IRANIAN JOURNAL OF BOTANY 24 (2), 2018
DOI: 10.22092/ijb.2018.122705.1211

RELATIONSHIPS BETWEEN GENOME SIZE, MORPHOLOGICAL AND ECOLOGICAL TRAITS IN SATUREJA (LAMIACEAE) SPECIES

A. Shariat, G. Karimzadeh, M. H. Assareh & J. Loureiro

Received 2018. 07. 22; accepted for publication 2018. 10. 17

Shariat, A., Karimzadeh, G., Assareh, M. H. & Loureiro, J. 2018. 12. 30: Relationships between genome size, morphological and ecological traits in *Satureja* (Lamiaceae) species. *-Iran. J. Bot. 24 (2): 163-173.* Tehran.

Savory as an aromatic plant has traditionally been used in folk medicine as well as a spice of foods, showing inhibition against bacteria, fungi, and yeasts. There is interest in providing a new focus to contribute, from the perspective of genomic content, towards a better understanding of the *Satureja* adaptation. Using flow cytometry (FCM), nuclear DNA content of five *Satureja* (Lamiaceae) species, collected from different locations were analyzed for the first time. Linear regressions of 2C values were evaluated with ecological and morphological parameters. Flow cytometry measurements showed that 2C DNA contents varied from 1.30 to 1.47 pg in diploid species, and with a 2C value of 2.54 pg being obtained for the tetraploid species, *S. spicigera*. There were significant relationships between genome size and 18 morphological traits and climatic characteristics. These relationships could be resulted from geometrical scaling constraints. The obtained results will enhance the knowledge of the genus *Satureja* and constitute an important source of information for future researches.

Anahita Shariat & Ghasem Karimzadeh (correspondence< karimzadeh_g@modares.ac.ir>), Department of Plant Genetics and Breeding, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran, P. O. Box 14115-336. - Mohammad Hassan Assareh, Research Institute of Forests and Rangelands of Iran, P. O. Box: 13185-116, Tehran, Iran, Agricultural research, education and extension organization (AREOO).- João Loureiro, Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal.

Key words: Flow cytometry; genome size; *Satureja* spp.; Ploidy level

ارتباط بین اندازه ژنوم و صفات مورفولوژیکی و اکولوژیکی در گونه‌های مرزه (Satureja spp.)

آناهیتا شریعت: دانش آموخته دکتری اصلاح نباتات دانشکده کشاورزی دانشگاه تربیت مدرس

قاسم کریم‌زاده: دانشیار گروه ژنتیک و به‌نژادی گیاهی، دانشکده کشاورزی دانشگاه تربیت مدرس

محمدحسن عصاره: استاد پژوهش، مؤسسه تحقیقات جنگلها و مراتع کشور، سازمان تحقیقات، آموزش و ترویج کشاورزی

ژوا لوریرو: استاد گروه علوم زیستی، مرکز اکولوژی کاربردی، دانشگاه کوئیمبرا، پرتغال

مرزه به عنوان یک گیاه دارویی و معطر در ادویه‌جات و در طب سنتی کاربرد دارد و دارای خواص بازدارندگی در برابر رشد باکتری ها، قارچ‌ها و مخمرها است. ارائه یافته‌های جدید، در زمینه محتوای ژنتیکی گونه‌های مرزه منجر به درک بهتر سازگاری این جنس می‌گردد. در تحقیق حاضر با استفاده از فلوسایتومتری، محتوای DNA هسته‌ای پنج گونه مرزه برای اولین بار مورد تجزیه و تحلیل قرار گرفت سپس با استفاده از گرسیون خطی رابطه اندازه ژنوم با پارامترهای اکولوژیک و مورفولوژیک مورد ارزیابی قرار گرفت. نتایج فلوسایتومتری نشان داد که مقدار C DNA۲ در گونه‌های دیپلوئید مرزه از ۱/۳۰ تا ۱/۴۷ و در گونه تتراپلوئید (S. spicigera) ۲/۵۴ پیکوگرم متغیر بود. بین اندازه ژنوم و ۲۴ ویژگی مورفولوژیک و آب

و هوایی رابطه معنی داری وجود داشت. نتایج به دست آمده از این تحقیق می‌تواند منبع مهمی از اطلاعات را برای محققان علاقمند به این جنس فراهم آورد.

INTRODUCTION

In the flowering plants, genome size (GS) varies more than 2,300-fold, from 64 Mbp (*Genlisea aurea*, Greilhuber & al. 2005) to approximately 148,880 Mbp (*Paris japonica*; Pellicer & al. 2010). Several ecological and genetic hypotheses have been proposed in order to disentangle the patterns of genome size variation in plants (Corradi & al. 2010; Garcia & al. 2013). It has been proposed that small genomes are associated with a short life-cycle and that rapid growth is adaptive in stressful environmental conditions, such as drought (Miller & Chambers 2006; Veselý & al. 2012). Also, other studies found a quadratic relationship between genome size and seed size. Species presented with small 1Cx-values (and 2C-values) had restricted range of seed sizes, while species with large nuclear DNA contents did not present small seeds (Beaulieu & al. 2007). Furthermore, a positive correlation between genome size and both epidermal and guard cell size was confirmed over a large set of angiosperms (Beaulieu & al. 2008; Hodgson & al. 2010). Moreover, Bainard & al. (2012) found that traits with significant correlation with DNA content were linked to plant competitive ability. *Satureja* L. (Savory), an important culinary herb in Iran, belongs to the Lamiaceae family and comprises about 284 species (Jamzad, 2010). Iran is one of the world's important sources of its germplasm, encompassing 16 species of the genus (Jamzad, 2010). The species of *Satureja* are recognized for their therapeutic properties (e.g., analgesic, antimicrobial, antiviral, antioxidant) because of rich content of essential oils, flavonoids, and triterpenoids (Shariat & al., 2016, 2018 a,b), but little information on the karyotype and genome size of *Satureja* spp. can be found in the literatures. In the genus, several basic chromosome numbers have already been reported, $x = 6$ for *Satureja multiflora* Briq (Krogulevich, 1978), $x = 9$ for *S. acinos* Scheele (Gill, 1981), $x = 10$ for *S. douglasii* Briq (Gill, 1981), $x = 11$ for *S. bulgarica* K. Malý *(*Markova & Goranova 1995), $x = 15$ for *S. bachtiarica* Bunge, *S. khuzistanica* Jamzad, *S. rechingeri* Jamzad, *S. sahendica* Bornm, and *S. spicigera* Boiss (Shariat & al. 2013), $x = 21$ for *S. robusta* Brenan (Morton 1993), and $x = 24$ for *S. hortensis* L. (Gill 1981). Regarding to genome size, as far as we know, only two estimations are available in the literature: 5.56 pg/2C ($2n = 30$) in *S. montana* (Ceccarelli & al. 1998) and 2.26 pg/2C ($2n = 30$) in *S. cuneifolia* (Siljak-Yakovlev & al. 2010). Considering this information, the main objective of this study were to increase our knowledge regarding the genome size of five of endemic *Satureja s*pecies from Iran and to explore possible correlations between genome size and morphological and environmental variables. The results of this study will lay a foundation for the genomics and the genetics of this important medicinal plant.

MATERIALS AND METHODS

Plant materials

Seeds of five native Iranian *Satureja* species were collected during the growing season from natural habitats in different locations in Iran, as described in table 1. Data on altitude, longitude, and latitude of each collecting site were collected with a GPS instrument (map 76CSx, GARMIN, Taiwan; table 1). Rainfall and temperature data from a weather station of each region were obtained from Iran Meteorological Organization's website (http://www.irimo.ir/). For genome size estimations and morphological analyses, seeds were germinated in pots (17 × 17 cm, 13 cm deep), containing a mixture of sand- clay- humus (1-1-1) under natural light conditions in the greenhouse. During the growing period, plants were irrigated weekly and kept free of weeds by hand hoeing. Six weeks after sowing, when the seedling height were 8-10 cm, leaves were collected for genome size examination.

Morphological measurements

The collected materials were studied morphologically, considering the characteristics of the flower parts, bracts, and stomata. In more detail, 46 morphological variables were measured as listed in table 2 & 3 Most characters were obtained through digital photographs acquired with a STEDDY-T 7300 stereo zoom microscope (Medline Scientific, UK)**,** equipped with a digital camera (COOLPIX P90, Nikon Co., Tokyo, Japan), and further analyzed, using MicroMeasure 3.3 Software. Stomata traits were measured with the same digital camera, interfaced to a BH2-RFCA Olympus microscope (Olympus Optical Co., Tokyo, Japan). A number of stomata were counted in five randomly chosen fields at 10x magnification. The width and the length of stomata and guard cells were measured at 100x. Five measurements and counts were performed in each mature leaf. These values were averaged and used as plant estimates for stomata size and density, respectively.

Genome size estimations using flow cytometry

Genome size estimations of the five *Satureja* species were obtained, using flow cytometry. For this purpose, nuclear suspensions were obtained by chopping plant material according to the method described by Tavan & al. (2015). In brief, 45-50 mg. of young leaf tissue of both the sample and of an internal reference standard (*Solanum lycopersicum* 'Stupicke', 2C = 1.96 pg DNA; Doležel & al. 1992) were added to a glass petri dish and subsequently chopped with a sharp razor blade for approximately 60 s in a one ml of Woody Plant Buffer (WPB; Loureiro & al. 2007). The nuclear suspension was then filtered through a 30 µm-nylon mesh to remove large debris and subsequently stained with 50 µl of propidium iodide (PI; one mg ml^{-1}, Sigma) solution added to each sample. As PI is an intercalating fluorescent dye that binds both to DNA and double-stranded RNA, samples were also treated with 50 µl of RNase stock solution (one mg ml^{-1}, Sigma) to prevent staining of double-stranded RNA. Samples were incubated at room temperature and were analyzed within 5 min (Loureiro & al. 2007), using a Cyflow Space flow cytometer (Partec, Münster, Germany), equipped with a 532 nm green solid-state laser, operating at 30 mW. For each species, the instrument settings were established at the beginning of the analyses and kept constant throughout the whole experiment. Relative fluorescence intensity (FL) histograms were obtained and evaluated, using the FloMax software (Version 2.4, Partec, Münster, Germany). Also, FL vs. time and FL vs. side light scatter (SSC) in logarithmic scale cytograms were also obtained. In the latter graphic, a region of interest comprising mostly the isolated nuclei was defined. The FL histogram in a linear scale was gated with this region. At least 1300 nuclei per G_1 peak were analyzed (Suda & al. 2007). Only histograms with a coefficient of variation (CV) lower than 3% for G_1 peaks of both the sample and the standard species were accepted. Samples with CV values higher than 3% were discarded and a new sample was prepared and analyzed. Five different individuals from each species were analyzed on three different days to avoid errors due to instrumental drift. The holoploid genome size in pg (2C; Greilhuber & al. 2005) was calculated according to the following formula: Sample 2C DNA (pg) content = (Sample G_1 peak mean / *Solanum lycopersicum* G_1 peak mean) × *Solanum lycopersicum* 2C DNA amount (pg).

Monoploid genome size (the amount of DNA of one chromosome set, 1Cx-value, with chromosome base number x) and holoploid genome size (the amount of DNA of the whole chromosome complement, 1C-value, with chromosome number n, irrespective of the degree of generative polyploidy, aneuploidies, etc.) (Greilhuber & al. 2005) were further calculated. The obtained values were expressed in picograms (pg) and/or in mega base pairs (Mbp), using the formula by Doležel & al. (2003): 1 pg = 978 Mbp.

Statistical analyses

In order to evaluate possible correlations between the estimated characteristics and genome size, firstly, 2C nuclear DNA values and other characteristics were standardized (by subtracting the mean 2C nuclear DNA content for a species and dividing it by the standard deviation for the species) in order to account for relative differences in the range of morphological and environmental variation experienced by the different species. Then, linear and quadratic regressions were used to estimate the relationship between the variation in standardized 2C nuclear DNA contents and the other standardized variables for the entire complex. This approach is similar to that used in phenotypic selection analysis reported by Kingsolver & al. (2001) and McIntyre (2012). All regressions were performed, using SPSS statistical software V.16 (SPSS Inc., Chicago IL, USA). Principal component analysis (PCA) was carried out to differentiate the studied species based on morphological characteristics. The PCA was done, using Minitab 16 statistical software (Ryan & Joiner 2001) based on the correlation matrix of variables.

RESULTS

Flow cytometric data revealed that the highest amount of 2C nuclear DNA content was 2.54 pg in *S. spicigera* (table 1; fig. 1). The species with the lowest genome size value was *S. sahendica* with 1.30 pg/2C. Monoploid genome size and holoploid genome size are calculated and shown in table 1. The mean comparison of 2C DNA value between four diploids (1.418 pg) and a tetraploid (2.548 pg) was statistically different ($P < 0.01$). Among the diploid species, there were also statistically significant differences in genome size ($P < 0.01$; table 1). In particular, *S. sahendica* presented a significantly lower value of genome size (1.30 pg/2C) than the remainder diploid species. The monoploid genome size (table 1) ranged from 622.98 Mbp (*S. spicigera*) to 718 Mbp (*S. bachtiarica*), with statistically significant differences being obtained between some species (table 1). The previous study revealed that four species (*Satureja bachtiarica*, *S. khuzistanica*, *S. rechingeri*, and *S. sahendica*) were diploids with a karyotype formula of either $2n=2x=30$m or 28m+2sm, while *S. spicigera* was tetraploid ($2n=4x=58$m+2sm) (Shariat & al. 2013).

Table 1. Collection data, 2C-value and flow cytometric DNA estimation data of *Satureja* species. Means with different symbol letters in columns are significantly different ($P < 0.01$) according to Duncan test.

Species	Collection data	Mean rainfall (mm)	Mean Temp (ºC)	Ploidy level	2C-value (pg) ± SE	1C-value (pg)	1C-value (Mbp)	1Cx-value (Mbp)
Satureja. bachtiarica	Yazd, Mehriz, 1920 m, 10596 (TARI)	61	19.4	2*x*	1.470 ± 0.008^{b}	0.735	718.83	718.83^{a}
S. khuzistanica	Lorestan, Khorramabad, 1190 m, 101597 (TARI)	529	16.4	2*x*	1.446 ± 0.005^{b}	0.723	707.09	707.09^{a}
S. rechingeri	Ilam, Mehran, Dehloran, 395 m, 101598 (TARI)	327	25.5	2*x*	1.456 ± 0.010^{b}	0.728	711.98	711.98^{a}
S. sahendica	Azarbaijane Sharqi, Sahand, 2200 m, 101599 (TARI)	254	13.0	2*x*	1.300 ± 0.008^{c}	0.650	635.70	635.70^{b}
S. spicigera	Gilan, Roodbar, 570 m, 101600 (TARI)	352	16.0	4*x*	2.548 ± 0.016^{a}	1.274	1245.97	622.98^{c}

A negative significant relationship was identified between 2C value and either peduncle length 2 (PL2), corolla color (CoC), abaxial guard cell density (AbGCD) and altitude (table 3), but significant positive relationships were detected between 2C DNA and calyx color (CC), calyx upper teeth length (CUTL), leaf color (LC), bracteole length (BL), chlorophyll (Chl), plant height (PH), number of main branch (NMB), maximum canopy cover (MAX CC), minimum canopy cover (MINCC), adaxial stomata length (ASL), adaxial stomata width (AdSW), adaxial stomata area (AdSA), abaxial stomata length (AbSL), abaxial stomata width (AbSW), and abaxial stomata area (AbSA) (table 3). Comparisons of genome size with geographic variables revealed significant but a low negative linear relationship between genome size variation and altitude for the complex as a whole ($R^2 = 0.29$, $b = -0.54^{**}$, table 3). The PCA based on mixed morphological parameters separated clearly most of the species. *Satureja sahendica* (2*x*) and *S. specigera (*4*x*) were close to each other. The first two principal components accounted for 56% of total variation. The first component accounted for 33% of the total variation, mainly attributable to the parameters of LA, SLW, CoW, CoL, and SL associated positively and InL and BL negatively. The second component accounted for 22.7%, attributable to the parameters of IL positively and CC negatively. A scatterplot of the component loadings (correlation between initial variables and principal components) gave more details on the traits shared by species appearing near to each other in fig. 2 and showed the traits responsible for separation between distant species (fig. 2b).

The morphological characteristics of diploid and tetraploid *Satureja* species were assessed (table 4 & 5) to determine which quantitative traits might be useful for identifying tetraploid plants (with larger genome size; table 1).

DISCUSSION

Our study revealed either negative or positive significant relationships between 2C nuclear DNA content with ecological, morphological traits of five *Satureja* species.

Genome size as a basic data of species is being used in a wide range of biological fields. Many studies have been carried out to evaluate the relationships between genome size and biological and ecological traits (*e.g.* Vinogradov, 2003; Leitch & Bennett, 2004; Chase & al. 2005; Beaulieu & al. 2007; Garcia & al. 2008; Jaume & al. 2009).

Table 2. The list of morphological traits and their abbreviations.

Traits (unit)	Abbr.	Traits (unit)	Abbr.
Quantitative traits			
Inflorescence length	IL	Chlorophyll (mg $cm^{-2)}$	Chl
Floral leaf length	FLL	Plant height (cm)	PH
Floral leaf width	FLW	Number of main branch	NMB
Calyx length	CL	Number of subbranch	NSB
Calyx lower teeth length	CLTL	Maximum canopy cover (cm)	MaxCC
Calyx upper teeth length	CUTL	Minimum canopy cover (cm)	MinCC
Peduncle length 1	PL1	Leaf area ($mm^{2)}$	LA
Peduncle length 2	PL2	Adaxial stomata length (µm)	ASL
Corolla length	CoL	Adaxial stomata width (µm)	AdSW
Corolla width	CoW	Adaxial stomata area (μm^2)	AdSA
Filament length	FL	Adaxial guard cell density	AdGCD
Style length	StL	Adaxial stomata guard cell length (µm)	AdSGCL
Stem leaf length	SLL	Abaxial stomata length (µm)	AbSL
Stem leaf width	SLW	Abaxial stomata width (µm)	AbSW
Bracteole length	BL	Abaxial stomata area (μm^2)	AbSA
Internode length	InL	Abaxial guard cell density (no.)	AbGCD
Stem diameter	SD	Abaxial guard cell length (µm)	AbSGCL
Qualitative traits			
Calyx color (code, varied from light to dark green)	CC	Leaf color (code, varied from light to dark green)	LC
Calyx hair (code, varied from lack to abundant)	CH	Leaf hair density (code, varied from lack to abundant)	LH
Corolla color (code, varied from white to violet)	CoC	Stem color (code, varied from light to dark brown)	SC
Anther color (code, white, milky, yellow, light violet, violet)	AC		

Although the obtained results are not congruent (Knight & al. 2005). Knight & al. (2005) proposed a "large genome constraint" hypothesis, where it is predicted that organisms with large genomes will be comparatively constrained, whereas organisms with small genomes will have a wide range of morphological characteristics and can be found in a diverse range of habitats (table 3). We studied the relations between genome size and 51 traits. Among those, 17 morphological and one ecological characters were significantly correlated with genome size. (table 3). Five and 12 characters had negative and a positive significant relationship with genome size respectively. Altitude is one of ecological factors with negative relationship with 2C DNA ($b= - 0.541^{**)}$.

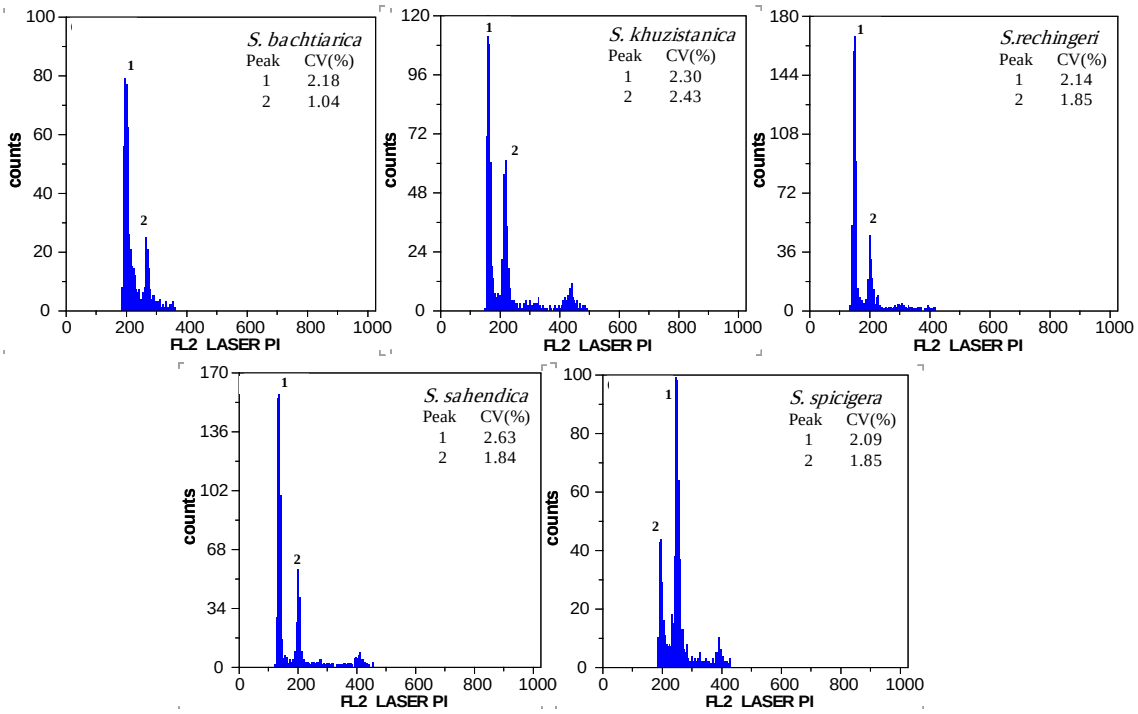

Fig. 1. Histograms of flow cytometric 2C DNA content of five *Satureja* species. The No. 1 peaks refer to the *Satureja* samples and No. 2 peaks refer to the *Solanum lycopersicum* cv. Stupick (2C DNA = 1.96 pg) internal reference standard.

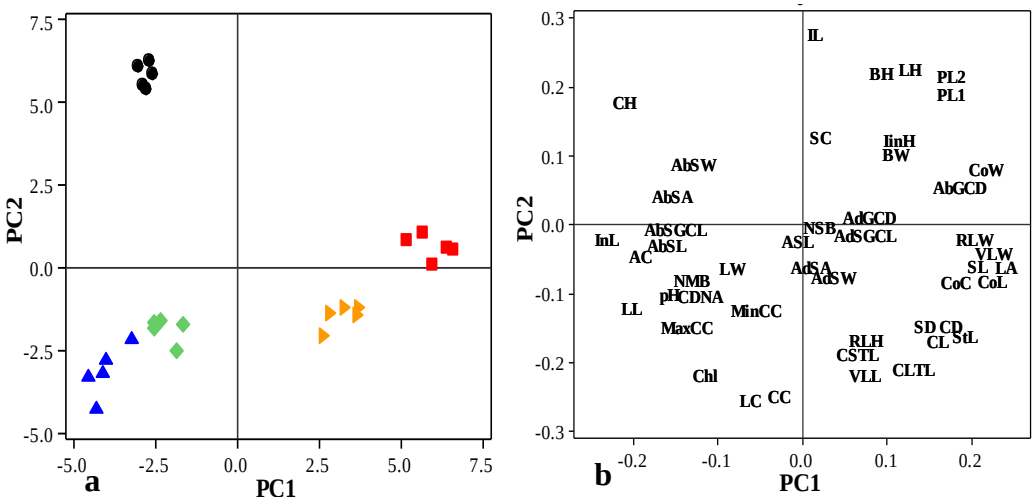

Fig. 2. Scatterplot of first two components from a PCA performed with 46 variables of 25 specimens. Different symbols indicate grouping by different functional classifications: a, five *Satureja* species, *S. bachtiarica* (●);*S. khuzistanica* (■); *S. spicigera* (◆); *S. sahendica* (▲) and *S. rechingeri* (►). b, Morphological traits (refer to table 2 for full names of all traits).

Table 3. Results of regressions of genome size on morphological and ecological parameters. Slope values (b) are standardized coefficients, in order to compare values of slope across all factors, ^{ns}, *, **non-significant ($P > 0.05$), significant at $P < 0.05$ and $P < 0.01$, respectively. Full names and abbreviations are listed in table 2.

Traits	R^2	b	Traits	R^2	B	Traits	R^2	b
IL	0.025	- 0.157^{ns}	FL	0.104	- 0.323^{ns}	MaxCC	0.242	0.492^{ns}
FLL	0.107	0.327^{ns}	StL	0.011	- 0.107^{ns}	MinCC	0.662	0.814^{ns}
FLW	0.001	0.019^{ns}	LC	0.185	0.431*	LA	0.109	- 0.330^{ns}
CL	0.048	- 0.218^{ns}	LH	0.459	- 0.677**	ASL	0.389	0.624**
CC	0.557	0.747**	SLL	0.020	- 0.141^{ns}	AdSW	0.342	0.584**
CLTL	0.012	0.108^{ns}	SLW	0.085	- 0.292^{ns}	AdSA	0.406	0.637**
CUTL	0.353	0.594**	BL	0.184	0.429*	AdGCD	0.002	0.046^{ns}
PL1	0.057	- 0.239^{ns}	InL	0.201	0.448^{ns}	AdSGCL	0.075	0.273^{ns}
PL2	0.210	- 0.458**	SC	0.051	0.226^{ns}	AbSL	0.371	0.609**
CH	0.126	0.355^{ns}	SD	0.001	- 0.028^{ns}	AbSW	0.566	0.752**
CoL	0.062	- 0.250^{ns}	Chl	0.235	0.485*	AbSA	0.650	0.806**
CoC	0.363	- 0.603**	pH	0.310	0.557**	AbGCD	0.237	- 0.487*
CoW	0.101	- 0.319^{ns}	NMB	0.818	0.905**	AbSGCL	0.049	0.222^{ns}
AC	0.061	0.247^{ns}	NSB	0.025	0.159^{ns}			
Ecological parameters								
Rainfall (mm)	0.024	0.156^{ns}	Temp. (ºC)	0.023	- 0.150^{ns}	Latitude	0.131	0.361^{ns}
Altitude (m)	0.293	- 0.541**	Longitude	0.220	0.150^{ns}			

Satureja sahendica had the smallest genome size, which was collected at an altitude of 1920 m, but *S. spicigera* with the largest genome size was collected from an altitude of 570 m. There was a clear gap among most characters and the estimated genome sizes of the species, since there was no relation between genome size and 29 other characters, hence we tried to discuss the significant relations. The pattern in morphological differences and genome size is rather complicated and sometimes it could be hardly explained by the nucleotypic effect (Bennett, 1972) (*e.g.* peduncle and leaflet length, corolla color, calyx short teeth length) (Šímová & Herben 2011; Hanušová & al. 2014).

The results of the recent study show the similar positive linear trends in the relationship between genome size and mentioned traits, probably suggest that variation in genome size can be found across a temporal gradient as well. The adaxial and abaxial stomata length, width and stomat area in tetraploid *S. spicigera* was significantly larger than diploid species (table 3). We determined the genome size was significantly and positively correlated with the stomata characteristics. Initially, small stomata can open and close more rapidly when compared to large ones. Therefore, in dry habitats, plants with small stomata could afford greater water use efficiency, whereas, in shaded, the cool and humid air conditions, large stomata may be an advantage (Hodgson & al. 2010). Genome size potentially represents a simple selection of large cells with large stomata and vacuoles in humid air (*e.g. S. spicigera*). Secondly, theoretically large stomata facilitate the efficiency of photosynthesis in deep shade, but small stomata size has limitations regarding photosynthetic capacity (Allen & Pearcy 2000; Hetherington & Woodward 2003). In the present study, *S. bachtiarica* from dry and high altitude habitats (mean rainfall: 61 mm; altitude: 1920 m) tends to have smaller stomata than the other species. Stomata size is not the only ecological and physiological characteristic but may often have the best subordinate impact upon genome size (Beaulieu & al. 2008). Similar to our findings, Beaulieu & al. (2008), Majdi & al. (2010), Jalili & al. (2013), and Tavan & al. (2015) reported significant positive relationships between genome size and both guard cell length and epidermal cell area, and a negative relationship with stomatal density. On the

other hand, Knight and Ackerly (2002) reported that species with large 2C-values represent stronger relationship between nuclear DNA content and environmental factors. In the present work, we found negative significant relationships among altitudinal distribution of species and genome size variation. The lowest genome size was related to *S. sahendica* that is distributed in the Sahand Mountains (2200 meters ASL). Phylogenetically informative DNA sequence data would allow discrimination among the five species in our study. Previous studies which focused on the taxonomy and genetic diversity of different Iranian species of *Satureja*, revealed the clustering of the species in three main groups, which were somewhat congruent with their geographical distributions (Hadian & al. 2010). Using Selectively Amplified Microsatellite Polymorphic Loci (SAMPL), the maximum degree of similarity was obtained between *S. rechingeri* and *S. khuzistanica*, the two species that are distributed in southwest of Iran. In our study, despite the fact that the species had similar genome size values, the PCA analysis (by using 46 traits) classified these species into completely separate groups (fig. 2). The genome size of *S. spicigera (*the tetraploid species detected in this study) was practically twice as that of *S. sahendica*, with some degree of genome downsizing, almost no degree of separation was observed in the PCA analysis for these two species. Also, the previous study on karyological comparison among *Satureja* species indicated that *S. sahendica* and *S. specigera* were classified in the same group when species plotted based on total length of haploid complement (CL) and centromeric index (CI) (Shariat & al. 2013) that support our hypothesis about taxonomic correlation of these two species.

Table 4. Mean morphological and ecological values for each *Satureja* species. *S. bach* (*S. bachtiarica*); *S. khu (S. khuzistanica*); *S. rech (S.rechingeri*); *S. sah (S. sahendica*); *S. spi (S. spicigera*); Full names and abbreviations are listed in table 2.

Traits	*S.bach*	*S. khu*	*S. rech*	*S. sah*	*S. spi*	Traits	*S.bach*	*S. khu*	*S. rech*	*S. sah*	*S. spi*
IL	23.20	18.20	8.40	8.20	10.80	SD	1.16	1.86	1.96	1.58	1.62
FLL	6.32	15.20	9.30	12.12	13.84	Chl	16.66	27.04	27.00	45.72	52.00
FLW	1.82	7.20	4.00	1.73	3.54	pH	24.56	20.60	22.12	31.60	39.60
CL	2.68	7.68	6.98	7.00	5.24	NMB	3.80	1.80	2.60	1.40	15.60
CC	1.00	2.00	2.00	2.00	3.00	NSB	21.20	25.00	24.20	16.60	25.40
CLTL	1.38	3.44	3.08	2.96	2.99	MaxCC	23.80	21.60	26.40	38.20	44.60
CUTL	0.66	1.82	1.40	1.15	2.01	MinCC	6.40	12.80	11.20	5.10	30.60
PL1	3.10	4.04	2.84	0.98	1.79	LA	13.80	168.00	170.00	49.80	37.00
PL2	6.62	9.00	4.84	2.16	1.90	ASL	16.50	19.00	15.25	14.75	20.50
CH	4.00	1.00	1.00	2.00	3.00	AdSW	8.75	11.00	10.63	7.13	12.75
CoL	7.12	19.20	15.04	11.78	10.50	AdSA	113.70	164.48	130.38	82.73	209.44
CoC	2.00	3.00	4.00	3.00	1.00	AdGCD	60.96	65.79	65.02	59.94	62.99
CoW	3.26	5.44	4.02	2.74	2.80	AdSGCL	26.50	31.63	26.75	26.00	29.75
AC	3.00	2.00	1.00	5.00	4.00	AbSL	17.50	14.13	17.13	16.13	20.00
FL	8.60	18.80	22.90	11.30	10.30	AbSW	13.38	9.38	8.63	6.75	15.13
StL	6.94	20.80	24.90	14.66	15.04	AbSA	184.35	104.20	116.89	85.25	237.71
LC	1.00	2.00	2.00	3.00	3.00	AbGCD	73.66	82.30	95.50	68.07	62.99
LH	4.00	5.00	2.00	3.00	1.00	AbSGCL	28.75	24.63	27.38	30.00	29.88
SLL	10.60	20.70	23.80	26.00	19.50	Rain	61	529	327	254	352
SLW	2.12	12.80	14.60	3.66	3.80	Altitude	1,920	1,190	395	2,200	570
BL	4.00	0.00	4.50	7.05	7.74	Temp	19.40	16.40	25.50	13.00	16.00
InL	14.00	5.10	7.80	16.40	17.60	Longitude	54.37	48.22	46.53	46.32	49.43
SC	3.00	4.00	1.00	2.00	3.00	Latitude	31.53	33.48	33.08	37.85	36.86

Table 5. Principal component analysis for karyotypic and morphological parameters of *Satureja* species. Full names and abbreviations are listed in table 2.

Traits	PC1	PC2	PC3	PC4	Traits	PC1	PC2	PC3	PC4
Eigenvalue	15.649	10.692	9.002	3.802					
Cumulative (%)	0.333	0.560	0.752	0.833					
C DNA	-0.120	-0.129	0.229	-0.120	BL	-0.2	-0.145	-0.081	-0.048
IL	0.017	0.253	0.129	0.065	InL	-0.229	-0.047	-0.012	0.075
FLL	0.076	-0.192	0.145	0.263	SC	0.023	0.102	0.249	0.273
FLW	0.206	-0.046	0.160	0.058	SD	0.146	-0.171	-0.014	-0.03
CL	0.161	-0.195	-0.052	0.113	Chl	-0.113	-0.243	0.003	0.168
CC	-0.026	-0.272	0.132	-0.005	pH	-0.151	-0.126	0.069	0.052
CLTL	0.132	-0.235	0.022	0.088	NMB	-0.129	-0.111	0.219	-0.14
CUTL	0.067	-0.212	0.189	0.011	NSB	0.023	-0.028	0.113	-0.134
PL1	0.177	0.165	0.120	-0.080	MaxCC	-0.136	-0.174	0.038	0.045
PL2	0.178	0.191	0.077	0.031	MinCC	-0.054	-0.15	0.234	-0.103
CH	-0.210	0.153	0.074	-0.021	LA	0.235	-0.076	-0.019	-0.081
CoL	0.225	-0.106	0.032	0.088	ASL	-0.005	-0.049	0.297	0
CoC	0.184	-0.109	-0.189	-0.030	AdSW	0.039	-0.089	0.241	-0.204
CoW	0.220	0.056	0.081	0.017	AdSA	0.011	-0.086	0.268	-0.123
AC	-0.191	-0.063	-0.018	0.307	AdGCD	0.081	-0.023	0.082	-0.059
FL	0.215	-0.083	-0.067	-0.177	AdSGCL	0.074	-0.034	0.215	0.052
StL	0.190	-0.178	-0.038	-0.134	AbSL	-0.159	-0.054	0.05	-0.24
LC	-0.060	-0.278	0.000	0.161	AbSW	-0.127	0.064	0.24	-0.174
LH	0.129	0.202	0.001	0.263	AbSA	-0.153	0.018	0.203	-0.205
SLL	0.076	-0.243	-0.130	0.101	AbGCD	0.187	0.028	-0.091	-0.266
SLW	0.229	-0.072	-0.023	-0.15	AbSGCL	-0.148	-0.033	-0.068	0.006

Their similar geographical distribution could suggest for a common evolutionary history, but *S. sahendica* belongs to east Azerbaijan, Sahand Mountains (north west of Iran) and *S. specigera* belongs to Gilan, Roodbar (north of Iran), this makes the conclusion and interpretation more difficult. Still, in the study of Hadian & al. (2010), these species clustered in two different groups. Further genetic and cytogenetic studies are needed to clarify this issue.

CONCLUSION

The present results provide an accurate estimate of the genome size in five Iranian native *Satureja* species for the first time. Furthermore, the relationship of genome size with morphological and ecological factors was elucidated. These findings provide a remarkable contribution to the revision of the genus specially for plant breeders to select appropriate methods. Furthermore these results could provide new insight for cultivation of economic and medicinal valuable species and the protection of gene reservoirs of endemic and threatened species and acts as the guide for future studies.

REFERENCES

Allen, M. T. & Pearcy, R. W. 2000: Stomatal behavior and photosynthetic performance under dynamic light regimes in a seasonally dry tropical rain forest. -Oecologia. 122 (4): 470–478.

Bainard, J. D., Bainard, L. D., Henry, T. A., Fazekas, A. J. & Newmaster, S. G. 2012: A multivariate analysis of variation in genome size and endoreduplication in angiosperms reveals strong phylogenetic signal and association with phenotypic traits. -New Phytol. 196 (4): 1240–1250.

Balao, F., Herrera, J. & Talavera, S. 2011: Phenotypic consequences of polyploidy and genome size at the

microevolutionary scale: a multivariate morphological approach. -New Phytol. 192 (1): 256–265.

Beaulieu, J. M., Leitch, I. J., Patel, S., Pendharkar, A & Knight, C. A: 2008. Genome size is a strong predictor of cell size and stomatal density in angiosperms. -New Phytol. 179 (4): 975–986.

Beaulieu, J. M., Moles, A. T., Leitch, I. J., Bennett, M. D., Dickie, J. B. & Knight, C. A. 2007: Correlated evolution of genome size and seed mass. -New Phytol. 173 (2): 422–437.

Bennett M. D. 1972: Nuclear DNA content and minimum generation time in herbaceous plants. -Proc Royal Soc Lond, Ser B, 181, 109–135.

Ceccarelli, M., Morosi, L. & Cionini, P.G. 1998: Chromocenter association in plant cell nuclei: determinants, functional significance, and evolutionary implications. -Genome 41 (1): 96-103.

Chase, M. W., Hanson, L., Albert, V. A., Whitten, W. M. & Williams, N. H. 2005: Life history evolution and genome size in subtribe Oncidiinae (Orchidaceae). -Ann. Bot. 95 (1): 191–199.

Corradi, N., Pombert, J. F., Farinelli, L., Didier, E. S. & Keeling, P. J. 2010: The complete sequence of the smallest known nuclear genome from the microsporidian Encephalitozoon Intestinalis. -Nat. Commun. 1 (6): 1–7.

Doležel, J., Bartoš, J., Voglmayr, H. & Greilhuber, J. 2003: Nuclear DNA content and genome size of trout and human. -Cytometry 51A (2): 127–128.

Doležel, J., Sgorbati, S. & Lucretti, S. 1992: Comparison of three DNA fluorochromes for flow cytometric estimation of nuclear DNA content in plants. -Physiol. Plant. 85 (4): 625–631.

Garcia, S., Canela, M. A., Garnatje, T., Mcarthur, E. D., Sanderson, S. C. & Vallè, S. J. 2008: Evolutionary and ecological implications of genome size in the North American endemic sagebrushes and allies (*Artemisia*, Asteraceae). -Biol. J. Linn. Soc. 94 (3): 631–649.

Garcia, S., Leitch, L. J., Anadon-Rosell, A., Canela, M. A., Galvez, F., Garnatje, T., Gras, A., Hidalgo, O., Johnston, E., Mas de Xaxars, G., Pellicer, J., Siljak-Yakovlev, S., Valle, J., Vitales, D. & Bennett, M. D. 2013: Recent updates and developments to plant genome size databases. -Nucleic Acids Res. 42 (D1): D1159–D1166. doi:10.1093/nar/gkt1195.

Gill, L. S. 1981: Taxonomy, Distribution and Ecology of the Canadian Labiatae. -Feddes Repert., 92 (1-2): 33–93.

Greilhuber, J., Doležel, J., Lysák, M.A. & Bennett, M. D. 2005: The Origin, evolution and proposed stabilization of the terms 'genome size' and 'C-value' to describe nuclear DNA contents. -Ann. Bot. 95: 255–260.

Hadian, J., Azizi, A., Fakhr Tabatabaei, M., Naghavi, M. R., Jamzad, Z. & Friedt, W. 2010: Analysis of the genetic diversity and affinities of different Iranian *Satureja* species based on SAMPL markers. -Planta Med. 76 (16): 1927–1933.

Hadian, J., Esmaeili, H., Nadjafi, F. & Khadivi-Khub, A. 2014: Essential oil characterization of *Satureja rechingeri* in Iran. -Ind. Crop. Prod. 61: 403–409.

Hanušová, K., Ekrt, L., Vít, P., Kolář, F. & Urfus, T. 2014: Continuous morphological variation correlated with genome size indicates frequent introgressive hybridization among *Diphasiastrum* species (Lycopodiaceae) in central Europe. -PLoS ONE 9 (6): e99552.

Hassan, H. M. 1968: Experimental taxonomy of oxalis section acetosellae and maianthemum. Ph.D. Thesis, Durham University, Durham, UK.

Hetherington, A.M. & Woodward, F.I. 2003: The role of stomata in sensing and driving environmental change. -Nature 424 (6951): 901–908.

Hodgson, J. G., Sharafi, M., Jalili, A., Díaz, S., Montserrat-Martí, G., Palmer, C., Cerabolini, B., Pierce, S., Hamzehee, B. & Asri, Y. 2010: Stomatal vs. genome size in angiosperms: the somatic tail wagging the genomic dog? -Ann. Bot. 105 (4): 573-584.

Jalili, A., Rabie, M., Azarnivandc, H., Hodgsond, J. G., Arzani, H., Jamzad, Z., Asri, Y., Hamzeheea, B., Ghasemia, F., Hesamzadeh Hejazi, S. M.& Abbas-Azimi, R. 2013: Distribution and ecological consequences of ploidy variation in *Artemisia sieberi* in Iran. -Acta Oecol. 53: 95–101.

Jamzad, Z. 2010: A new species of *Satureja* (Lamiaceae) from Iran. -Iranian Journal of Botany 2: 213-217.

Pellicer, J., Garcia, S., Garnatje, T. & Vallès, J. 2009: Changes in genome size in a fragmented distribution area: the case of *Artemisia crithmifolia* L. (Asteraceae, Anthemideae). -Caryologia 62 (2): 152–160.

Kingsolver, J. G., Hoekstra, H. E. & Hoekstra, J. M. 2001: The strength of phenotypic selection in natural populations. -Am. Nat. 157 (3): 245–261.

Knight, C. A. & Ackerly, D. 2002: Variation in nuclear DNA content across environmental gradients: a quantile regression analysis. -Ecol. Lett. 5 (1): 66–76.

Knight, C. A., Molinari, N. A. & Petrov, D. A. 2005: The large genome constraint hypothesis: evolution, ecology, and phenotype. -Ann. Bot. 95 (1): 177–190.

Krogulevich, R. E. 1978: Karyological analysis of the species of the flora of eastern Sayana. In Flora of the Prebaikal. Edited by L.I. Malyshev and G.A. Peshlcova. -Novosibirsk, Russia, pp. 19–48.

Leitch, I. J. & Bennett, M. D. 2004: Genome downsizing in polyploid plants. -Biol. J. Linn. Soc. 82 (4): 651–663.

Levan, A., Fredga, K. & Sandbreg, A. 1964: Nomenclature for centromeric position on chromosome. -Hereditas 52 (2): 201–220.

Loureiro, J., Rodriguez, E., Doležel, J. & Santos, C. 2007: Two new nuclear isolation buffers for plant DNA flow cytometry: A test with 37 species. -Ann. Bot. 100 (4): 875–888.

Majdi, M., Karimzadeh, G., Malboobi, M. A., Omidbaigi, R. & Mirzaghaderi, G. 2010: Induction of tetraploidy to feverfew (*Tanacetum parthenium* Schulz-Bip.): Morphological, physiological, cytological and phytochemical changes. –Hort. Science, 45 (1): 16–21.

Markova, M. & Goranova, V. 1995: Mediterranean chromosome number reports 5 (435-473). -Fl. Medit. 5: 289–317.

McIntyre, P.J. 2012: Cytogeography and genome size variation in the *Claytonia perfoliata* (Portulacaceae) polyploid complex. -Ann. Bot. 110 (6): 1195–1203.

Miller, J. M. & Chambers, K. L. 2006: Systematics of *Claytonia* (Portulaceae). -Syst. Bot. Monogr. 78: 1–236.

Morton, J. K. 1993: Chromosome numbers and polyploidy in the flora of Cameroon Mountain. -Opera Bot. 121: 159–172.

Paszko, A. 2006: A critical review and a new proposal of karyotype asymmetry indices. -Plant Syst. Evol. 258 (1-2): 39–48.

Pellicer, J., Fay, M. F. & Leitch, I. J. 2010: The largest eukaryotic genome of them all? . -Biol. J. Linn. Soc. 164 (1): 10–15.

Peruzzi, L. & Eroğlu, H.E. 2013: Karyotype asymmetry: again, how to measure and what to measure? -Comparative Cytogenetic 7 (1): 1–9.

Ryan, B. & Joiner, B. L. 2001: Minitab Handbook, 4th edn. Duxbury Press, California, USA.

Shariat, A., Karimzadeh, G. & Assareh, M. H. 2013: Karyology of Iranian endemic *Satureja* (Lamiaceae) species. -Cytologia 78 (3): 305–312.

Shariat, A., Karimzadeh, G., Assareh, M. H. & Zandi_Esfahan, E. 2016: Drought Stress in Iranian Endemic Savory (*Satureja rechingeri*): In vivo and In vitro Studies. -Journal of Plant Physiology and Breeding 2016, 6 (1): 1–13.

Shariat, A., Karimzadeh, G., & Assareh, M. H. and Hadian, J. 2018a: A promising application of drought stress for increasing product quality of Iranian endemic *Satureja sahendica* Bornm, medicinal plant. -Iran. J. Field Crop Sci. 49(1): 167-177.

Shariat, A., Karimzadeh, G., Assareh, M. H., & Hadian, J. 2018b: Metabolite profiling and molecular responses in a drought-tolerant savory, *Satureja rechingeri* exposed to water deficit. -3 Biotech 8(11), 477.

Siljak-Yakovlev, S., Pustahija, F., Åolic, E.M., Bogunic, F., Muratovic, E., BaÅ¡ic, N., Catrice, O. & Brown, S.C. 2010: Towards a genome size and chromosome number database of Balkan flora: C-values in 343 taxa with novel values for 242. -Adv. Sci. Lett. 3 (2):190–213.

Šímová, I. & Herben, T. 2011: Geometrical constraints in the scaling relationships between genome size, cell size and cell cycle length in herbaceous plants. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 279 (1730): 867–875.

Sokal, R. R. & Rohlf, F. J. 2012: Biometry: the principles and practice of statistics in biological research. 4th edition. Freeman, W.H. and Co. New York, USA. 937 pp. ISBN: 0-7167-8604-4 or 978-0-7167-8604-7.

Suda, J., Kron, P., Husband, B. C. & Trávníček, P. 2007: Flow cytometry and ploidy: applications in plant systematics, ecology and evolutionary biology. In: Doležel, J., Greilhuber, J., Suda, J. (eds.). Flow Cytometry with Plant Cells. Analysis of Genes Chromosomes and Genomes. Wiley - VCH, Weinheim, pp. 103–130.

Tavan, M., Mirjalili, M. H. & Karimzadeh, G. 2015: *In vitro* polyploidy induction: changes in morphological, anatomical and phytochemical characteristics of *Thymus persicus* (Lamiaceae). -Plant Cell Tiss. Org. 122 (3): 573–583.

Veselý, P., Bures, P., Smarda, P. & Pavlícek, T. 2012: Genome size and DNA base composition of geophytes: the mirror of phenology and ecology? -Ann. Bot. 109 (1): 65–75.

Vinogradov, A. E. 2003: Selish DNA is maladaptive: evidence from the plant Red List. -Trends Genet. 19 (11): 609–614.