Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: Rasekh, S.Z., & Karimzadeh, G. (2023). Chromosomal and genome size variations in Opium poppy (*Papaver somniferum* L.) from Afghanistan. *Caryologia* 76(4): 15-22. doi: 10.36253/caryologia-1955

Received: December 25, 2022

Accepted: December 18, 2023

Published: March 14, 2024

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Chromosomal and genome size variations in Opium poppy (*Papaver somniferum* L.) from Afghanistan

Sayed Zia Rasekh, Ghasem Karimzadeh*

Department of Plant Genetics and Breeding, College of Agriculture, Tarbiat Modares University, Tehran P. O. Box 14115-336, Iran
*Corresponding author. E-mail: karimzadeh_g@modares.ac.ir

Abstract. The genus *Papaver* classified in the Papaveraceae family, is a valuable, non-alternative medicinal plant which has illustrated a massive variety of pharmacologically important alkaloids. Chromosomal and monoploid genome size diversity of seven populations collected from different districts of Balkh Province in northern parts of Afghanistan were studied. All populations were diploid, six of which (P1-P6) had 22 chromosomes, while P7 had 20 larger chromosomes. The mean chromosome length (CL) of P1-P6 populations was 1.32 µm (0.91-1.74 µm), but that of P7 population was 2.24 µm. The results of flow cytometric analysis showed that the mean monoploid 2Cx DNA of P1-P6 populations was 5.701 pg (5.574-5.901 pg), whereas that of P7 population was 5.795 pg, confirming intraspecific variation. This study is being reported for the first time from the northern part of Afghanistan's opium cultivation area, and P7 population is also being reported for the first time in terms of chromosome number. Valuable information on Cytogenetics can be used in some research fields, including polygenetic analysis, taxonomic relationships, evolutionary characteristics, and plant breeding.

Keywords: *Papaver somniferum*, chromosome, monoploid genome size, 2Cx DNA, flow cytometry, Balkh province, Afghanistan.

1. INTRODUCTION

Opium poppy (*Papaver somniferum* L., $2n = 2x = 22$) is one of the oldest cultivated medicinal plants that has been used for thousands of years (Askitopoulou *et al*., 2002; Vu *et al*., 2022). Its origin and domestication is not clear, but archeological findings and references prove that the Mediterranean is the origin of the poppy plant from the middle of the 6th millennium BC (Askitopoulou *et al*., 2002; Salavert *et al*., 2018; Vu *et al*., 2021; Jesus *et al*., 2021). Recently, poppy has been cultivated, both as a licit and illicit crop, in Asia, Europe, Oceania and South America as a main source of benzylisoquinoline alkaloids (BIAs) (Askitopoulou *et al*., 2002; Beaudoin and Facchini, 2014; Guo *et al*., 2018; Vu *et al*., 2021). The word poppy has been used for many species of the Papaveraceae family, while opium word has been used for the air-dried latex extraction obtained from *Papaver somniferum* L. cap-

sules, one of the most useful plant species belonging to this family (Labanca *et al*., 2018). Opium poppy is one of the non-alternative sources of morphine, codeine, noscapine (Khan *et al*., 2011), and semisynthetic derivatives, including oxycodone and naltrexone (Carlin *et al*., 2020; Pei *et al*., 2021). Morphinan-based sedatives are obtained from opium poppy (Guo *et al*., 2018). There are about 600 species in the Papaveraceae. The majority of those are cultivated in gardens and their karyotypes are studied. In comparison, the meiotic chromosome numbers differ from 7 to 11(Sugiura, 1939). According to the latter report, the first karyological studies of Papaveraceae were done by Nemec (1910) on *Corydalis pumila* and next Tahara on *Papaver rhoeas, oriental*, and *somniferum*. Afterwards, Yasui (1921) and Ljungdahl (1922) reported that *Papaver* species are the chief studies in the Papaveraceae family. From which, meiotic karyotype studies of *Papaver somniferum* revealed that all populations were diploid with 11 chromosomes (Kaul *et al*., 1979; Rezaei *et al*., 2014). Estimation of *Papaver somniferum* genome size by flow cytometry is an easy and rapid technique that allows accurate value of nuclear DNA content (Kyrylenko *et al*., 2005). Some studies were carried out on the Papaveraceae family for genome size estimation, e.g. it was reported that the genome size of *P. somniferum* was 6.46 pg (Kyrylenko *et al*., 2005) and this amount for *P. bracteatum* was 6.15 pg (Tarkesh Esfahani *et al*., 2016). Hence, the key objective of the current study was to investigate the chromosomal and genome size variations in seven populations of opium poppy (*Papaver somniferum* L.) medicinal plant.

2. MATERIALS AND METHODS

2.1. Plant materials

The ripped seeded capsules of seven populations of *Papaver somniferum* were collected from seven different districts of Balkh Province, Afghanistan in 2020. Seeds were provided from mature capsules and the seeds of each individual plant were collected separately and kept in small plastic bags. The characteristics of the local information of the collected sites and geographic distribution are presented in Table 1 and Figure 1.

Table 1. Collected sites locations of *Papaver somniferum* populations of Northern part of Afghanistan in this study.

Population codes	Locality	Latitude (N)	Longitude (E)	Altitude (m)
P1	Chahar Kint, Balkh	36°20'33.43"	67°31'23.38"	1822
P2	Balkh, Balkh	36°45'54.62"	66°53'19.24"	341
P3	Kishindeh, Balkh	35°48'11.83"	67°05'38.92"	1862
P4	Chimtal, Balkh	36°28'27.42"	66°57'3.47"	553
P5	Khulm, Balkh	36°42'50.09"	66°57'7.92"	365
P6	Sholgara, Balkh	36°22'12.24"	66°53'29.67"	551
P7	Chahar Bolak, Balkh	36°48'5.64"	66°57'3.68"	335

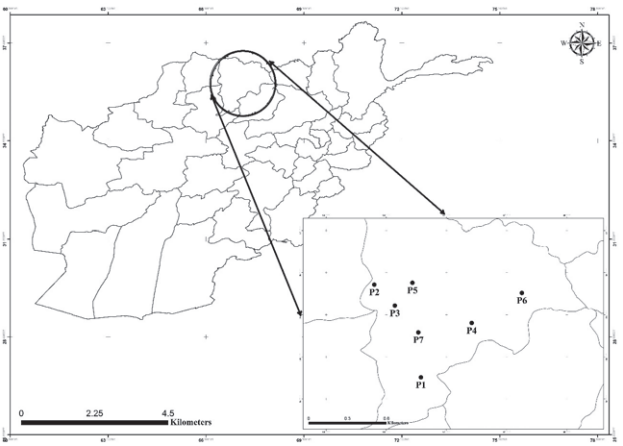

Figure 1. Geographic distribution of sampled *Papaver somniferum* on the map of Afghanistan using ArcGIS.

2.2. Cytological preparation

Seeds of seven *Papaver somniferum* L. populations were germinated on thick layers of petri paper in a glass petri dish at 23 ± 2 °C (Kaul *et al*., 1979; Tarkesh Esfahani *et al*., 2020). Seeds were sterilized by immersing in 70% (v/v) ethanol three times and 30s each time, followed by sodium hypochlorite 5% (v/v) for 6 min, and rinsed by distilled water for 3 times. The sterilized seeds were then transferred to two layers of moisturized filter paper in glass petri dishes and irrigated regularly by distilled water until germination (Tarkesh Esfahani *et al*., 2016). The seeds started germinating after 72 h in 16 h light and 8 h dark conditions at 23 ± 2 °C. Since pretreatment is necessary (Rezaei *et al*., 2014), so the 2 cm-long roots were first cold pretreated for 1.5 h at 4 °C, followed by chemical pretreatment in 0.05 M aqueous colchicine solution for 2.5 h (Ahmadi-Roshan *et al*., 2016). They were then fixed in acetic acid: alcohol (1:3) for 1 h. The roots were hydrolyzed for 15 min in 1M HCl, followed by staining with aceto-orcein 2% (w/v) for 2 h (Chowa *et al*., 2020; Sayadi *et al*., 2021; Najafi *et al*., 2022). Squash method was applied at 45% HCl (v/v) for preparing slides. Photomicrographs were captured by a DP12 digital camera (Olympus Optical Corporation, Tokyo, Japan) appointed to a BX50 Olympus microscope (Olympus Optical Corporation, Ltd., Tokyo, Japan).

Figure 2. Growing stage of seven population of *Papaver somniferum* in plate culture after 70 d (a). Transfer of every single population in separate pots in age 95 d (b). Growing stage of *Solanum lycopersicum* cv. Stupicke (2C = 1.96 pg DNA) the reference standard plant (c).

The chromosome length (CL) was measured using MicroMeasure software version 3.3.

To estimate the genome size, the seeds were cultured in a plate culture, containing sterile perlite and coco-peat under room temperature conditions. After two months, the grown plants having four developed leaves were transferred to separate pots. One cm^2 of young and well developed leaves of both *Papaver somniferum* plants and *Solanum lycopersicum* cv. Stupicke; 2C = 1.96 pg DNA (Doležel *et al*., 1998) as an internal reference standard plant were chopped simultaneously by a sharp razor blade in a glass petri dish, containing one ml of Woody Plant Buffer (WPB) (Loureiro *et al*., 2007; Tarkesh Esfahani *et al*., 2020; Sayadi *et al*., 2022). The resultant nuclear suspension was filtered through a green Partec 30 µm-nylon mesh (Partec, Munster, Germany), followed by treating with 50 µg ml^{-1} RNase (Sigma-Aldrich Corporation, MO, USA) and 50 µg ml^{-1} Propidium Iodide (PI, Fluka) as DNA staining agent, and then incubated for 2 min at room temperature. To determine the nuclear monoploid 2Cx DNA, the nuclei suspension was analyzed by a BD FACSCanto II flow cytometer (BD Biosciences, Bedford, MA, USA), using BD FACSDivaTM Software. Output data were then transferred to FloMax Software for Partec Flow Cytometer 2.4.1. The measurements of relative fluorescence intensity of stained nuclei were performed on a linear scale, analyzing at least 5,000 nuclei for each sample. The absolute DNA amount of a sample was calculated based on the values of the G1 peak means (Doležel *et al*., 1998; Bennett *et al*., 2000; Brown and Wittwer, 2000; Loureiro *et al*., 2007; Abedi *et al*., 2015; Tarkesh Esfahani *et al*., 2020; Abbasi-Karin *et al*., 2022; Sayadi *et al*., 2022) as follows:

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

Table 2. ANOVA of chromosome length (CL) and monoploid genome size (2Cx DNA; pg) of *Papaver somniferum* Populations.

S.O.V.	Df	MS CL	Df	MS 2C DNA (pg)
Population	6	36.215**	6	0.04174ns
Error	373	0.426	14	0.02597
Total	379		20	
CV%		21.7		8.93

ns Non significant difference ($P > 0.05$); ** significant difference ($P < 0.01$)

2.3. Statistical analyses

The normality test was first applied to chromosome length (CL) and genome size data, followed by *ANOVA*, using a completely randomized design (CRD) with five and three replications, respectively. The least significant difference (LSD) mean comparisons were carried out, using the general linear model (GLM) procedure in SAS 9.1 software (SAS Institute Inc 2009).

3. RESULTS

Karyotypic study results show that all of the examined seven opium poppy (*Papaver somniferum* L.) populations of Balkh Province, Afghanistan were diploids; six among which possess $2n = 2x = 22$ chromosomes, while the other one had $2n = 2x = 20$ chromosome (Figure 3, Table 3). This study has being reported for the first time

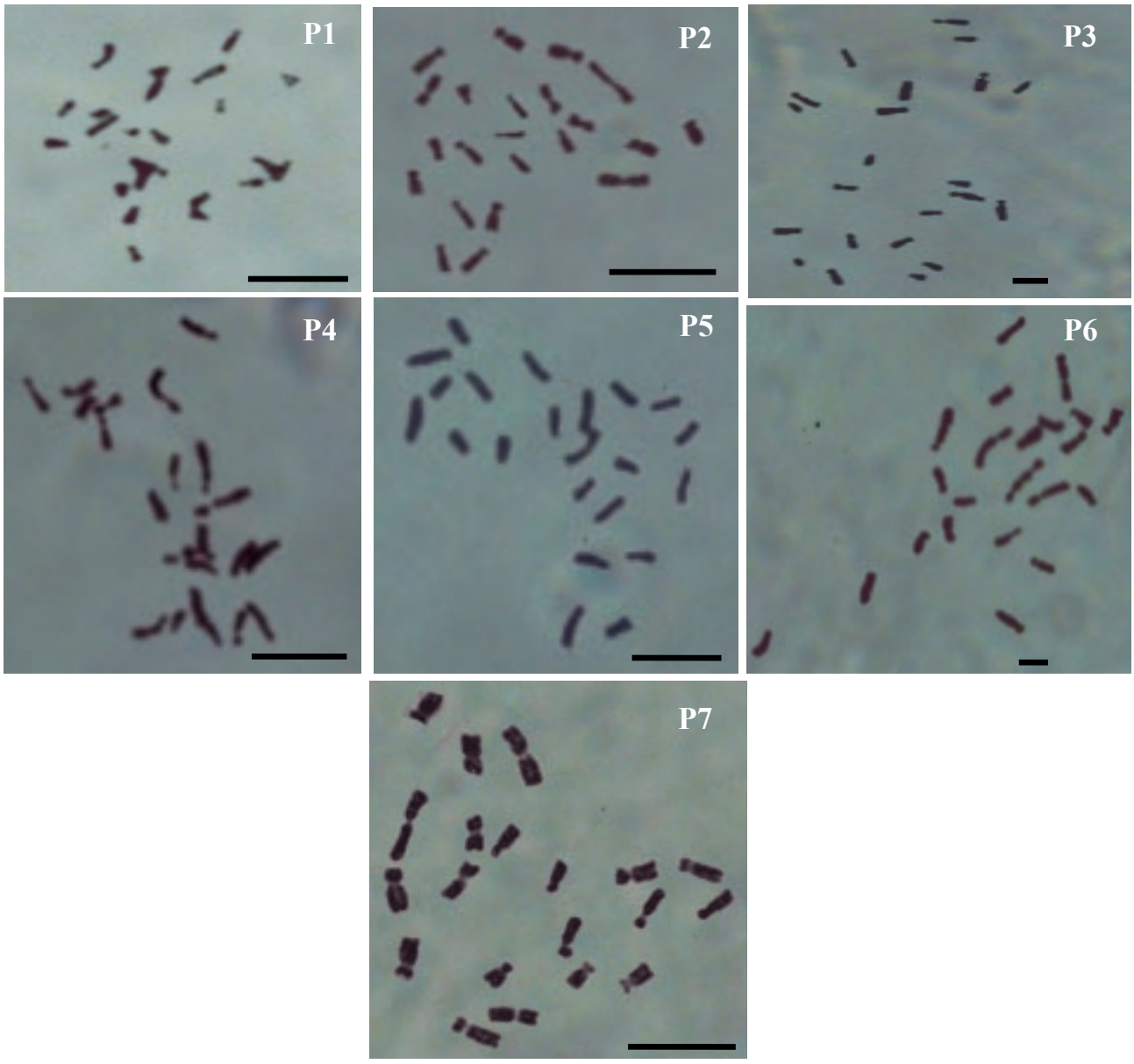

Figure 3. Karyotypes of somatic chromosomes of *Papaver somniferum* populations. Scale bars = 5 µm.

on opium cultivation area in Balkh Province, the northern part of Afghanistan. *ANOVA* results indicate significant differences ($P < 0.01$) for CL between the studied populations, showing intraspecific diversity (Table 2). The mean CL of all populations are shown in (Table 3). The mean CL of the first six populations (P1-P6) with $2n = 2x = 22$ chromosomes was 1.32 µm, ranging from 0.91 µm (P1) to 1.74 µm (P6), but that of the P7 population was 2.24 µm (Table 3). The monoploid nuclear DNA contents of seven studied populations are shown in figure 4. The mean 2Cx DNA amount of the P1-P6 populations with 22 chromosomes was 5.701 pg, ranging from 5.574 pg (P5) to 5.901 pg (P1), while that of P7 population was 5.795 pg (Table 3). The coefficients of variation for G_0/G_1 peaks of all species were less than 5%. The *ANOVA* of genome size indicates non-significant differences ($P > 0.05$). Hence, to make sure of any possible difference, furthermore, LSD mean comparisons were carried out at 0.05 probability level, indicating significant differences between P1 and P3 and P5 (Table 3). The results of histogram analysis are complementary and confirm the karyotypic studies, indicating the diploid nature of the examined populations.

Table 3. Means (± SE) comparisons of chromosome length (CL) and monoploid genome size (2Cx DNA; pg) of *Papaver somniferum* populations from Balkh-Afghanistan

Population	Locality	2*n*	CL	2Cx DNA (pg) Mean ± Se	1Cx genome size (pg)	1Cx genome size (Mbp)
P1	Chahar Kint	22	$0.91^{f} \pm 0.030$	$5.901^{a} \pm 0.11$	2.95	2885.10
P2	Balkh	22	$1.50^{c} \pm 0.047$	$5.779^{ab} \pm 0.04$	2.89	2826.42
P3	Kishindeh	22	$1.08^{e} \pm 0.039$	$5.613^{b} \pm 0.03$	2.81	2748.18
P4	Chimtal	22	$1.41^{cd} \pm 0.045$	$5.716^{ab} \pm 0.08$	2.86	2797.08
P5	Khulm	22	$1.27^{d} \pm 0.033$	$5.574^{b} \pm 0.18$	2.79	2728.62
P6	Sholgara	22	$1.74^{b} \pm 0.062$	$5.625^{ab} \pm 0.08$	2.81	2748.18
P7	Chahar Bolak	20	$2.24^{a} \pm 0.074$	$5.795^{ab} \pm 0.06$	2.89	2826.42
Means		P1-P6	1.32	5.701	--	--
		P7	2.24	5.795		
$LSD_{1\%}$			0.32	0.28	--	--

Means followed by the same letter within (CL) and "2Cx DNA (pg)" columns indicate they are not significantly different at ($P > 0.01$) and ($P > 0.05$), respectively, using LSD test.

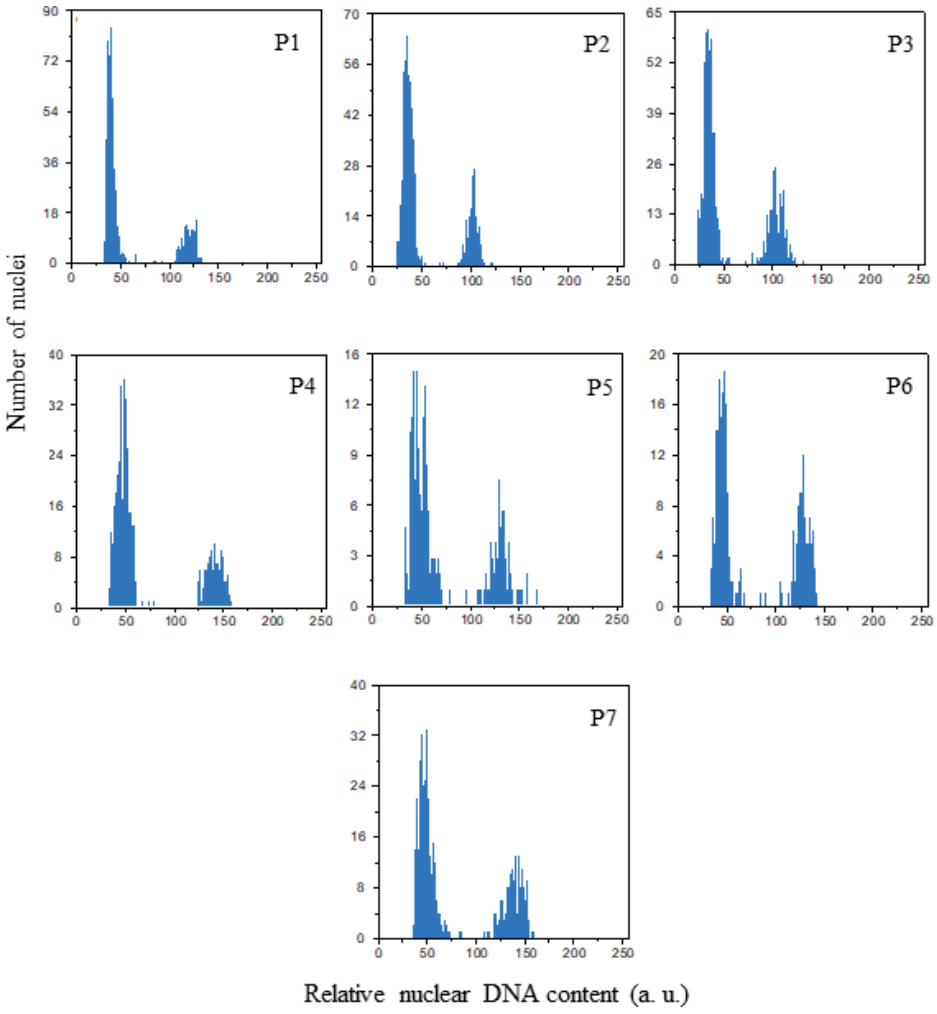

Figure 4. Flow cytometric histograms of 2Cx DNA content of seven *Papaver somniferum* populations. The left peaks refer to G_1 of the *Solanum lycopersicum* cv. Stupicke; 2C = 1.96 pg DNA internal reference standard and the right peak is G1 of the sample (*Papaver somniferum* L.).

4. DISCUSSION

Many pharmaceutical components and medical benefits have been reported for Opium poppy (Khan *et al*., 2011; Heydari *et al*., 2013; Labanca *et al*., 2018). Opium poppy is an important drug plant used in the manufacture of benzylisoquinoline and phenanthrene groups of alkaloids (Gümüşçü *et al*., 2008). For using the potential applicability, this plant still requires more research on its genetic characteristics as well as developing breeding methods. In the current study, we studied seven populations of opium poppy (*Papaver somniferum* L.) in terms of the chromosomal and genome size variations. Somatic chromosome morphology of *Papaver somniferum* shows that their chromosomes are numbered from 1 to 11 in order of differentiation in chromosome length (Kaul *et al*., 1979). The karyotype of cultured poppy plant root tips showed 22 chromosomes in all well spread root tip and shoot tip cells with more variation in length and centromere positions (Wakhlu and Bajwa, 1987). The karyotypic study in the present research showed that all populations were diploid and, in terms of chromosome numbers, P1-P6 had 22 and P7 had 20 chromosomes. The 22-chromosome number is in agreement with the studies conducted by Kaul *et al*. (1979) and Wakhlu and Bajwa, (1987), but not for the 20-chromosome P7 population. Considerable variation in somatic chromosome numbers of many plants, especially in the root tips, has been reported (Winterfeld., 2020; Mehravi *et al*., 2022). The first karyological studies of the Papaveraceae were done by Tahara on *Papaver somniferum, P. orientale*, and *P. rhoeas* which reported $2n = 22$ for poppy (*Papaver somniferum*) species (Sugiura, 1940). That is in exact conformity with P1-P6 populations in the current study, but differed from those in P7. On the other hand, in recent studies, the chromosome number of Iranian poppy (*Papaver bracteatum* L.) in diploid and in induced tetraploids showed 14 and 28, respectively (Tarkesh Esfahani *et al*., 2020), showing massive difference with that in the present study. Based on the obtained results of the current study, the P1-P6 populations were diploid with the base chromosome number of $x = 11$, the same base chromosome number of 11 was reported by previous studies (Sugiura, 1940; Kaul *et al*., 1979; Wakhlu and Bajwa, 1987; Tetenyi, 1994; Rezaei *et al*., 2014). Flow cytometry describes the use of this technique for the estimation of genomic DNA amount in cell nuclei (Doležel and Bartoš, 2005). In a research, the average 2C DNA content of all Persian poppy plants (*Papaver bracteatum*) was estimated as 6.15 ± 0.03 pg (Tarkesh Esfahani *et al*., 2016, 2020), indicating differences with that in the present study on *P. somniferum* populations. It can be noted that the 2Cx DNA content of *P. somniferum* species, having less variation was previously reported by researchers (Kyrylenko *et al*., 2005; Rezaei *et al*., 2014; Tarkesh Esfahani *et al*., 2016; Vu *et al*., 2021; *Pei et al*., 2021). The genome size of the first six populations (P1-P6) with 22 chromosomes was 5.701 pg (5.574-5.901 pg), which is similar to the previous report of Kyrylenko *et al*. (2005) in poppy species (*P. somniferum*) in terms of chromosome numbers possess 6.46 pg genomes size, showing a difference of 0.76 pg (13% reduction than that in the present study). By division of genome size by the number of chromosomes pg/chr, which was previously done in Mahdavi and Karimzadeh (2010) study on *Thymus* species (Lamiaceae), was also conducted in the current research. Hence, the genome size of the P1-P6 populations on the chromosome was equal to 0.259 pg/chr. Such statistics was calculated in the previous report by Kyrylenko *et al*. (2005) for *P. somniferum* with the same chromosome number to be 0.294 pg/chr, revealing 13% more than that in our six populations in the current study. More interestingly, 0.439 pg/chr was calculated in the Iranian poppy species (*P. bracteatum*) in the study of (Tarkesh Esfahani *et al*., 2020) which showed about 1.69 and 1.52 times increases compared to that in the first six populations and in the 7th population in the present study, respectively. The current study provides brand new information about genome size content diversity and karyotype in *P. somniferum* populations for the first time from the northern parts of Afghanistan that will help next researchers to consider whether other populations exist in other parts of this country.

ACKNOWLEDGEMENT

Authors acknowledge the Tarbiat Modares University for financial supporting of this research project.

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**: 129-135.

Abedi R, Babaei A, and Karimzadeh G. 2015. Karyological and flow cytometric studies of *Tulipa* (Liliaceae) species from Iran. Plant Syst. Evol. **301(5)**: 1473-1484.

Ahmadi-Roshan M, Karimzadeh G, Babaei A, and Jafari H. 2016. Karyological studies of *fritillaria* (Liliaceae) species from Iran. Cytologia **81(2)**: 133-141.

Aragane M, Watanabe D, Nakajima J, Yoshida M, Yoshizawa M, Abe, T, Nishiyama R, Suzuki J, Moriyasu T, Nakae D, Sudo H, Sato H, Hishida A, Kawahara N, Makabe S, Nakamura I, and Mii M. 2014. Rapid identification of a narcotic plant *Papaver bracteatum* using flow cytometry. J. Nat. Med. **68(4)**: 677-685.

Askitopoulou H, Ramoutsaki IA, and Konsolaki E. 2002. Archaeological evidence on the use of opium in the Minoan world. Int. Congr. Ser. **1242(C)**: 23-29.

Beaudoin GAW and Facchini PJ. 2014. Benzylisoquinoline alkaloid biosynthesis in opium poppy. Planta **240(1)**: 19-32.

Bennett MD, Bhandol P, and Leitch IJ. 2000. Nuclear DNA amounts in angiosperms and their modern uses - 807 New estimates. Ann. Bot. **86(4)**: 859-909.

Brown M and Wittwer C. 2000. Flow cytometry: Principles and clinical applications in hematology. Clin. Chem. **46(8 II)**: 1221-1229.

Carlin MG, Dean JR, and Ames JM. 2020. Opium alkaloids in harvested and thermally processed poppy seeds. Front. Chem. **8(737)**: 1-9.

Chowa J, Puangpairoteb T, Anamthawat-Jónssonc K, and Umpunjuna P. 2020. Karyotypic and molecular cytogenetic characterization of diploid and polyploid accessions of medicinal herbs in the genus *Paris* from northern Thailand. Sienceasia **46(3)**: 297-307.

Dittbrenner A, Lohwasser U, Mock HP, and Börner A. 2008. Molecular and phytochemical studies of *Papaver somniferum* in the context of intraspecific classification. Acta Hortic. **799**: 81-88.

Doležel J, Greilhuber J, Lucretti S, Meister A, Lysák 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, and Bartoš J. 2005. Plant DNA flow cytometry and estimation of nuclear genome size. Ann. Bot. **95(1)**: 99-110.

Gümüşçü A, Arslan N, and Sarıhan EO. 2008. Evaluation of selected poppy (*Papaver somniferum* L.) lines by their morphine and other alkaloids contents. Eur. Food Res. Technol. **226(5)**: 1213-1220.

Guo L, Winzer T, Yang X, Li Y, Ning Z, He Z, Teodor R, Lu Y, Bowser TA, Graham IA, and Ye K. 2018. The opium poppy genome and morphinan production. Science **362(6412)**: 343-347.

Jesus A, Bonhomme V, Evin A, Ivorra S, Soteras R, Salavert, A, Antolín F, and Bouby L. 2021. A morphometric approach to track opium poppy domestication. Sci. Rep. **11(1)**: 1-11.

Heydari M, Hashempur MH., and Zargaran A. 2013. Medicinal aspects of opium as described in Avicenna's Canon of Medicine. Acta Med Hist Adriat. **11(1)**: 101-12.

Kaul BL., Tandon V, and Choudhary DK. 1979. Cytogenetic studies in *Papaver somniferum* L. Proc. Indian National Sci. Acad. **88(4)**: 321-325.

Khan ZH, Khan M, Aftab T, Idrees M, and Naeem M. 2011. Influence of alginate oligosaccharides on growth, yield and alkaloid production of opium poppy (*Papaver somniferum* L.). Front Agric China, **5(1)**: 122-127.

Kyrylenko TK, Martynenko OI, and Alkhimova OG. 2005. Nuclear genome size and karyotype analysis in *Papaver* for BAC library construction. Biopolym. Cell **21(2)**: 145-150.

Labanca F, Ovesnà J, and Milella L. 2018. *Papaver somniferum* L. taxonomy, uses and new insight in poppy alkaloid pathways. Phytochem. Rev. **17(4)**: 853-871.

Loureiro J, Rodriguez E, Doležel J, and Santos C. 2007. Two new nuclear isolation buffers for plant DNA flow cytometry. Ann. Bot. **100(4)**: 875-888.

Mahdavi S, and Karimzadeh G. 2010. Karyological and nuclear DNA content variation in some Iranian endemic *Thymus* species (Lamiaceae). J. Agric. Sci. Technol **12**: 445-458.

Mehravi S, Karimzadeh G, Kordenaeej A, and Hanifei, M. 2022. Mixed-ploidy and dysploidy in *Hypericum perforatum*: a karyomorphological and genome size study. Plants **11(22)**: 3068.

Najafi S, Altuner F, Ülker M, Oral E, Özdemir B, and Şelem E. (2022). Karyological analysis on wheat Tir (*Triticum aestivum* var. *Aestivum* L. spp. Leucospermum Körn.) ecotypes in Lake Van Basin, Turkey. Int. J. Agric. Technol. **18(3)**: 1093-1102.

Pei L, Wang B, Ye J, Hu X, Fu L, Li K, Ni Z, Wang Z, Wei Y, Shi L, Zhang Y, Bai X, Jiang M, Wang S, Ma C, Li S, Liu K, Li W, and Cong B. 2021. Genome and transcriptome of *Papaver somniferum* Chinese landrace CHM indicates that massive genome expansion contributes to high benzylisoquinoline alkaloid biosynthesis. Hortic. Res. **8**: 1-13.

Rezaei M, Naghavi MR, Hoseinzadeh AH, Abbasi A, and Jahangiri B. 2014. Study of karyological characteristics in *Papaver bracteatum* and *Papaver somniferum*. Cytologia **79(2)**: 187-194.

Salavert A, Martin L, Antolín F, and Zazzo A. 2018. The opium poppy in Europe: exploring its origin and dispersal during the Neolithic. Antiquity **92(364)**: 1-5.

Sayadi V, Karimzadeh G, Naghavi MR, and Rashidi Monfared S. 2021. Karyological studies and chromosome variation among Iranian endemic *Allium* species (Amaryllidaceae). J. Plant Physiol. Breed. **11**: 97-108.

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.

SAS Institute Inc. 2009. SAS/STAT 9.2 User's Guide. SAS Publishing. Cary, NC.

Sugiura T. 1939. Chromosome studies on Papaveraceae with special reference to the phylogeny. Cytologia **10(3)**: 448-476.

Sugiura T. 1940. Chromosome studies on Papaveraceae with special reference to the phylogeny. Cytologia **10(4)**: 558-576.

Tarkesh Esfahani S, Karimzadeh G, and Naghavi MR. 2016. 2C DNA value of persian poppy (*Papaver bracteatum* Lindl.) medicinal plant as revealed by flow cytometry analysis; a quick effective criteria for distinguishing unidentified papaver species. Int. J. Adv. Biotechnol. Res. **7(2)**: 573-578.

Tarkesh Esfahani S, Karimzadeh G, and Naghavi MR. 2020. *In vitro* polyploidy induction in persian poppy (*Papaver bracteatum* lindl.). Caryologia **73(1)**: 133-144.

Tetenyi P. 1994. Biodiversity of *Papaver Somniferum* L.(opium poppy). Int. J. Med. Aromat. Plants **390**: 191-202.

Vu U, Hong T, Tamiru-oli M, Hurgobin B, and Christopher R. 2022. Insights into opium poppy (*Papaver* spp.) genetic diversity from genotyping-by-sequencing analysis. Sci. Rep. **12(1)**:1-15.

Wakhlu AK and Bajwa PS. 1987. Cytological analysis embryogenic callus culture and regenerated plants of *Papaver somniferum* L.(Opium poppy). Cytologia **52(3)**: 631-638.

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(2)**: 1-17.