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Chromosome, ploidy analysis, and flow cytometric genome size estimation of *Datura stramonium* and *D. innoxia* medicinal plant

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Abstract. *Datura stramonium* and *D. innoxia* are among the important species of *Datura* genus. They have many uses in traditional and modern medicine. Since Iran is located in the origin area of *Datura*, it is expected that Iranian germplasms are factors of global genetic diversity of *Datura*. Ploidy level, chromosome number and length, and genome size estimation were studied on 15 populations of both *Datura* species mostly collected from different parts of Iran and a few from abroad. For chromosomal preparations, root tip was squashed and stained with 1% (w/v) aceto-orcein. For genome size estimation, flow cytometric analysis was conducted on fresh developed leaves of *Datura* samples along with those of internal standard reference (*Solanum lycopersicum* cv. Stupick, 2C = 1.96 pg DNA), using PI fluorochrome. All the studied populations were diploids ($2n = 2x = 24$). The mean chromosome length in *D. stramonium* and *D. innoxia* was determined as 1.97 µm and 2.39 µm, respectively; the latter had 21% larger chromosomes. The mean monoploid genome size was determined as 3.80 pg in *D. stramonium* (ranged 3.65 pg to 3.93 pg) and 3.91 pg in *D. innoxia* (ranged 3.68 pg to 4.30 pg). The present study provides completely new information about cytogenetics in *D. stramonium* and *D. innoxia* populations from Iran for the first time, which is useful for whole genome sequencing and the construction of genetic and physical maps in the future.

Keywords: chromosome, DNA C-value, monoploid genome size, *Datura,* Iran.

INTRODUCTION

Solanaceae is a large plant family that includes economically species and having still many members cytologically unexplored (Zhang *et al.*, 2023). The genus *Datura* from the Solanaceae family produces various secondary metabolites, for example tropane alkaloids, terpenoids, and glycoalkaloids

to defense against natural enemies such as herbivorous insects, pathogenic agents (bacteria, fungi, viruses) and different abiotic stresses (De-la-Cruz *et al.,* 2021). The classification of *Datura* species is organized into two primary groups. The first group, Ceratocauli, consists solely of the species *D. ceratocaula*. The second group encompasses a variety of other species and is further divided into two sections. The first section includes *D. arenicola*, *D. discolor*, *D. ferox*, *D. kymatocarpa*, *D. leichhardtii*, *D. quercifolia*, and *D. stramonium*, while the second section (termed polyphyletic) comprises *D. innoxia*, *D. lanosa*, *D. metel*, *D. reburra*, and *D. wrightii* (Bye and Sosa, 2013) which are native to North America (De-la-Cruz *et al*., 2021), distributed in subtropical regions of the world (Hassan and Amer, 2019; Papagrigoriou *et al.,* 2019). On the other hand, Karimi (2001) believed that the origin of *D. stramonium* are India and the western shores of the Caspian Sea. Hence, the two species *D. stramonium* and *D. innoxia* are found abundantly in Iran; *D. innoxia* is less distributed than *D. stramonium* in coastal areas, but it is more distributed in the outskirts of cities (Ghahraman, 1998; Muzafarian, 2000). Among the Iranian names of *Datura* weed, Tatore weed, Tatoleh, and Jozmash can be notified (Kirimi, 2001). In which, *D. stramonium* also known as the Thorn Apple, Jimson Weed, and Angel's Trumpet (Disel *et al.,* 2016). Both *D. stramonium* and *D. innoxia* are important species of *Datura* genus (Batool *et al.,* 2020; Al-Zharani *et al.,* 2021), having several traditional and modern medicinal uses (Mohammed *et al.,* 2021). Morovati *et al*. (2023) showed that the essential oil of the aerial parts of *D. stramonium* is rich in monoterpenoid derivatives such as camphor and borneol, which are widely used as therapeutic agents against the proliferation of cancer cells for the treatment of neurological and antiviral disorders (Salakhutdinov *et al.,* 2017).

Genome size, chromosome number and structure changes play an important role in speciation events, adaptation and the development of new genetic networks during evolution (Pellestor and Gatinois, 2020; Winterfeld *et al.,* 2020). Accordingly, analysis and chromosome observation and genome size estimates, elucidate phylogenetic relationships, structure, function, organization, and evolution (Amosova *et al.,* 2019). Such cytogenetic studies may be useful in establishing systematic and evolutionary relationships, resolving taxonomic ambiguities, and achieving a better understanding of the branching pattern of *Datura* genera (Dobigny *et al.,* 2004; Knight *et al.,* 2005; Bancheva and Greilhuber, 2006; Guerra, 2008; Bainard *et al.,* 2013). Hence, for those reasons, many studies are conducted to genome size estimates and chromosomes studies (Burchardt *et al.,* 2020).

Variation of chromosome number in the *Datura* genus can indicate intra- and inter-specific differences in genomic DNA quantities and also, variation of intra/interspecific genome size may reflect karyotypic differences, such as differences in the case of chromosome number and size (Bennett *et al.,* 2008). Previously, Blakeslee (1921) reported various chromosome number in *D. stramonium* as $2n$ = 12, 25, 26, 36, and 48 in the USA, but in recent years Hassan and Amer (2019) stated that the commonly chromosome number in this species was $2n$ = 24. Confirming the latter report, Badr *et al.* (1997) verified the chromosome base number in *D. innoxia* and *D. stramonium* as $n = x = 12$. Moreover, recently, Sadeghian and Hatami (2022) clarified that *D. innoxia* is diploid with $2n$ = 24. Monoploid genome size (1Cx-value) as the amount of DNA of one basic chromosome set (with chromosome base number x), regardless of the degree of generative polyploidy, aneuploidies, etc. (Greilhuber *et al.,* 2005; Karimzadeh *et al.,* 2011; Abedi *et al.,* 2015).

In previous study, the 2C DNA of *D. stramonium* was reported as 4.18 pg. (Kubešova *et al.,* 2010). Also, in the report of Bennett and Smith (1976) who evaluated the absolute amounts of nuclear DNA for 753 species of angiosperms, using Feulgen microdensitometry. The 2C DNA of *D. innoxia* was reported as 4.60 pg (Bennett and Smith, 1976). Due to shortcomings in some of the used cytogenetic techniques and lack of access to detailed information on DNA C value, karyology, and ploidy levels of *Datura* genus and since Iran is located in the center of the origin of diversity, so it is expected that Iranian *Datura* germplasm indicates much of the worldwide genetic diversity of *Datura*. On the other hand, there is no reliable report regarding the number of chromosomes and genome size regarding *Datura* genus in Iran. Thus, reliable conclusions cannot be drawn on the actual range of chromosomal variation in *Datura* without considering the Iranian germplasm. Hence, the current study, for the first time, was aimed to provide a detailed survey of chromosomal and genome size variation in the Iranian *D. stramonium* and *D. innoxia* by focusing on populations that were not studied before. For this purpose, several Iranian populations of *D. stramonium* and *D. innoxia* were investigated.

MATERIALS AND METHODS

Seed collection site

The seeds of 13 Iranian endemic populations of *Datura stramonium* (9 populations) and *D. innoxia* (4 populations) were collected from different sites of Iran

Table 1. Locality collection characteristics of *D. stramonium* and *D. innoxia.*

Altitude (m)	Latitude (N)	Longitude (E)	Local Collection locations	Population codes
1723	35°43'57"	53°37'49"	Semnan, Semnan, Iran	S1P1
1612	32°36'12"	51°26'01"	Isfahan, Isfahan, Iran	S1P2
120	39°29'18"	48°07'49"	Mughan plain, Ardabil, Iran	S1P3
1500	36°42'15"	48°21'31"	Zanjanrood, Zanjan, Iran	S1P4
1362	36°26'17"	45°56'43"	West Azerbaijan, Iran	S1P5
65	38°06'44"	41°07'33"	Saravan, Gilan, Iran	S1P6
30	33°95'04"	41°55'89"	Venous Rezvanshahr, Gilan, Iran	S1P7
1505	29°34'80"	52°35'26"	Shiraz, Fars, Iran	S1P8
1880	11°16'46"	51°49'27"	RuBland (RUS)	S1P9
1880	11°16'46"	51°49'27"	Brasitieh (BRA)	S1P10
1269	35°44'17"	51°10'23"	Tehran, Tehran, Iran	S1P11
1800	37°12'11"	44°52'21"	Turgor, Urmia, Iran	S2P1
1914	34°27'00"	46°80'76"	Mahidasht, Kermanshah, Iran	S2P2
981	34°35'17"	50°49'02"	Qom, Qom, Iran	S2P3
838	29°49'17"	51°33'48"	Kazerun, Fars, Iran	S2P4

S1: *Datura stramonium*, S2: *Datura innoxia.*

during the October and November of 2021, also, two populations (P9, P10) of *Datura stramonium* species were prepared from Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany. The species code and geographical descriptions, including latitude, longitude, and altitude are shown in Table 1 and Figure 1.

Figure 1. Location of the sampling sites of 13 Iranian endemic *Datura* populations on the map of Iran.

Plant material and growing conditions for genome size estimation

For study the genome size, the collected seeds were planted in grow bags with 10 kg soil (sandy loam) and placed for three months in greenhouse of agricultural faculty of Tarbiat Modares University in Tehran. Under greenhouse conditions, average air temperature was 25 °C. Finally, the developed leaves at the four-leaf stage were collected to determine their genome size.

Flow cytometric genome size estimation

The 2C-value of each *Datura* species was determined by flow cytometric analyses. FCM (Flow cytometric) analysis was carried out by PI (Propidium Iodide) staining technique and *Solanum lycopersicum* cv. Stupicke; 2C = 1.96 pg DNA (Doležel *et al.,* 1998) as an internal reference standard plant (Figure 2). About 2 cm^2 of healthy fresh young leaves of *Datura* and internal reference standard were co-chopped with a sharp razor blade in a glass petri dish, containing one ml of ice-cold WPB buffer (Woody Plant Buffer, Loureiro *et al.,* 2007). The crude nuclei suspension was filtered through a 30 µm green nylon mesh (Partec, Münster, Germany). Then RNase (Sigma-Aldrich Corporation, MO, USA) and propidium iodide (PI; ach 50 µg ml^{-1}) was added. For the resulting sample, the relative fluorescence intensity was calculated. After incubation for two min at RT, to determine the amount of genomic 2C DNA, the nuclei suspension was examined by BD FACSCantoTM-KE flow cytometer (BD Biosciences, Bedford, MA, USA), equipped with an

Figure 2. Two species of *Datura* in grow bags in greenhouse (a). *Datura innoxia*, (b) *D. stramonium* (c). *Solanum lycopersicum* cv. Stupicke (2C = 1.96 pg DNA) the internal reference standard plant (d).

argon ion laser (488 nm) via BD FACSDivaTM software. At least 5,000 nuclei were typically analyzed for each sample in three replications (Sayadi *et al.,* 2022; Zarabizadeh *et al.,* 2022). For create a histograms, the range of gating zone was calculated by using the Partec FloMax ver. 2.4e. (Partec, Münster, Germany). The measurements of relative fluorescence intensity of stained nuclei were performed on a linear scale. By calculating the values of the means of G1 peak, the absolute DNA amount of each sample was estimated (Doležel *et al.,* 2003, 2007; Greilhuber *et al.,* 2005; Karimzadeh *et al.,* 2011) as follows:

Sample 2Cx DNA (pg) = (Sample G1 peak mean/Standard G1 peak mean) × Standard 2C DNA (pg).

Value was calculated based on a conversion formula where 1 pg of DNA represents 978 Mbp (Doležel *et al.,* 2003).

Chromosome analysis

Initially, the scraped seeds were placed in Petri dishes with sandpaper and germinated on moist filter paper at 20 - 25 °C under light conditions in a growth chamber. For the cytological preparations, each root tip (0.5 - 1 cm long) was removed and pretreated with 0.002 M 8- hydroxyquinoline at 25 °C for 2.5 h in the dark to induce cell cycle delay in metaphase. The roots were washed by distilled H_2O in several times and fixed in 3:1 (v/v) of ethanol and glacial acetic acid (Carnoy solution) at 4 °C for 17 h. The fixed roots were washed in distilled H_2O, hydrolyzed in 1 M HC1 at 60 °C (11 min for *D. stramonium* and 13 min for *D. innoxia*) in a water bath, and washed in water, then stained by aceto-orcein 1% (w/v) at 25 °C (50 min for *D. stramonium* and 60 min for *D. innoxia*) in darkness (Reference). Finally, for microscopic studies, the five root tips from different individuals were squashed in a drop of 45% (v/v) acetic acid and analyzed per *Datura* populations. Slides were examined and High-resolution microscopic digital photographs (Super High Quality; SHQ; Tiff format images) were acquired, using an Olympus BX50 (Olympus Optical Co., Ltd., Tokyo, Japan) microscope equipped with an Olympus DP12 digital camera. It is reminded that each replicate is a cell from the meristem of the plant and five slides from the terminal meristem of five different plants were prepared from each population.

Statistical analysis

The karyotypic and flow cytometric data was analyzed according to analysis of variance based on a completely randomized design with five and three replications, using SAS Statistical Package Program version 9.0 and SPSS software version 20. The PROC UNIVARIATE within SAS was used to test the assumptions of *ANOVA*, and residuals were normally distributed. The means were compared through the least significant difference (LSD) posthoc test at the 5% probability level. Moreover, the standard errors of the means were calculated.

RESULTS

Chromosome counts and length and ploidy level

Figures 3 show the somatic complement karyotypes in the 15 *D. stramonium* and *D. innoxia* populations. All cells studied of the examined *Datura* populations consistently had ploidy levels and chromosome number of $2n = 2x = 24$ were with small chromosomes. Based on *ANOVA* results, among populations *D. stramonium* for Chromosome length (CL), were significant differences ($P < 0.05$; Table 2). The mean chromosome length (CL) was determined as 1.966 µm, varied from 1.627 µm (S1P2) to 2.286 µm (S1P8, Table 3). On the other hand, the ANOVA results, among populations *D. innoxia* verified significant differences ($P < 0.01$; Table 2) in Chromosome length (CL). The highest and the least values of Chromosome length (CL) in S2P1 (2.819 µm) and S2P2 (1.967

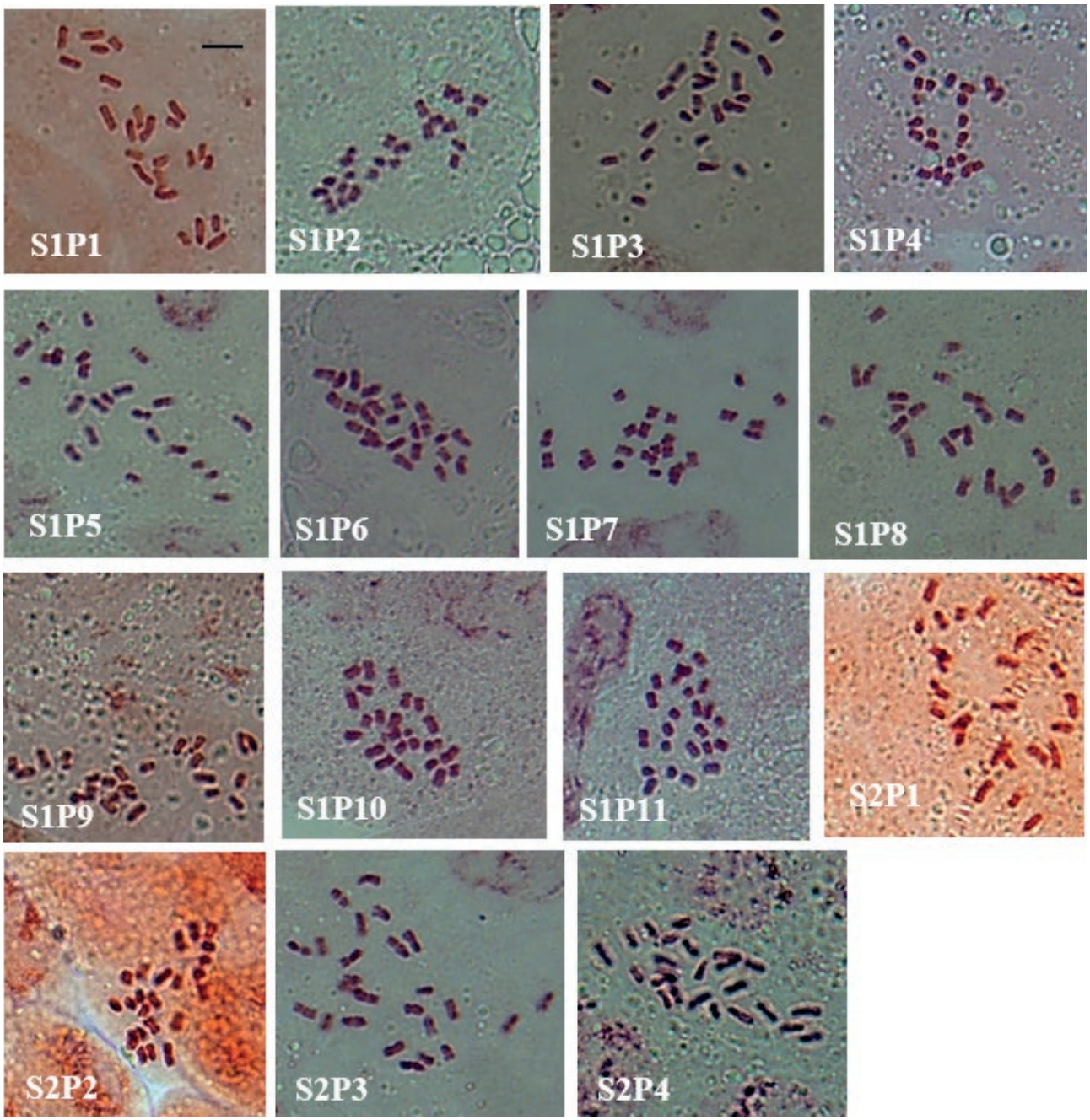

Figure 3. Somatic chromosomes ($2n = 2x = 24$) of 11 *Datura stramonium* populations and four *D. innoxia* populations. Scale bar = 5 μm.

μm), respectively and the mean Chromosome length in this species was 2.388 μm (Table 3).

Flow cytometric analysis of monoploid genome size

The nuclear DNA values of 15 populations of two species of *Datura* genus were estimated by flow cytometry. In the process of estimating the DNA content of the nuclei in the leaf tissue, two peaks were observed in the obtained histograms. In all populations under study, the left peak corresponds to the *Solanum lycopersicum* cv. Stupicke (2C value = 1.96 pg DNA) internal reference standard plant, and the right peaks refer to the *Datura* populations (Figures 4). Based on the *ANOVA* results (Table 4), no significant difference in the comparison

Table 2. *ANOVA* of chromosome length (µm) of *Datura stramonium* and *D. innoxia* populations.

S.O.V.	*D. stramonium*		*D. innoxia*	
	df	MS	df	MS
Population	10	0.17039*	3	0.6176**
Error	44	0.06506	8	0.1081
Total	54		11	
CV%		12.97		13.77

*Significant (P<0.05); **Significant (P<0.01).

Table 4. *ANOVA* of monoploid genome size (2Cx DNA, pg) of *Datura stramonium* and *D. innoxia* populations.

S.O.V.	*D. stramonium*		*D. innoxia*	
	df	MS	df	MS
Population	10	0.03099ns	3	0.25142*
Error	44	0.05217	8	0.04042
Total	54		11	
CV%		6.0		5.12

ns non-significant (P>0.05); * Significant (P<0.05)..

Table 3. Means (±SE) and the range comparisons of chromosome length (µm) of *Datura stramonium* and *D. innoxia* populations.

Population codes	CL (µm) *D. stramonium*	Population codes	CL (µm) *D. innoxia*
S1P1	2.032 ± 0.212abc	S2P1	2.819 ± 0.076a
S1P2	1.627 ± 0.071d	S2P2	1.967 ± 0.142b
S1P3	2.133 ± 0.132ab	S2P3	2.323 ± 0.119ab
S1P4	1.757 ± 0.061cd	S2P4	2.441 ± 0.216ab
S1P5	1.883 ± 0.086bcd	---	---
S1P6	2.006 ± 0.100abc	---	---
S1P7	2.005 ± 0.092abc	---	---
S1P8	2.286 ± 0.119a	---	---
S1P9	1.985 ± 0.068abc	---	---
S1P10	2.092 ± 0.160ab	---	---
S1P11	1.820 ± 0.040bcd	---	---
Mean	1.966		2.3876
Range	1.627-2.286		1.967-2.819
$LSD_{5\%}$	0.325		0.607

CL: chromosome length (µm), S1: *Datura stramonium*, S2: *D. innoxia.*

Table 5. Means (±SE) and the range monoploid genome size (DNA 2Cx value, pg) of *D. innoxia* populations.

Population codes	2Cx genome size (pg)	1Cx genome size (pg)	1Cx genome size (Mbp)
S2P1	4.305 ± 0.032a	2.152	2104.66
S2P2	3.682 ± 0.122b	1.841	1800.50
S2P3	3.708 ± 0.119b	1.854	1813.21
S2P4	3.963 ± 0.155ab	1.982	1938.40
Mean	3.914	1.957	1914.19
Range	3.682-4.305	1.841-2.152	1800.50-2104.66
$LSD_{5\%}$	0.377		

of genome size among populations *D. stramonium* was observed. However, the mean monoploid genome size was determined as 3.8 pg, varied from 3.650 pg (S1P5) to 3.934 pg (S1P4). Also, the *ANOVA* results, between four populations *D. innoxia* verified significant differences in genome size (Table 4). The mean genome size (Table 5) was determined as 3.91 pg, varied from 3.682 pg (S2P2) to 4.305 pg (S2P1).

DISCUSSION

Fifteen *Datura* populations we studied, among which nine populations of *Datura stramonium* and four populations of *D. innoxia* were of Iranian endemic origin. The results of the current study, which were used to examine karyotype diversity and estimate genome size from the new and unworked populations of *Datura* plant, are being reported for the first time in the world. Our results provide basic cytogenetic information for these two species, which are helpful for the whole-genome sequencing and the construction of genetic and physical maps in the future. Cytogenetic investigations carried out on the populations of *Datura stramonium* and *D. innoxia* showed that all the studied populations were diploid with chromosome number of 24, which was completely consistent with the results of previous reports (Badr *et al.,* 1997; Hassan and Amer, 2019; Sadeghian and Hatami, 2022). Because of short chromosomes' lengths, the locations of the centromeres could not be identified clearly, hence chromosome length (CL) parameter was measured, as reported for different species by researchers (e.g. Morales Valverde, 1986; Karimzadeh *et al*., 2010; Abbasi-Karin *et al*., 2022; Rasekh and Karimzadeh, 2023, Yari *et al*., 2024). According to the results of the current study, the mean chromosome length (CL) in *D. stramonium* and *D. innoxia* populations was 1.97 µm and 2.39 µm, respectively. In other words, *D. innoxia* populations had 21% larger chromosomes. Moreover, in the present study, the leaf materials were used for the estimation of genome size, using

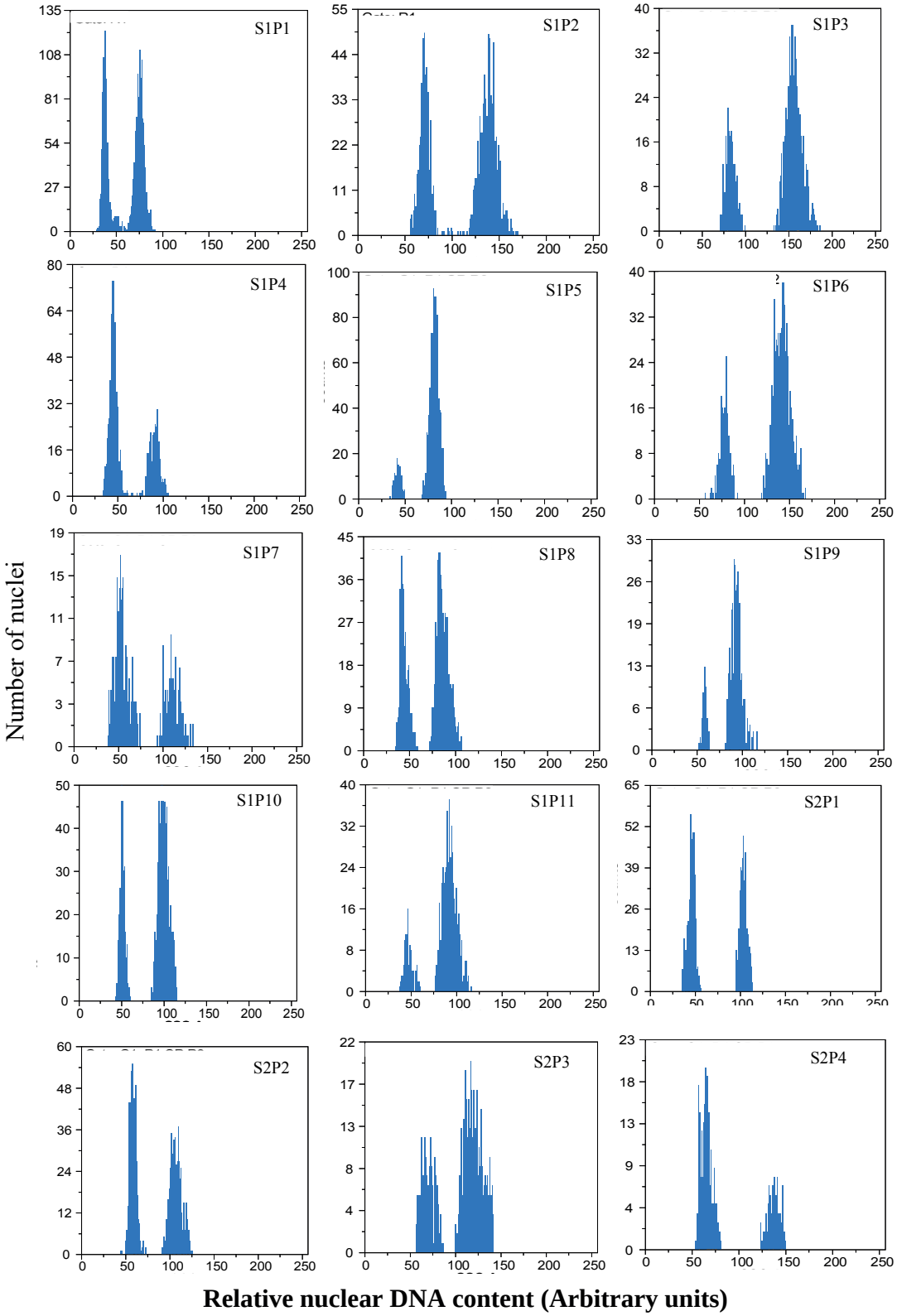

Figure 4. Histograms of monoploid genome size (2Cx DNA content) of *Datura stramonium* and *D. innoxia* populations. The left peaks refer to the G1 peaks of *Solanum lycopersicum* cv. Stupicke (2C = 1.96 pg DNA) as an internal reference standard plant and the right peaks refer to the G1 peaks of the samples.

flow cytometric analysis (Mohammadpour *et al.,* 2022; Rasekh and Karimzadeh, 2023; Yari *et al.,* 2024). The mean monoploid genome size in *D. stramonium* and *D. innoxia* populations was 3.799 pg and 3.914 pg, respectively. The cytogenetic information obtained from this research is more than the mean chromosome length and the mean genome size reported in the previous studies (Bennett and Smith *et al.,* 1976; Badr *et al.,* 1997). The reason for this is unknown, but this difference could be related to the cell cycle, the rate of cell division, ecological behavior in plant communities and life forms, and differences between the methods of nuclear DNA content analysis (Bennett *et al.,* 2000). On the other hand, previous studies have only been conducted on one population. In general, it can be concluded that the average chromosome length and average monoploid genome size in *D. innoxia* species are 0.40 and 0.11 times higher than those in *D. stramonium* species, respectively.

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REFERENCES

Abbasi-Karin Sh, Karimzadeh G, and Mohammadi-Bazargani M. 2022. Interspecific chromosomal and genome size variations in *in vitro* propagated willow herb (*Epilobium* spp.) medicinal plant. Cytologia 87(2): 129-135.

Abedi R, Babaei A, and Karimzadeh G. 2015. Karyological and flow cytometric studies of Tulipa (Liliaceae) species from Iran. Plant Syst. Evol. l301: 1473-1484.

Al-Zharani M, Nasr FA, Alqahtani AS, Cordero MAW, Alotaibi AA, Bepari A, Alarifi S, Daoud A, Barnawi IO, and Daradka HM. 2021. *In vitro* cytotoxic evaluation and apoptotic effects of *Datura innoxia* grown in Saudi Arabia and phytochemical analysis. Appl. Sci. 11(6): 2864.

Amosova AV, Zoshchuk SA, Rodionov AV, Ghukasyan L, Samatadze TE, Punina EO, Loskutov IG, Yurkevich OY, and Muravenko OV. 2019. Molecular cytogenetics of valuable Arctic and sub-Arctic pasture grass species from the Aveneae/Poeae tribe complex (Poaceae). BMC Genetic 20(1): 1-16.

Badr A, Khalifa SF, Aboel-Atta AI, and Abou-ElEnain MM. 1997: Chromosomal criteria and taxonomic relationships in the Solanaceae. Cytologia 62(2): 103-113.

Bainard JD, Forrest LL, Goffinet B, and Newmaster SG. 2013. Nuclear DNA content variation and evolution in liverworts. Mol. Phylogenet. Evol. 68: 619-627.

Blakeslee AF. 1921. Types of mutations and their possible significance in evolution. Am Nat. 55: 254-267.

Bancheva S and Greilhuber J. 2006. Genome size in Bulgarian Centaurea s.l. (Asteraceae). Plant Syst. Evol. 257: 95-117.

Batool A, Batool Z, Qureshi R, and Raja NI. 2020. Phytochemicals, pharmacological properties and biotechnological aspects of a highly medicinal plant: *Datura stramonium*. J. Plant Sci., 8(2): 29-40.

Bennett MD, Bhandol P, and Leitch I J. 2000. Nuclear DNA amounts in angiosperms and their modern uses- 807 new estimates. Ann. Bot. 86: 859-909.

Bennett MD and Smith JB. 1976. Nuclear DNA amounts in angiosperms. Philosophical Transactions of the Royal Society B-Biological Sciences 274: Issue 933.

Bennett MD, Price HJ, and Johnston JS. 2008. Anthocyanin inhibits propidium iodide DNA fluorescence in Euphorbia pulcherrima: implications for genome size variation and flow cytometry. Ann. Bot. 101: 777-790.

Burchardt P, Buddenhagen CE, Gaeta ML, Souza MD, Marques A, and Vanzela ALL. 2020. Holocentric karyotype evolution in *Rhynchospora* is marked by intense numerical, structural, and genome size changes. Front. Plant Sci. 11: 1390.

Bye R, and Sosa V. 2013. Molecular phylogeny of the jimsonweed genus *Datura* (Solanaceae). Systematic Botany, 38(3): 818-829.

De-la-Cruz IM, Hallab A, Olivares-Pinto U, Tapia-López R, Velázquez-Márquez S, Piñero D, Oyama K, Usadel B, and Núñez-Farfán J. 2021. Genomic signatures of the evolution of defense against its natural enemies in the poisonous and medicinal plant *Datura stramonium* (Solanaceae). Sci. Rep. 11(1): 1-19.

Disel NR, Yılmaz M, Kekec Z, and Karanlık M. 2016. Poisoned after diner: Dolma with *Datura stramonium*. Turkish Journal of Emergency Medicine, 15(1): 51-55.

Dobigny G, Ducroz JF, Robinson TJ, and Volobouev V. 2004. Cytogenetics and cladistics. Syst. Biol., 53: 470-484.

Doležel J, Bartos J, Voglmayr H, and Greilhuber J. 2003. Nuclear DNA content and genome size of trout and human. Cytometry 51: 127-128.

Doležel J, Greilhuber J, Lucretti S, Meister A, Lysak M. A, Nardi L, and Obermayer R. 1998. Plant genome size

estimation by flow cytometry: Inter-laboratory comparison. Ann. Bot., 82(Suppl. A): 17-26.

Doležel J, Greilhuber J, Suda J. 2007. Estimation of nuclear DNA content in plants using flow cytometry. Nat. Protoc., 2: 2233-2244.

Ghahraman A. 1998. Flora of Iran. Research Institute of Forests Rangelands. Tehran, Iran, pp. 17. (In Persian)

Greilhuber J, Doležel J, Lysák MA, and Bennett MD. 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.

Guerra M. 2008. Chromosome numbers in plant cytotaxonomy: concepts and implications. Cytogenet. Genome. Res. 120: 339-350.

Hassan RA and Amer WM. 2019. Biosystematic study of the Egyptian *Datura stramonium* (Solanaceae). Phytotaxa 408(3): 178-194.

Karimi H. 2008. Weeds of Iran. Iran University Press, Tehran, Iran, 419 p. (In Persian).

Karimzadeh G, Danesh-Gilevaei M, and Aghaalikhani M. 2011. Karyotypic and nuclear DNA variations in *Lathyrus sativus* (Fabaceae). Caryologia 64: 42-54.

Karimzadeh G, Mousavi SH., Jafarkhani-Kermani M, and Jalali-Javaran M. 2010. Karyological and nuclear DNA variation in Iranian endemic muskmelon (*Cucumis melo* var. Inodorus). Cytologia 75: 451-461.

Knight CA, Molinari NA, and Petrov DA. 2005. The large genome constraint hypothesis: evolution, ecology and phenotype. Ann. Bot. 95: 177-190.

Kubešova M, Moravcova L, Suda J, Jarošik V, and Pyšek P. 2010. Naturalized plants have smaller genomes than their non-invading relatives: a flow cytometric analysis of the Czech alien flora. Preslia 82(1): 81-96.

Loureiro J, Rodriguez E, Doležel J, and Santos C. 2007. Two new nuclear isolation buffers for plant DNA flow cytometry: a test with 37 species. Ann. Bot. 100: 875-888.

Mohammadpour S, Karimzadeh G, and Ghaffari SM. 2022. Karyomorphology, genome size, and variation of antioxidant in twelve berry species from Iran. Caryologia 75(4): 133-148.

Mohammed FS, Kına E, Sevindik M, Dogan M, and Pehlivan M. 2021. *Datura stramonium* (Solanaceae): Antioxidant and antimicrobial potentials. Turk. J. Agric. Food Sci. Technol. 9(4): 818-821.

Morales Valverde R. 1986. Taxonomía De Los Géneros *Thymus* (Excluida De La Sección Serpyllum) Y Thymbra En La Península Ibérica). CSIC - Real Jardín Botánico (RJB), Ruizia. Monografias del Jardín Botánico 3: 324 p.

Muzafarian V. 2000. Plant Classification. Amirkabir Publications, Tehran, Iran, pp. 393 (In Persian).

Papagrigoriou G, Papazoglou D, Lazari D, Zorić L, and Tsialtas J. 2019. Hybridization effects on seed traits of annual *Datura* accessions focusing on oil concentration and composition. Ind. Crops Prod. 132: 69-75.

Pellestor F and Gatinois V. 2020. Chromoanagenesis: A piece of the macroevolution scenario. Mol. Cytogenet. 13: 3.

Rasekh SZ and Karimzadeh G. 2023. Chromosomal and genome size variations in opium poppy (*Papaver somniferum* L.) from Afghanistan. Caryologia 76(4): 15-22.

Sadeghian S and Hatami A. 2022. Chromosome number reports and karyotype analysis of seven species from the flora of Iran. Iran. J. Bot. 28(2): 165-169.

Salakhutdinov NF, Volcho KP, and Yarovaya OI. 2017. Monoterpenes as a renewable source of biologically active compounds. Pure. Appl. Chem. 89(8): 1105-1117.

Sayadi V, Karimzadeh G, Naghavi MR, and Rashidi Monfared S. 2022. Interspecific genome size variation of Iranian endemic *Allium* species (Amaryllidaceae). Cytologia 87(4): 335-338.

Winterfeld G, Ley A, Hoffmann MH, Paule J, and Röser M. 2020. Dysploidy and polyploidy trigger strong variation of chromosome numbers in the prayer-plant family (Marantaceae). Plant Syst. Evol. 306: 36.

Zarabizadeh H, Karimzadeh G, Rashidi Monfared S, and Tarkesh Esfahani S. 2022. Karyomorphology, ploidy analysis, and flow cytometric genome size estimation of *Medicago monantha* populations. Turk. J. Bot. 46: 50-61.

Zhang Y, Guo W, Yuan Z, Song Z, Wang Z, Gao J, Fu W, and Zhang G. 2023. Chromosome-level genome assembly and annotation of the prickly nightshade *Solanum rostratum* Dunal. Sci. Data 10: 341.

Yari A, Karimzadeh G, Rashidi Monfared S, and Sayadi S. 2024. Mixed-ploidy in Iranian endemic *Cymbopogon olivieri* (Boiss.) Bor: A chromosomal and holoploid genome size study. Cytologia 89(2): 1-5.