Artículo científico

Cytogenetics characterization of *Minthostachys mollis* (Kunth.) Griseb. Populations from Tucumán province (Argentina)

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Abstract

Minthostachys mollis (Kunth.) Griseb. (Lamiaceae) is a native species from the central and northwest Argentinean region. Reported a chromosome number are 2n = 24 and 2n = 42. In this work, two populations of *M. mollis* were cytogenetically characterized in Tucumán province, Argentina, by means of mitosis and meiosis analysis, with material from Río Nío and Villa Padre Monti (Burruyacú, Tucumán). For the mitosis and meiosis analysis, the preparations were made with 2 % haematoxylin as colorant. The pollen grain viability was assessed by using cotton blue in lactophenol technique. Results showed a chromosome number 2n = 48 for both studied populations. In meiosis analysis, normal as well as abnormal divisions in different percentage were observed. Among the abnormalities, multivalent, dyads, triads and polyads of different degree and size were observed. Also, configurations like abnormal spindle orientation, laggard chromosomes and micronucleus in variable percentage were observed. The pollen viability analysis showed 87.2 % of viable pollen grains. The new chromosomal number found in *M. mollis* could be owing to: a) Formation of gametes (n = 24) from population of Villa Padre Monti and Rio Nio. b) In sympatric zones, with species 2n = 24 and 2n =42, could take place the union of non-reduced gametes (4x = 24), and gametes 4x from those of heptaployds 2n = 42, resulting the populations with 2n = 48. These results constitute a basis in order to perform further studies about the reproductive biology of this species.

Key words: chromosomes, meiosis, mitosis, reproduction, genetic variability.

Resumen

Minthostachys mollis (Kunth.) Griseb. (Lamiaceae) es una especie nativa de la región central y noroeste de Argentina. Se informaron para la especie números cromosómicos 2n = 24 y 2n = 42. Se caracterizaron citogenéticamente dos poblaciones de *M. mollis* de la provincia de Tucumán, Argentine, mediante el análisis de mitosis y meiosis, con material proveniente de Río Nío y de Villa Padre Monti (Dpto. Burruyacú, Tucumán). Para mitosis y meiosis, se realizaron preparados con hematoxilina al 2 %. La viabilidad de grano de polen se determinó con azul de algodón en lactofenol. Los resultados mostraron un número cromosómico 2n = 48 para las dos poblaciones. En meiosis, se observaron divisiones normales y anormales, en diferente porcentaje. Se presentaron multivalentes, díadas, tríadas y políadas de diferente grado y tamaño. También se observaron configuraciones tales como orientación anormal del huso, rezagados y micronúcleos en porcentaje variable. El estudio de la viabilidad de polen dio un 87,2 % de granos de polen viable. El nuevo número cromosómico encontrado en *M. mollis* puede deberse a: a) formación de gametas de poblaciones de n = 24 de Villa Padre Monti y Rio Nio. b) En zonas simpátricas, con especies 2n = 24 y 2n = 42, podría tener lugar la unión de gametas no reducidas (4x = 24) y gametas 4x de heptaploides 2n = 42, resultando poblaciones con 2n = 48. Estos resultados constituyen una base para realizar estudios más profundos de la biología reproductiva de esta especie.

Palabras clave: cromosomas, meiosis, mitosis, reproducción, variabilidad genética.

Introduction

Minthostachys mollis (Kunth.) Griseb. (Peperina) is a native species from *Lamiaceae* family. Numerous studies reported about its distribution in Latin America, from Venezuela, Ecuador, Perú, Bolivia, Colombia, Brazil and Argentina. It is widely distributed in the central and northwest region of Argentina. From the morphological point of view, it is a perennial sub shrub reaching until 2 m height with climbing stems, branched, pubescence, thicker in the upper part. Its leaves are in opposite position, petiolated, varying from 1-5 cm length to 1-3 cm wide. It has white flowers in axillar pedunculate

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clusters grouped in discontinuous bunches. It is reproduced by seeds with some levels of cross-fecundation by casmogamy or protogyny (Ordoñez *et al*., 2002; Ojeda, 2004; Scandaliaris *et al*., 2007; Elechosa, 2009). It is frequent in protected areas and on the mountain slopes.

In Tucumán province, populations of *M. mollis* observed by the authors of this paper differ morphologically from others found in near provinces. The morphologic variation, especially that related to its leaf morphology, could be associated to genetic variability, but also can be the result of the environmental conditions where the species grows. Some factors like humidity and altitude above sea level would have a greater incidence, which could affect the species behavior, mainly in the reproductive aspect and its persistence along the time. It was reported the action of biotic and abiotic factors in the reproduction of some species (Fuzinatto *et al*., 2008).

In Tucumán province, it was observed an excessive extraction of this species in natural populations. It was detected an important loss of the biodiversity by the extraction of the entire plants, which puts into risk the natural multiplication of the native germplasm. Owing to that it is important to know the existing variability, generating information for propose *in situ* conservation plans.

From the agronomical point of view, in the last years it has been produced an important increase in the demand of this species, so is it necessary to realize a reasonable and sustainable use of this resource. For this, the processes of domestication before the incorporation into the crop of the species are valuable.

In relation to the genetics of the species, it is important to know in first term, the chromosome number of the genus as well as the species, degree of ploidy and other derivations. The study of the chromosome complement allows a deep karyotype study and its particularities (Stebbins, 1971).

From a cytogenetic point of view, the first works have been registered for the species variable chromosome numbers 2n = 24 and 2n =42 (x = 6) in populations from Córdoba and Tucumán provinces (Ordoñez and Ojeda, 1997; Ordoñez *et al.,* 2002, and Ojeda, 2004). In natural populations, meiotic abnormalities were observed, such as, cytomixis and anaphasic bridges, triades, hexades, and diades. The different chromosomal number observed could be explained by the occurrence of cytomixis. In populations with 2n = 24 irregularities of meiosis were reported, varying from 0.4 to 0.8 % (univalents, laggard chromosomes and triads). For the populations with 2n = 42, irregularities reached until a 23.5 % (Ojeda, 2004).

Studies of meiosis explain some reproductive phenomena of the species. The comprehension of this mechanism as genetic variability source makes clearer some aspects of the environmental adaptation of the organisms and consequently the perpetuation through the offspring (Caetano, 2003).

On the basis of the phenotypic variability observed, it is interesting to know about the cytogenetic behavior of this species in order to determine the present genetic variability in the populations.

The objective of this work is to carry out a cytogenetic characterization in two populations of *M. mollis* from Tucumán province, through the mitosis and meiosis study, evaluation of chromosome behavior in the male gametogenesis and determination of pollen grain viability.

Materials and methods

Plant material. Stakes of *M. mollis* were collected from Río Nío (26º 26’ 25, 62’’ S – 65º 01’ 30,9’’ W) and Padre Monti Village (26º 30’ 54’’ S – 64º 59’ 21’’ W), Burruyacú Departament, Tucumán province, Argentina. For the mitosis study they were put into pots until obtaining roots.

Slide preparation. The meristems were pretreated with paclosol (paradichlorobenzene), fixed in absolute ethylic alcohol/acetic acid solution (3:1). Further, the hydrolysis was carried out with HCl 1N for 4 min at 60 °C and kept in 45 % acetic acid. Finally, preparations were made by macerating and squashing the material in a drop of 2 % haematoxylin as colorant and 1 % ferric citrate as mordant (Pastoriza, 2001; Singh, 2002). For the meiosis study, flower buds from *in situ* plants were collected in each population. Young flower buds close to anthesis were used and fixed in Newcomer solution (isopropyl alcohol:glacial acetic acid:petroleum ether:acetone: dioxane; 6:3:1:1:1). Squashing of anthers was performed by applying the same colorants used in mitosis technique.

Viability assessment. The pollen grain viability study was carried out by using cotton blue in lactophenol as colorant (D’Ambrogio de Argüeso, 1986), considering the turgid and stained grains as normal and the colorless and/or deformed as abnormal ones. The microphotographs were taken with a Carl Zeiss Primo Star optical microscope with an integrated digital camera.

Results

Results showed for mitosis a chromosome number 2n = 48 for both populations studied, with a chromosome size varying from 0.2 to 0.4 µm. This makes difficult the karyotype study (Figure 1).

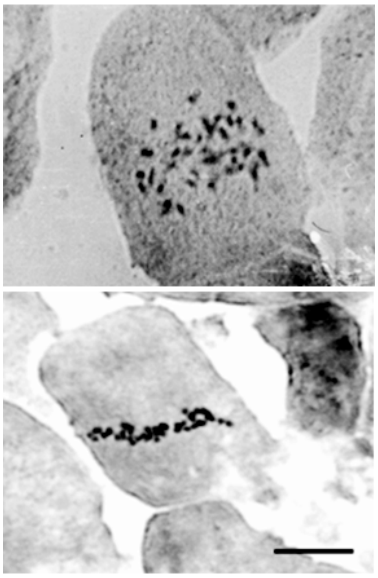

Fig. 1. Somatic chromosomes obtained from radicle meristems of *Minthostachys mollis* (Kunth.) Griseb. (2n = 48). (Bar represents 10 µm).

With regard to meiosis chromosome analysis, in the Pollen Mother Cells (PMC) a differential chromosome behavior was found (Figure 2).

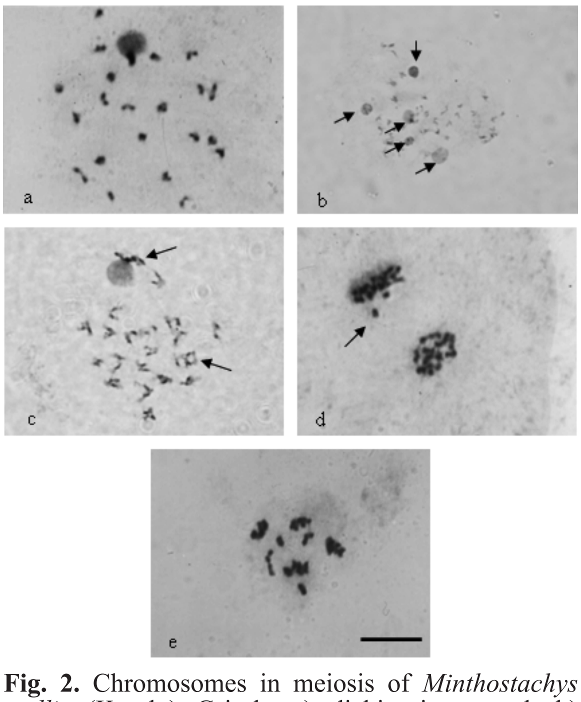

Fig. 2. Chromosomes in meiosis of *Minthostachys mollis* (Kunth.) Griseb. a) diakinesis normal, b) diakinesis with polinuclear cells (arrows indicates nucleolus), c) diakinesis with trivalents and quadrivalents; d) laggard chromosomes; e) abnormal distribution of spindle and laggard chromosomes. (Bar represents 10 µm).

It was frequent the presence of normal divisions, observed in diakinesis (Fig. 2d) and tetrads (Fig. 3d). In the other hand, the irregular chromosome behavior during the first meiosis division was observed for the presence of polinuclear cells in diakinesis (Fig. 2a), formation of trivalent and multivalent in diakinesis (Figs. 2b and c), anaphase segregation with laggard chromosomes (Fig. 2e), and abnormal distribution of spindle with no chromosomes being incorporated to the cellular poles causing the further microcyte formation (Fig. 2f). Cytomictical bridges in triads were observed, which indicate an incomplete or abnormal cytokinesis (Fig. 3).

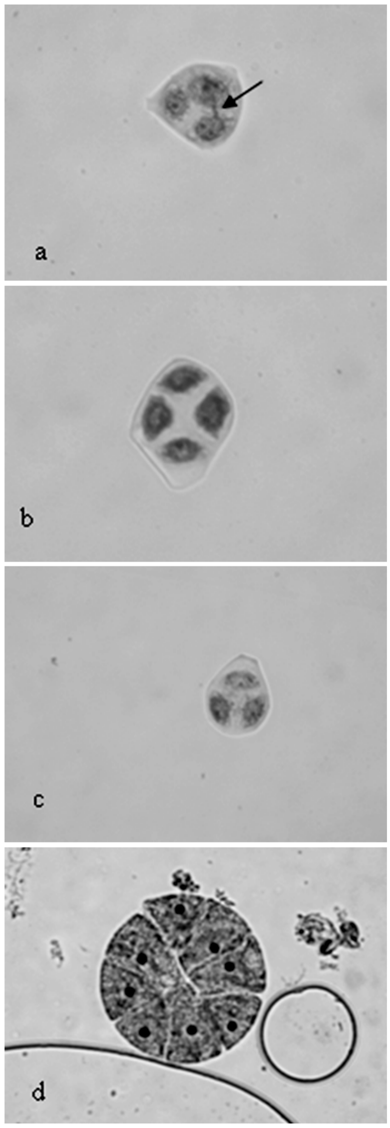

Fig. 3. Tetrads and other configurations with differential behavior in the formation of gametes in *Minthostachys mollis* (Kunth.) Griseb. a) cytomictical bridges (arrow), b) tetrad normal, c) triad, d) polyad.

At the end of division II of meiosis, particularly in the tetrad formation, dyads and polyads of different number were observed (Fig. 3), in addition to normal tetrads. Eight and more microspores besides microcytes were found

(Figs. 4a-c). Type of abnormalities, in number and percentage are shown in Table 1.

The pollen grain viability analysis resulted in 87.2 % of viable grains.

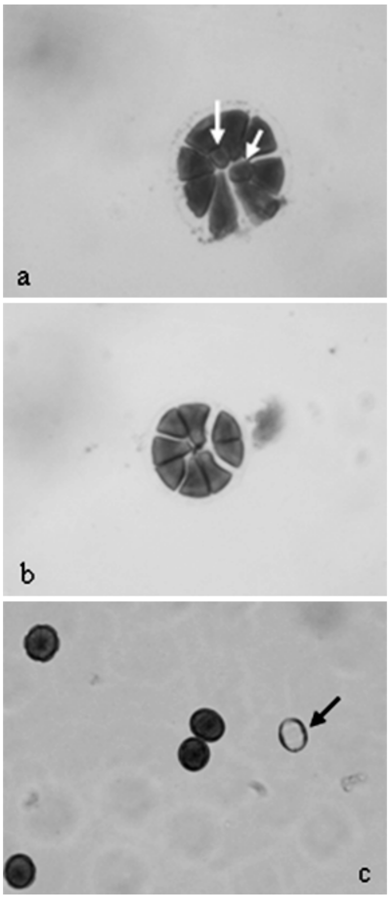

Fig. 4. Microspores in *Minthostachys mollis* (Kunth.) Griseb. a) polyads with microcytes (arrow), b) octads, c) abnormal pollen grains (arrow).

Discussion and conclusion

The number of chromosomes observed is different from those previously reported (Ordoñez and Ojeda, 1997; Ojeda, 2004). The variations could correspond to different cytotypes. In nature, *M. mollis* has sexual reproduction, with crossed fecundation by casmogamy or protoginy (Ojeda, 2004). This can produce intra and inter-specific combinations within and among accessions of a given species (Camadro *et al*., 2004). Morphological, genetic and molecular evidences are necessaries for supporting the assertion that the new chromosomal number is a cytotype.

It is probably that a genetic flow exists among populations of different chromosomal number, because there are morphological–plants which respond to the biotype of 2n = 24 living in sympatry with those 2n = 48 (octoploid), as reported in this paper. According to Fuzinatto *et al.* (2008), the ploidy level could be associated to morphological variations, especially height and leaf morphology that could affect this species reproduction.

In relation to the abnormalities registered at male gametogenesis, some are coincident with those reported by Ojeda (2004); these abnormalities are common in the species and could result in the formation of unbalanced gametes. Another aspect was the presence of polinuclear cells, even in a low number, which could have affected the nucleoli organization.

Considering that this species has a wide distribution in Argentina, in different environmental conditions, some non-reduced gametes (4x) could hve been formed in populations with 2n = 24. Moreover, the abnormalities in polyploids with an odd chromosomal number, as heptaployd (2n = 42) reported by Ojeda (2004), could produce some differential anaphasic segregation that may lead to 4x gametes. The new chromosomal number found in *M. mollis* could be due to: a) Formation of gametes n = 24, from population of Villa Padre Monti and Rio Nio. These locations could be an appropriate environment to establish themselves

Table 1. Configurations registered in male gametogenesis of *Minthostachys mollis* (Kunth.) Griseb. with specification of the observed phase, number and percentage of analyzed cells.

Phase	Type of configuration	Number of cells	Percentage of cells
Diakinesis	Normal	10	5.74
Diakinesis	Polinuclear cells	4	2.29
Diakinesis	Trivalent and quadrivalent	6	3.44
Telophase I	Normal	12	6.89
Telophase I	Laggards	3	1.72
Metaphase II	Abnormal distribution of spindle	2	1.14
Tetrads	Tetrads with binuclear cells	15	8.62
Tetrads	Normal	61	35.05
Dyads	Dyads	5	2.87
Polyads	Polyads of different grade and size (triads and octads)	52	29.88
Triads	With cytomictical bridges	4	2.29
Total cells analyzed		174	

as population, conferring adaptive advantages. b) In sympatric zones, with species 2n = 24 and 2n = 42, could take place the union of non-reduced gametes (4x = 24), and gametes 4x from those of heptaployds 2n =42, resulting the populations with 2n =48.

The irregular chromosome segregation and abnormal cytokinesis in some cells, leading to polyads formation, can affect the formation and viability of pollen grain, producing unbalanced gametes, pollen grains of different size, some with *n* genetic constitution, others with *2n* and others with incomplete genetic information which produces infertile pollen. One consequence of this could be new cytotypes that explain the morphological and chemical differences.

The irregular behavior detected in *M. mollis* produces an important negative effect in the normal gamete formation as well as in fertility of the studied populations. The meiotic abnormalities may explain the formation of heterogeneous pollen size in these populations.

Because of the reproductive limitations detected, added to the loss of variability by the anthropic action (genetic erosion), it is important to consider alternatives that could promote the preservation and sustainable production of this genetic resource that has a significant importance in the Argentinean northwest region.

These results constitute a basis in order to perform further studies about the reproductive biology of this species, considering the different chromosomal numbers found.

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