears to be beneficial for plants under water-deficit conditions, because it increases light reflectance from the leaf surface (Ehleringer and Werk 1986; Vogelmann *et al.* 1996; Farooq *et al.* 2009), which would decrease the overall leaf temperature by decreasing heat load. Higher leaf hair density could also increase the leaf boundary layer (Schuepp 1993), which affects leaf temperature by modifying the rate of heat transfer from the leaf (Ehleringer and Mooney 1978; Farooq *et al.* 2009). Leaf hair density increase was correlated with a greater decrease in aboveground biomass. These results show a multiplicity of responses of *C. ciliaris* genotypes under limited water availability. The capacity of this species to regulate its growth in different ways as an adaptive mechanism under water deficit indicates significant genetic variation that could affect its productivity and, in consequence, the vegetation cover of ecosystems during an increase of aridity (Kharrat-Souissi *et al.* 2012a). Furthermore, Mansoor *et al.* (2002) demonstrated that genotypes of *C. ciliaris* have not shown uniformity for any single phenotypic character in response to stressed environments and each genotype appears to present a unique suite of adaptations helping its survival under drought conditions.

The large amount of variation in several traits in *C. ciliaris* may enhance its potential for responding to new selection pressures in variable environments. Furthermore, the spatial heterogeneity in the environment affects the degree of phenotypic plasticity displayed by plants (Van Kleunen and Fischer 2005; Volis *et al.* 2005). Phenotypic plasticity may be adaptive with phenotypes that are matched to their environment having relatively higher fitness than other phenotypes (Ruiz-R. *et al.* 2006; Ofir and Kigel 2010). *Cenchrus ciliaris* is a particularly good candidate for phenotypic plasticity and local adaptation because (1) it is highly genetically differentiated, (2) it is very phenotypically plastic and (3) it can support extreme variation of rainfall and temperature in arid environments, and is likely to be under considerable selective pressure for local adaptation.

A key characteristic of invasive species is their ability to colonise and persist in a broad range of environmental conditions (Buswell *et al.* 2011). Plasticity in *C. ciliaris* can play an important role in biological invasions by allowing individuals to colonise and establish in environmentally diverse habitats. On the other hand, Kawecki and Ebert (2004) suggest that, if sufficient heritable variation is available and alternative genotypes are favoured in different habitats, natural selection in an introduced range may create a mosaic of locally adapted populations. Such local adaptation can lead to increased local abundance and, therefore, can be a key mechanism of invasion success (Parker *et al.* 2003). The invasive *C. ciliaris*, therefore, may also spread across diverse habitats by undergoing local adaptation, evolving ecotypes with distinctive traits and/or patterns of individual plasticity. Invasion biologists could use controlled experimental gardens or reciprocal transplant experiments to investigate whether *C. ciliaris* relies on phenotype plasticity to colonise new areas.

Conclusion: insights for conservation and invasion biology

According to Visser *et al.* (2008), dealing with seed collection strategies for restoration projects of degraded arid areas, it would be more appropriate to use a seed mixture of native genotypes of *C. ciliaris* than to use cultivars composed of a single genotype. Whalley *et al.* (2013) made a similar recommendation for restoration using Australian native grasses, which are predominantly inbreeding using a range of different mechanisms. This corresponds to the objective of maximising the possibility of local adaptation of restored populations by selecting the most adapted genotypes. According to our current knowledge, this strategy is insufficient particularly for *C. ciliaris*. Further molecular and cytogenetic investigations are needed to understand the genetic structure of *C. ciliaris* and, in particular, the relationship between environmental and genomic variation. Indeed, studies of genetic diversity of *C. ciliaris* underline the possibility of sexual reproduction, recombination and gene flow within and between populations (Kharrat-Souissi *et al.* 2011). This poses questions about the methods of collection, culture and harvest of seeds of *C. ciliaris*. For instance, in order to guarantee the genetic constitution of native seeds at the time of reseeded, we suggest that the following ground rules should be respected: (1) seeds should be collected from different mother plants, separately, in order to maximise the representation of different genotypes and, (2) when multiplying the genotypes of *C. ciliaris*, the possibility of genetic exchange in the nursery, the possible phenomena of homogenisation and reduction of the genetic diversity caused by the methods of cultivation and *ex situ* conservation, should be taken into account.

The association of morphological, cytological and molecular data is of particular interest at both local and broad scales for various aspects concerning *C. ciliaris*. Indeed, the lack of strong correlations observed between geographical structure and either the AFLP data or the heritable phenotypes, the existence of genotypic variations within offspring and the various origins of pentaploids, suggest that long-distance gene exchange, hybridisation and genomic evolution processes all occur in *C. ciliaris*. This new evidence provides insights into the genetic diversity of *C. ciliaris* supporting the complex and highly dynamic nature of polyploid plants (Soltis and Soltis 2000), which is a key element in restoration ecology, and also in the context of invasion biology (Pandit 2006; Ainouche *et al.* 2009; Hull-Sanders *et al.* 2009; Treier *et al.* 2009; Pandit *et al.* 2011). In this context, te Beest *et al.* (2012) demonstrated that polyploidy might increase the adaptive potential of invasive species in their novel habitat. Many studies have compared the growth of plants from native and invasive populations (Daehler 2003; Ehrenfeld 2003; Callaway and Ridenour 2004; Colautti and MacIsaac 2004; Hierro *et al.* 2005), but few have considered the role of ploidy.

We reiterate that *C. ciliaris* has been considered as an invasive species that poses serious threats to biodiversity conservation when introduced in Australia, America and Mexico (Marshall *et al.* 2012). Many studies have been undertaken to understand the behaviour of *C. ciliaris* as an invader for the purpose of managing invasion (Franklin *et al.* 2006; Friedel and Wycott 2007; Eyre *et al.* 2009; Smyth *et al.* 2009; Miller *et al.* 2010). However, none of these studies have taken into account the impact of ploidy on the invasive capacity of this species. We suggest, therefore, that knowledge of polyploidy of the germplasm of *C. ciliaris*

introduced in these areas is essential in comparative studies of invasive and native populations. Critical analysis of the relationship between invasiveness and polyploidy will require study of the polyploid complex, in which one may directly compare polyploids and their diploid relatives under field conditions (Kubátová *et al.* 2008; Treier *et al.* 2009; Schlaepfer *et al.* 2010).

Finally, the likelihood of genome evolution in the polyploid *C. ciliaris*, shown in this review, underlines the potential for adaptation in this facultative apomictic species. We conclude that three genetic mechanisms may contribute to the evolution of *C. ciliaris* complex, namely hybridisation, polyploidy and apomixis. Hybridisation and polyploidy generate the genetic variability in this taxon and, thanks to apomixes, the new hybrid types adapted to different environments can be maintained and perpetuate. Since apomixis is facultative, natural selection would also act on sexually reproduced genotypes, accelerating the evolution process.

Perspective: from research on the apospory to restoration ecology

Field (morphometry) and laboratory work (cytogenetic and molecular analysis) has shed light on the genetic reasons for the variation and the potential of adaptation of *C. ciliaris* in response to increasing aridity. At a time of unprecedented speeds of climate change, the identification and preservation of such potential will be essential for maintaining biodiversity. Conservation and restoration programs of *C. ciliaris* continue to be developed and new questions and debates are to be expected. First, unequivocal confirmation of the degree of apomixis of each ploidy level requires simultaneous examination of both genetic and cytological evidence. Second, further genetic and phenotypic examinations of *C. ciliaris* focussed on genome evolution will require more comprehensive sampling throughout its distribution area, in order to increase our knowledge of variation. Ultimately, experimental studies of polyploidy series are needed to test rigorously a hypothesis about ecological adaptation of polyploids and its consequence for *C. ciliaris* invasiveness.

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