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Genetic structure of *Zephyranthes fosteri*, specie with ornamental and medicinal potential in Mexico

Estructura genética de *Zephyranthes fosteri*, especie con potencial ornamental y medicinal en México

Martha Isabel Torres-Morán[1], Ana Paulina Velasco-Ramírez[1]*, Norma Almaraz-Abarca[2], Julio Yesua Anaya-Covarrubias[1], Alejandro Velasco-Ramírez[1]

[1] Instituto de Manejo y Aprovechamiento de los Recursos Fitogenéticos. Centro Universitario de Ciencias Biológicas y Agropecuarias. Universidad de Guadalajara. Camino Ramón Padilla Sánchez No. 2100 Nextipac, Zapopan, Jalisco. 45110. México

[2] Centro Interdisciplinario de Investigacion para el Desarrollo Integral Regional (CIIDIR-IPNUnidad Durango), Departamento de Biotecnologia, Sigma s/n, Fraccionamiento 20 de Noviembre II, 34220, Durango, Durango, Mexico.

ABSTRACT

Zephyranthes fosteri is a wild species distributed in Mexico, it is popular for its ornamental quality. Anthropogenic activities perturbed its natural habitats and threaten its survival. At present time, there is not enough information about the genetic relationships and structure among *Z. fosteri* distributed in Mexico. The objective of this investigation was to elucidate the genetic relationships and structure among accessions of *Z. fosteri* collected in western and southeastern Mexico. ISSR markers were used to establish the genetic variability and genetic structure among 60 accessions randomly collected in western Mexico and one population found in southeastern Mexico. Jaccard's coefficient and AMOVA analysis determined the similarity and variability among and within accessions, and Bayesian model was used to assess the genetic structure. A mean heterozygosity of 0.49 was found indicated a moderate variability. The AMOVA analysis showed that 75 % of this variability was within accessions, and 25 % among accessions. A 56.75 % polymorphism was detected with ISSR markers, and genetic structure analysis identified four genetic groups.

Keywords: ornamental plants; ISSR; geophytes; Amaryllidaceae

RESUMEN

Zephyranthes fosteri es una especie silvestre que se encuentra en diferentes partes del territorio Mexicano y que es popular por su calidad ornamental. Su hábitat ha sido constantemente perturbado por actividades antropogénicas amenazando su supervivencia. Existe poca información sobre las relaciones genéticas y la estructura entre *Z. fosteri* en México. El objetivo de esta investigación fue dilucidar las relaciones y la estructura genética entre las accesiones de *Z. fosteri* recolectadas en el occidente y sureste de México. Los marcadores ISSR se usaron para establecer la variabilidad y estructura genética entre 60 accesiones tomadas al azar en el occidente y una población encontrada en el sureste de México. El coeficiente de Jaccard y el Análisis Molecular de Varianza (AMOVA) determinaron la similitud y la variabilidad entre y dentro de las accesiones. Se utilizó el modelo bayesiano para determinar estructura genética. Una heterocigosidad de 0.49 indicó una variabilidad moderada y los resultados del análisis AMOVA mostraron que 75 % y 25 % de esta variabilidad se encontraban inter e intrapoblacional respectivamente. Se detectó polimorfismo de 56.75 % y el análisis de la estructura genética identificó cuatro grupos genéticos, asignando cada muestra a un grupo.

Palabras claves: plantas ornamentals; ISSR; bulbosas; Amaryllidaceae

INTRODUCTION

The genus *Zephyranthes* (Amaryllidaceae) comprises approximately 70 species with neotropical distribution (Hutchinson 2003; Judd et al., 1999). *Zephyranthes* species has potential as geophytes within landscape design since it benefits from the natural vegetation of the region optimizes the plating design both aesthetically and functionally (Seyidoglu et al., 2009).

Zephyrantes fosteri Traub is a wild perennial herbaceous plant with beautiful flowers and reproductive bulbs. It is commonly known as mayito because its flowers appear in May (Padilla-Sánchez et al., 2016). Its elegant appearance gives this species the potential to be appreciated as a decorative landscape element or as a potted plant (Seyidoglu et al., 2009). It is mainly found in forest areas and has been reported in Mexico's western and southern regions as well as in the State of Jalisco, Mexico (Tapia-Campos et al., 2012). Nowadays this species can be found as part of some people's collection, as a potted ornamental plant. Populations of the plants in the wild, are being fragmented by anthropogenic activities such as new road construction and housing developments and consequently it genetic variability could be threatened and make its conservation more difficult (Barrett and Kohn, 1991; Withlock et al., 2011).

Multi-locus markers such as *Inter Simple Sequence Repeats* (ISSR) have been successfully used to assess the genetic variability of several plants (González et al., 2005; Takrouni and Boussaid, 2010; Vargas-Ponce et al., 2011). Each ISSR fragment represents a locus between two microsatellites, and its amplified profile provides a fingerprint for independent

*Autor para correspondencia: Ana P. Velasco-Ramírez
Correo electrónico: pauvela73@hotmail.com
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individuals (González and Aguirre, 2007; Basha et al., 2009; Wu et al., 2014). In addition, Bayesian population analysis can assess genetic coincidences and similarities among populations and can assist in documenting the population genetic status. This method relates the allele frequencies, amongst a set of samples that define a population, to the frequencies found in individuals of differentiated populations (Porras-Hurtado et al., 2013).

The lack of reports describing the genetic variability of *Z. fosteri*, its industrial potential medicinal use, since this species contains inhibitory alkaloids to treat mild to moderate Alzheimer's symptoms disease (Bastida et al., 2006), and endanger status makes the assessment of its genetic variability a priority in ornamental and plant resources. This new information, will contribute to the development of rational conservation, exploitation as well as breeding and domesticating strategies for the species. The objective of this investigation was to elucidate the genetic structure and the genetic variability among accessions of *Z. fosteri* collected in western Mexico and compare with one population found in Southeastern Mexico.

MATERIALS AND METHODS

Plant material and sampling sites

Bulbs and plants were collected in four mexican localities, three located in the western states of Jalisco, Michoacan (Highland and Valleys zones in Michoacan) and Nayarit, as well as a locality in the southeast of Mexico (Yucatan) where it had not been reported in this locality (Table 1, Figure 1). Plants were identified using Mc Vaugh (1989) taxonomic key, and a herbarium voucher of each group deposited at the Herbarium of the Instituto de Botánica Universidad de Guadalajara (IBUG). Bulbs of plants were conserved as *in vivo* accessions in a nursery at Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA), Universidad de Guadalajara located at 20°45'N and 103° 31'W with altitude of 1650 above the sea level.

Bulbs were individually planted (Figure 2) in pots containing a humus soil-pumice substrate (1:1) and placed in the CUCBA nursery. A nutritive formula containing N, P, and K (20-20-20) was added to each pot every two weeks until leaves were fully developed and ready for being collected for DNA extraction.

Table 1. Origin and classification of accessions of *Zephyranthes fosteri*.
Tabla 1. Origen y clasificación de accesiones de *Zephyranthes fosteri*.

Location	Code	Geographical position	Distinguishable trait (Flowers color)
Michoacán	M1 to M8	West	Violet
Nayarit	N1 to N22	West	Violet
Jalisco (Highland zone)	ZA01 to ZA27	West	Violet
Jalisco (Valleys zone)	Za1 to Za 6	West	White
Yucatán (Mérida)	MY1 to MY7	South	White

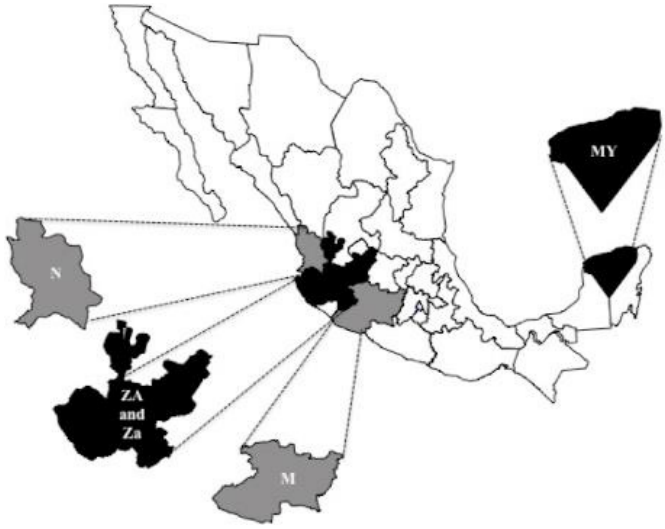

Figure 1. Geographic location of the wild populations of collected *Zephyranthes fosteri*. M: Michoacan; N: Nayarit (Western zone); ZA: Jalisco (Highland zone); Za: Jalisco (Valleys zone); MY: Yucatan Merida (Southeastern zone).
Figura 1. Ubicación geográfica de las poblaciones silvestres de *Zephyranthes fosteri* recolectadas. M: Michoacán; N: Nayarit (zona occidental); ZA: Jalisco (zona de las tierras altas); Za: Jalisco (zona de los Valles); MY: Yucatán, Mérida (zona sureste).

DNA extraction

Foliar DNA was isolated according to the CTAB (*Cetyl Trimethyl Ammonium Bromide*) procedure by Keb-Llanes et al. (2002), and then analyzed for purity and concentration by standard electrophoretic and spectrometric methods (Sambrook and Russell, 2001).

Amplification conditions

Five ISSR primers from 15 sequences selected from the University of British Columbia (2015) and University State the Ohio (2000) (843, 899, 16, 901, BECKY) databases were chosen for amplification (Table 2). Amplification was carried out in 20 µL of 1X PCR buffer containing 2.5 mM $MgCl_2$, 0.8 µM of primer, 0.25 mM dNTPs, 4 ng of DNA, and 0.05 U of Taq DNA polymerase (Promega®). PCR cycling included 3 min at 95 °C followed by 40 cycles of 45 s each at 95 °C (DNA denaturalization), 45 s at 52 °C (annealing), 1 min 30 s at 72

Figure 2. *Zephyranthes fosteri*. (a) Individuals in plant nursery. (b) Flowers. (c) Herborized voucher specimens.
Figura 2. *Zephyranthes fosteri*. (a) Individuos establecidos en vivero. (b) Flores. (c) Especimenes ejemplares de herbario.

Table 2. ISSR primers sequences and polymorphism based on resulting banding patterns of *Zephyranthes fosteri.*
Tabla 2. Secuencia de cebadores ISSR y polimorfismo basados en los patrones de bandas para *Zephyranthes fosteri.*

Primer	Sequence (5´-3´)	Amplicons	Polymorphism (%)
843	CTC TCT CTC TCT CTC TRA	124	53.22
899	CAT GGT GGT GGT CAT TGT	134	55.22
16	YRG ACA GAC AGA CA	111	43.24
901	GTC TGT GTG TGT YR	122	59.01
BECKY	CAC ACA CAC ACA CAY C	26	73.07
	Global	517	56.75

ºC (extension), and a final extension at 72 ºC for 10 min. The amplified fragments were separated by electrophoresis on 6 % polyacrylamide gels and stained with silver salts according to Sanguinetti et al. (1994) and, Sambrook and Russell (2001).

Data analysis

Binary matrixes of presence/absence (1/0 respectively) were codified from the amplified fragment data and Jaccard's similarity coefficient was determined. Analysis of molecular variance (AMOVA) and the variance components, among and within accessions, and their statistical significance was calculated using GenAIEX v. 6.5 (Peakall and Smouse, 2012) and Infogen (Balzarini and Di Rienzo, 2016), respectively. A cluster analysis, using the Unweighted Paired Group Method with Arithmetic Averages (UPGMA). Bootstrap as support for stastical sampling was used, with 10^{10} iterations. Analyses were performed using FreeTree, TreeView and NTSYS-pc 2.21 softwares (Hampl et al., 2001; Reif et al., 2004; Rohlf, 2009).

Genetic variability and informativeness of the ISSR markers were tested through the Polymorphic Information Content (*PIC*), heterozygosity per locus (*He*), average heterozygosity (*Hav*), the multiplex ratio (*MR*) and marker Index (*MI*) (Roldán-Ruiz et al., 2000). The genetic structure was estimated using the STRUCTURE software (v. 2) (Pritchard et al., 2000) four simulations were performance using *K=2* to *K=5* by an admixture model with10^5 iterations, and 10^5 burn-in periods (*K* = number of simulated groups). The differentiation coefficient (*Fst*) based on allelic frequencies, as well as the average heterozygosity for each population were calculated (IPGRI, 2003). Finally, the Evanno method was estimated in order to determine the adequate number of simulated groups (*K*) using Structure Harvester online software (Evanno et al., 2005; Earl and VonHoldt, 2012).

RESULTS

Taxonomic

The taxonomic analysis confirmed that all the individuals collected belonged to the species *Zephyranthes fosteri* (Figure 2).

Molecular marker informativeness

A total of 517 fragments were amplified. The number of amplicons per primer, percentage of polymorphism and amplicon sizes varied from 26 to 134, 43 to 73, and 150 to 2500 pb, respectively (Table 2). Table 3 contains both, the ISSR markers effectiveness values and the average heterozygosity (*Hav*).

Table 3. Marker effectiveness. Parameters for ISSR markers used in *Zephyranthes fosteri* wild populations.
Tabla 3. Efectividad del marcador. Parámetros del marcador ISSR utilizados en poblaciones silvestres de *Zephyranthes fosteri.*

Parameters for marker efficiency	ISSR
Number of individuals	60
Total number of bands (*L*)	517
Polymorphic bands (*p*)	279
Total number primer combinations (*T*)	5
Multiplex ratio (*MR*) (*L*/*T*)	103.4
Polymorphism percentage *(%p)*	56.75
Polymorphic information content (*PIC*)	0.59
Average heterozygosity (*Hav)*	0.49
Marker index (*MI*) *Hav* × *MR*	50.66

Genetic variability and structure

The dendrogram in Figure 3 shows that individuals from the various regions are grouped into one of four groups (S1, S2, S3, and S4), according to the UPGMA cluster analysis. Group S1 included individuals collected in Michoacán (M) and Nayarit (N), group S2 group consisted of individuals from

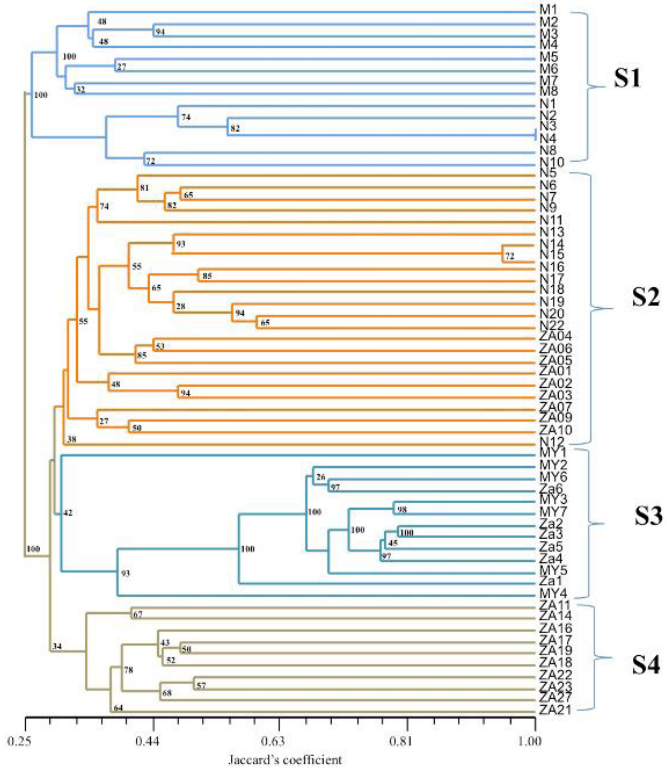

Figure 3. Clustering analyses (UPGMA) and boostrap, based on ISSR markers, showing the genetic relationships among *Zephyranthes fosteri* individuals .
Figura 3. Análisis de agrupamiento (UPGMA) y remuestreo, basado en marcadores ISSR, que muestras las relaciones genéticas entre los individuos de *Zephyranthes fosteri*.

Nayarit with some from Jalisco (Za) (Valleys zone), group the S3 included individuals collected in Yucatan (Merida) and Jalisco (Valleys zone) and group S4 individuals from Jalisco (ZA) (Highland zone).

Results of similarity analyses show the highest similarity (0.80) between the Yucatan and Jalisco Valleys populations, and the lowest (0.25) between the Michoacan and Nayarit. Individuals in the S3 group (MY and Za) were genetically similar. Figure 4 shows the results of the genetic structure with simulations of *K=2* to *K=5* indicated with a different color and the assignment of individuals to a particular group was indicated too. The outcome of both, the STRUCTURE analysis (probabilistic value LnP (D) = -2213) and the Evanno test (highest Delta *K* value = 5.76) indicated that *K* = 4 was the optimal number of groups. The AMOVA analysis demonstrated significant differences in the variability among and within accessions (25% and 75 % respectively) (Table 5).

Bayesian probability analysis differentiated in every simulation, the MY plus Za from all others groups and the results were consistent with those obtained in the cluster analysis (Figure 3).

Table 4 contains the result of the genetic differentiation analysis (*Fst*). The highest genetic differentiation (*Fst*) value was found for the S3 group (0.5738), whereas, the S2 group had the lowest differentiation value (0.2319). Genetic differentiation (*Fst*) and Heterozigosity (*He*) values for the four groups are also included in Table 4. Major heterozygosity was found in the S1 group (0.3309).

Table 4. Genetic differentiation (*Fst*) and expected heterozigosity (*He*) for *K=4* simulation for individuals of *Zephyranthes fosteri.*
Tabla 4. Diferenciación genética (*Fst*) y Heterocigocidad esperada (*He*) para simulación de *K= 4* para individuos de *Zephyranthes fosteri.*

Cluster identification	Similarity within groups	Assigned group in simulation	Differentiation coefficient (*Fst*)	Expected heterozygosity
S1	0.28 – 0.55	Yellow	0.4228	0.3309
S2	0.28 – 0.96	Yellow and blue	0.2319	0.3111
S3	0.32 – 0.81	Green	0.5738	0.1536
S4	0.35 – 0.51	Yellow and red	0.2905	0.2898

Table 5. Analysis of molecular variance (AMOVA) for ISSR markers in 60 individuals of *Zephyranthes fosteri*.
Tabla 5. Análisis de la varianza molecular (AMOVA) para el marcador ISSR en 60 individuos de *Zephyranthes fosteri*.

Source of variation	df	SS	MS	Est. Var.	% of Var.	PhiPT	P
Among Pops	4	1753.846	438.461	30.532	25%	0.249	0.001
Within Pops	56	5143.466	91.848	91.848	75%		
Total	60	6897.311		122.380	100%		

df: degree of freedom, SS: sum of square, MS: square medium, Est. Var: standard variation, % of Var: percentage of variation, PhiPT: estimator of the average genetic differentiation, P: probability

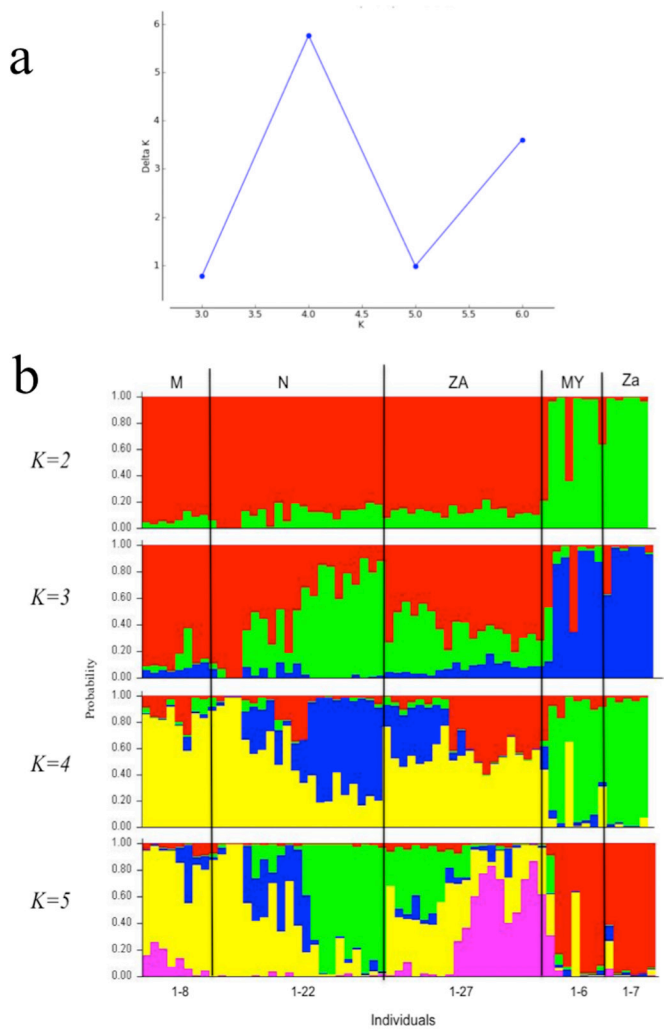

Figure 4. (a) Magnitude of ΔK as a function of *K* from Evanno method. The peak value of ΔK was at *K=4*, suggesting four groups for *Zephyrnthes fosteri* wild populations. (b) Simulations *K=2* to *K=5*, to determine genetic structure of *Zephyranthes fosteri* wild populations based on ISSR markers. M, N and ZA from Western zone, Za from Valley zone and MY from Southeaster zone.
Figura 4. (a) Magnitud de ΔK como una función de *K* del método de Evanno. El valor máximo de ΔK fue *K=4*, sugiriendo cuatro grupos para las poblaciones silvestres de *Zephyrnthes fosteri*. (b) Simulaciones *K=2* a *K=5*, para determinar la estructura genética de poblaciones silvestres de *Zephyranthes fosteri* basadas en marcadores ISSR. M, N y ZA de la zona occidental, Za de la zona del Valle y MY de la zona del sureste.

DISCUSSION

The outcome of this study is based on 517 loci from 60 individuals and meets the requirements for a reliable sample (Nei, 1978; Brown et al., 1983). The total loci detected with ISSR markers in this study are reliable for genetic analysis (Ng and Tan, 2015). As Ferraro et al. (2013), reported ISSR markers have been proven to be an efficient marker, and result to be appropriate to detect high levels of polymorphism among *Z. fosteri*.

The results obtained supports that ISSR markers are useful for detecting genetic variability and genetic relationships among wild plant species (Valdés De La Cruz et al., 2010; Onaum et al., 2015; Jae-Han et al., 2012). The level of poly-

morphism found in this study (56.75 %) (Table 3) is similar to that of lingonberry (*Vaccinium vitis-idaea* L.) (Debnath, 2006) and orange (*Citrus indica*) (Kumar et al., 2010) detected with the same marker.

A PIC value of 0.59 (Table 3), calculated with >20 individuals and >50 detected loci, indicates that the ISSR primers identified a modest number of informative loci (Nei, 1978; Bussell et al., 2005). PIC values obtained with other species such as sugar cane (0.45) (Devarumath et al., 2012) and *Opuntia* (0.27) support this fact (Valadez-Moctezuma, 2015).

The *Hav* value obtained was similar to those of some wild species of *Dioscorea* (0.45) (Velasco-Ramírez et al., 2014) but higher than for *Tribulus terrestris* (0.31) (Sarwat et al., 2008). A *Hav* of 0.4 suggests the presence of enough variability for not considering this species under threat of extinction. Intra and inter population variability were also found in the *Z. fosteri*.

The group patterns obtained from the cluster analysis indicated the presence of two genotypes in Nayarit, two genotypes in Jalisco and another one to the Michoacán cluster, that could be sources of worthy alleles for the development of commercial varieties with violet flowers. Genetically, the Yucatan (MY) and Jalisco Valley populations (Za) were the closest populations (Figure 3) this was unexpected given the significant geographical distance and the orographic barriers between locations, that difficults the genetic flux between the them. The similarity between the populations would have been more clear if they were distributed from south to north as is the case for several other crops and wild ornamentals (Heiser and Nelson, 1974; Eid et al., 2011; Piperno, 2011). On the other hand, individuals from these regions shared white flowers and had a high probability of belonging to the same genetic population. Conversely, the cluster comprised of Michoacan (M), Nayarit (N) and Jalisco (ZA) populations was consistent with its geographical origin (western region). The use of the genus *Zephyranthes* in traditional medicine, in Mexico and Latin American, and its recognition as a medicinal plant among local people could explain its distribution and establishment in Yucatan and Jalisco (Martínez and Cúneo, 2009; Ávila, 2012).

The genetic variability results (Table 4) indicates that group S2 had the largest range of similarity values while the S4 cluster had the shortest, possibly due to different adaptation processes to environmental conditions including soil and climate or a concordance among morphological characters such as flower color. Assessment of the genetic variability in wild species offers the possibility of identifying and selecting valuable alleles for the development of commercial varieties. Also, it offers the potential to modify important economic attributes for ornamental plants like propagation easiness, color, and shapes of leaves and flowers, as well as plant architecture (Dandekar, 2003).

Genetic structure results fits with the AMOVA analyses (Figure 4). Even with a few individuals in the MY and Za groups, the simulations consistently assigned both accessions to a unique simulated group (*K*). This outcome is reliable based on the principles established by Porras-Hurtado et al. (2013) for the Bayesian method and for assigning mixed individuals to clusters, based on the number of loci detected with ISSR (Ng and Tan, 2015; Nelson and Anderson, 2013).

CONCLUSIONS

ISSR were useful markers in assessing the genetic variability of wild populations of *Z. fosteri* and can be used to discriminate between genotypes. As the main natural propagation of this species is asexual, determination of genetic variability is relevant. The genetic variability found in this study, the climate change, and the accelerated growth of buildings and roads are good reasons to establish conservation and breeding programs for wild ornamental plants. This study is a starting point for further research targeting both, the selection of alleles associated with morphological features for developing economically important ornamental varieties and initiating conservation efforts.

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