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Genetic, cytological and molecular characterization of chia (*Salvia hispanica* L.) provenances

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ABSTRACT

Chia (*Salvia hispanica* L.) is a native plant to southern Mexico and northern Guatemala although in these days also is cultivated in others South American countries such as Bolivia and Argentina. This study describes a genetic, cytological and molecular characterization of nine provenances of *Salvia hispanica*. The genome size was estimated to be C = 0.84 pg of DNA. Also a quantitative description of the karyotype is presented. The karyo-idiogram analysis shows that pairs 5 and 6 of the chromosomes are in the limit between submetacentric and subtelocentric types. When the nine provenances were studied using ISSR markers we were able to observe a very low genetic variability for the ISSR loci analysed (h = 0.08–0.15; I = 0.10–0.23; P = 9.3–23.5%). Selfing and reduced genetic background produced by genetic drift and human selection, might explain in part the scarce genetic differentiation found among provenances.

These genetic characterizations are part of the basic genetic information, which should be considered for improvement and germplasm conservation programs.

1. Introduction

Chia, *Salvia hispanica* L., is an annual herbaceous plant that belongs to the family Lamiaceae. Several studies have shown the distribution of the plant (Martínez-Gordillo et al., 2013) as well as cytological characterization (Masoud et al., 2010; Masoud and Alijanpoo, 2011; Ranjbar et al., 2015). It's native to southern Mexico and northern Guatemala although has achieved great development in neighboring countries such as Bolivia and Argentina (Beltran-Orozco and Romero, 2003; Ayerza and Coates, 2004; Capitani et al., 2012). The plants can reach 1–1.5 m in height. It has opposite leaves, 4–8 cm long and 3–5 cm wide. The flowers are hermaphrodite, presenting colors purple to white. They form terminal clusters which bloom from July to August in the northern hemisphere and late summer in the southern hemisphere (Baginsky et al., 2016). After the summer, the flowers give rise to a fruit in the form of indehiscent achene. The seed is rich in mucilage, starch and oil. It is about 2 mm long by 1.5 wide and is oval and shiny with black and grayish white color (Hernández-Gómez et al., 2008). The flowers are pedicellate and are clustered in groups of six or more, in whorls on the rachis of the inflorescence. The calyx is persistent and pubescent bilabiate. The corolla is purple or blue and bilabiate monopetalous; lip expands out and down and the top arches up in the form of helmet or galea (Ramamoorthy, 1985). The mechanism of pollination in chia is not known precisely. It has been suggested that it is allogamous (Hernández-Gómez et al., 2008) and insect pollinated because of the petal color, the “landing pad” shape of the lower lip of the corolla, the articulation of the stamens to the corolla and the presence of nectar at the base of ovary. However, *S. hispanica* is self-compatible and probably self-pollinated, since the flowers are very small and homostylic and even isolated plants produce seed being chia a predominant self-pollinated species (s = 92%; Cahill, 2004).

This species grows in light to medium soils, well-drained, not

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too humid; it tolerates acidity and drought (Silva et al., 2016), but cannot stand frost (Baginsky et al., 2016). It requires abundant sunshine, and does not develop fruits in the shade (Ramamoorthy, 1985; Cahill, 2004). Its economic potential lies in its seed, which produces oil for human and industrial use (Ayerza, 2009). The historical importance of the culture go back to the pre-Columbian populations of Central America where it was one of the basic foods, however was displaced by crops brought to America by the Spaniards. Nevertheless, at the end of the 20th century started again an interest in this crop, mainly due to the fact that the seeds are an important source of fiber, B-group vitamins and poly-unsaturated essential fatty acids, such as linolenic and linoleic acids that the human being cannot synthesize and must be ingested in the diet.

Although it is estimated that the greatest genetic diversity of this species is present in Mexico, there is little information available about basic genetic attributes such as the karyotype, nuclear genome size and molecular markers variability.

The number of chromosomes described for *Salvia hispanica* is $2n = 2x = 12$ (Estilai and Hashemi, 1990), but the genome size (C-value) is unknown. However in previous investigations carried out in other species of the genus, has been suggested that the mean of C value is 0.62 pg (Bennett and Leitch, 2011). On the other hand, genetic studies using molecular markers have been scarce. Cahill (2004) reported a high level of RAPD diversity among wild and cultivars of chia from Mesoamerica.

This study describes a preliminary genetic, cytological and molecular characterization of *Salvia hispanica*, using nine provenances of chia. The genetic variability was evaluated using inter simple sequence repeat (ISSR; Zietkiewicz et al., 1994); the cytological characterization were done through chromosome morphology studies using a karyio-idiogram that assign a value to the ratio "short arm/total chromosome length" and the genome content was estimated using the Feulgen reaction with tomato genomic DNA as standard through quantitative microdensitometry. These studies are part of the basic genetic characterization, which should be considered in breeding and germplasm conservation.

2. Materials and methods

2.1. Plant material

The following nine provenances of chia were used: Atlixco, Mexico (18°54′45N); Puebla, Mexico (20°39′58″N); Jalisco, Mexico (20°39′58″N); Arequipa, Peru (16°23′56″S); Arica, Chile (18°31′25″S); Antumapu, Santiago, Chile (33°27′S); Salta, Argentina (24°47′S) and Santa Cruz de la Sierra, Bolivia, white and black lines (17°47′S). These seeds represent the distribution of cultivars through Mexico and South America. Seeds were germinated and grown using the following greenhouse conditions: 20/15 °C day/night (±2 °C) with a light regime of 14/10 h day/night and 900 μmol m^{-2} s^{-1} light intensity.

2.2. Plant treatments

The root tips from germinated seeds were pre-treated for 12 h with 8-hydroxyquinoline 0.002 M at 8-10 °C, fixed in ethanol - glacial acetic acid (3:1) at 4 °C for 24 h, and stored in 70% ethanol at 4 °C until chromosome processing was done. The root tips were stained with the Feulgen reaction (hydrolyzed for 7 min in 1N HCl at 60°C, stained with Schiff reagent for 60 min and washed in sulphurous water) (Navarrete et al., 1983; Schulte, 1991). Then slides were made by squashing root meristems.

2.3. Cytological analysis

Chromosomes were observed with a Nikon Eclipse E-400 microscope equipped with a Moticam 2500 digital camera. In photomicrographs of at least five best metaphase plates (without overlapping) per provenance, the chromosomes were measured and arranged according to length and shape. The measurements of the short arms (SA) and long arms (LA) and the total relative length of each chromosome pair were expressed as a percentage of the total length of the haploid chromosome set. To obtain a detailed description of chromosome morphology, means and confidence intervals of relative arm lengths of each chromosome pair of all nine provenances of *S. hispanica* were plotted in a Karyo-idiogram according to Spotorno (1985) which assigns the chromosome shape on the basis of the centromeric index proposed by Levan et al. (1964) (ratio short arm/total chromosome length).

2.4. Genome content

Slides made by squashing root tips of chia germinated seeds not treated with antimitotic were prepared. These were stained with Feulgen reaction along with root tips of germinated seeds of commercial tomato (*Lycopersicon esculentum*). The genome size of tomato is known to be 2C = 2.06 pg DNA (Bennett and Smith, 1991) therefore we used it as a comparison standard to establish the genome size of *Salvia hispanica* by quantitative microdensitometry (Spencer et al., 1999; Vilhar et al., 2001; Jara-Seguel et al., 2008; Palma-Rojas et al., 2012). *L. esculentum* was used because its genome size is close to that reported for most species of the *Salvia* genus (Johnston et al., 1999; Bennett and Leitch, 2011). Slides were prepared as for the antimitotic treatments.

The Nikon Eclipse E-400 microscope, equipped with a Cohu 4912-200 black and white digital camera and image analyser software Image Pro-Plus (Media Cybernetic) was used to capture equivalent images of compact chia and tomato prophase. The software captures black and white images from the microscope and analyses the different structures visible on the images. Nuclear optic density (OD) is calculated by the software according to the formula $OD = \log_{10}(1/T) = -\log_{10}T$; where T = intensity of transmitted light/intensity of incident light. From this estimation, the computer integrates the values of OD obtained for each one of the pixels and it calculates the integrated optical density (IOD = ΣOD). For *Salvia hispanica* IOD values of 10 compact prophase from five individuals per provenances (two samples per individual) were determined. To evaluate the existence of significant differences between the IOD values of the nine provenances, a one-way ANOVA was performed. The IOD values were converted to absolute mass of DNA by comparison with 10 compact prophase from five individuals (50 in total) of *Lycopersicon esculentum* (2C = 2.06 pg). The 2C-value was determined using the equation CVu = CVs x (IODu/IODs). In the equation CVu = 2C-value of *S. hispanica*; CVs = 2C value of *L. esculentum*; IODu = average IOD of *S. hispanica*; IODs = average IOD of *L. esculentum*. (Vilhar et al., 2001; Hardie et al., 2002; Moscone et al., 2003).

2.5. Provenances characterization through the use of ISSR markers

Ten plants from each provenance were used for the ISSR genotyping. Genomic DNA was extracted from young leaves (0.5 g) of each plant using the method described in Tittarelli et al. (2009). The purified total DNA was quantified by spectrophotometry and gel electrophoresis (Klagges et al., 2013). DNA samples were stored at −80° C until use.