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Karyotype study in several populations of *Papaver dubium* from North West of Iran

Rozita MADADİ [1], Rasool ASGHARİ-ZAKARİA [*1], Mojtaba FATHİ [2]

[1]Department of Crop Production and Breeding, Faculty of Agriculture, University of Mohaghegh Ardabili, Ardabil, Iran
[2] Agricultural Support System Company, Ardabil, Iran

Abstract

Karyotype and morphology of mitotic chromosomes in five populations of medicinal plant *Papaver dubium* collected from various geographical locations of northwest of Iran were studied. Chromosome characteristics were measured from 10 complete metaphase cells using Micromeasure software. Results showed that *P. dubium* is a diploid species with 2n=2x=14 chromosomes. The karyotype consisted of seven pairs of submetacentric chromosomes with one pair of SAT chromosome (chromosome 1) had a fair secondary constriction at the end of its short arm. Karyological characteristics of all materials studied were similar to each other; however, there were some variations on chromosome arm ratios and relative lengths among different populations. All of the populations placed on 3A class of Stebbin's asymmetry categories.

Key words: Biodiversity, Chromosome analysis, Medicinal plants, *Papaver dubium, Papaveraceae*

1. Introduction

Ardabil province is located in the north west of Iran from 37°, 45' to 39°, 42' North latitude and 47°, 2' to 48°, 55' east longitude with a range of altitude between 20 m in the riverside of Araxes River to 4811 m at the top of Sabalan Mountain. The existing climatic and ecological variation has resulted in growth of various medicinal plants including a number of poppy species in the region.

Genus *Papaver*, containing the highest level of botanical and phytochemical variability, has embraced many species with numerous subspecies and varieties yields approximately 170 alkaloids from 13 important alkaloid groups. *Papaver* genus has been divided by different authors into five to eleven sections based on morphological traits, primarily on the characteristics of the capsules (Kadereit, 1993). Chromosome numbers in the family are mainly multiples of x=6, 7, 8, 9, 10, 11 (Mihalik, 1998). The main trait of the family is its capacity to synthesize various and very complex alkaloids (Mihalik, 1998).

In conjunction with data from other approaches, chromosome information and numerical analysis based on karyotype data continues to be useful in assessing generic and tribal relationships in large and complicated families of plants. *P. dubium* is an annual diploid (2*n*=2x=14) species belongs to the section Rhoeadium in *Papaver* genus with various pharmaceutical uses. There are a few reports on the chromosome number of *P. dubium* (Lavania and Srivastava, 1999). In the present study, an attempt was made to develop detailed karyotype of 5 diverse populations of this species.

2. Materials and methods

Seed of five populations of *P. dubium*, collected from various geographical locations from northwest of Iran (Table 1) was soaked for germination in Petri plates, on filtered paper, moistened with distilled water in darkness, at room temperature (22-24˚C). The root tips were pretreated in 0.05% solution of colchicine for 2.5 h at room

* *Corresponding author* / Haberleşmeden sorumlu yazar: rrasghari@yahoo.com

temperature before fixing in cromic acid -Formalin (1: 1v.v) at 4°C for 25 h. Staining with Hematoxylin was done as described earlier by Asghari-Zakaria *et al.* (2002).

Chromosome measurements including long arm, short arm, chromosome lengths, arm ratio index and relative chromosome length were taken from 10 enlarged well-spread metaphase cells for each population using Micromeasure software developed by the Biology department of Colorado State University, available on Internet at http://www.colostate.edu.Depts.Biology. Chromosomes were named as 1, 2, 3, …, 7 in descending order of length following Levan *et al.* (1964) method for nomenclature of chromosomes. Karyotype asymmetry was estimated using the ratio of the shortest/longest pair (R), the total form percent (TF), difference between relative length of the longest and the shortest chromosomes (DRL), Stebbin's (1971) asymmetry category (ST), the intra-chromosomal (A1) and the inter-chromosomal asymmetry index (A2) indices according to Romero Zarco (1986).

Table 1. Origin of *P. dubium* populations under present observation.

Population	Origin of collection	Latitude	Altitude
G1	Germi region, Ardabil province, northwest of Iran	N 38, 53, 04 E 47, 57, 29	1792
G2	Meshkin-Shahr region, Ardabil province, northwest of Iran	N 38, 42, 26 E 48, 02, 08	1451
G3	Pars-Abad region, Ardabil province, northwest of Iran	N 39, 00,34 E 48, 01, 40	1100
G4	Meshkin-Shahr region, Ardabil province, northwest of Iran	N 38, 21, 16 E 47, 31, 56	1304
G5	Ivrigh region, Ardabil, Ardabil province, northwest of Iran	N 38, 10, 22 E 48, 30, 54	1419

3. Results

Mitotic chromosomes of the five *P. dubium* populations are shown in Figure 1, and karyotypic characters of the seven mitotic chromosomes are shown in Table 2. While data on karyotype formulae, difference between relative length of the longest and the shortest chromosomes, total form percentage, ratio of the longest to the shortest chromosome, intra-chromosomal and inter-chromosomal asymmetry indices for each population are presented in Table 3.
Results showed that *P. dubium* is a diploid species with 2n=2x=14 chromosomes. This is in agreement with finding of Lavania and Srivastava (1999). Mean arm ratio index of chromosomes ranged from 1.81 in chromosome 2 to 2.27 in chromosomes 1 and 5. Karyological characteristics of all materials studied were similar to each other; however, there were some variations among populations on chromosome arm ratios and relative lengths. For example, chromosome 3 in the population G3, chromosome 6 in the population G4 and chromosomes 7 in populations G2 and G4 had arm ratio values smaller than 2.00, However; the respected chromosomes in other populations had arm ratio values greater than 2.00 in all of the populations (Table 2).

According to relative length of chromosomes, there were some variations among populations. Chromosome 1 was the longest chromosome in all of the populations except for G1 and G3 whereas chromosome 7 was the smallest one in all of the populations (Table 2). The NOR region was seen on the end of short arm of chromosome 1 in a few metaphase cells probably due to extra chromosome contraction.
In general, the karyotype of this species consisted of seven pairs of submetacentric chromosomes with arm ratio values greater than 1.81. Lavania and Srivastava (1999) also reported seven pairs but four were of metacentric and three were of submetacentric with the arm ratio values between 1.41 and 2.56 in the karyotype of this species.
According to asymmetry indices, the category of all populations was same and placed on 3A class of Stebbin's (1971) asymmetry categories with minor variations among the populations (Table. 3) which was similar with previous reports (Lavania and Srivastava, 1999).

4. Conclusions

It can be concluded that these populations show diversity in karyological characteristics with other populations and this biodiversity may be existed in pharmaceutical and medicinal characters of these populations, that needs to be conserved and utilized properly.

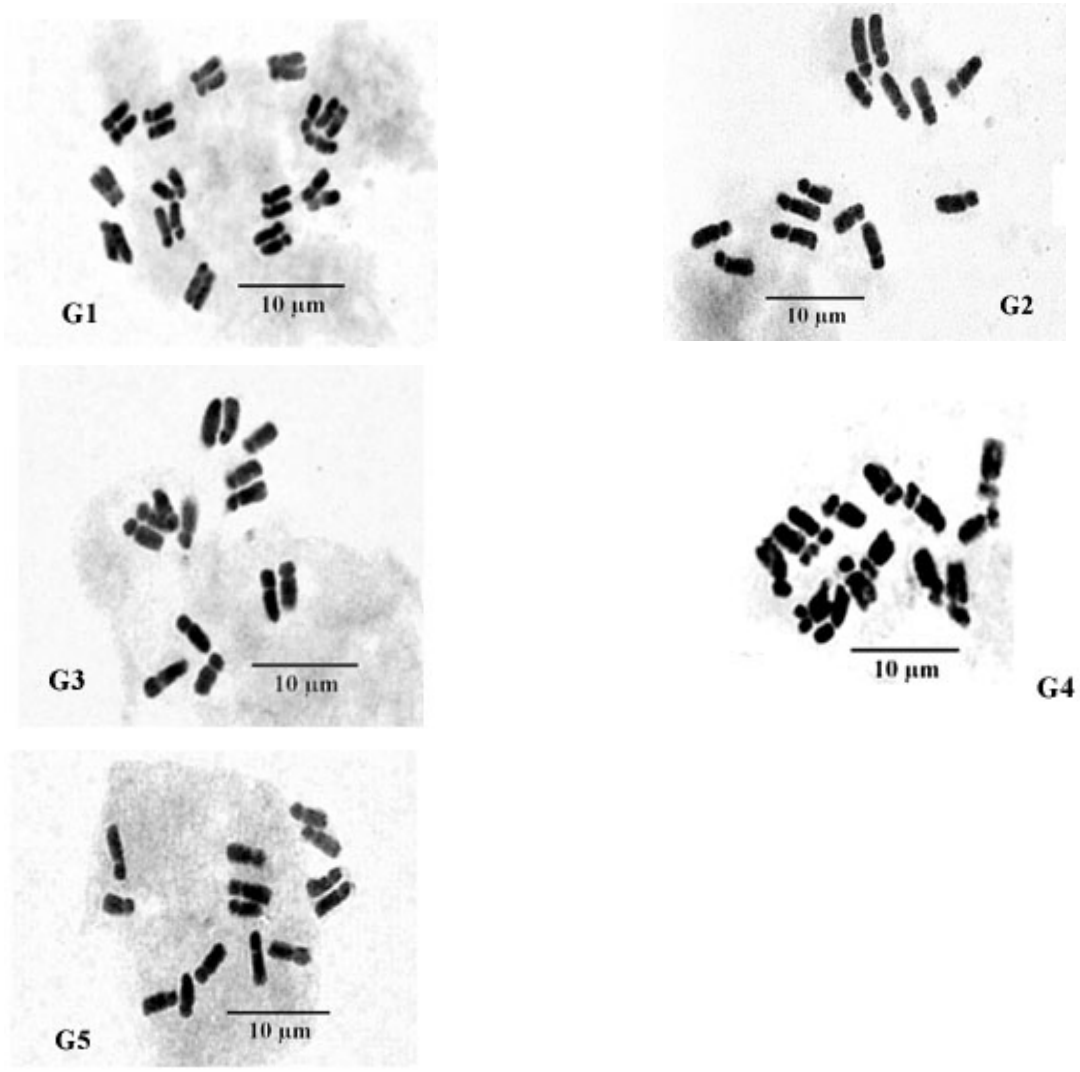

Figure1. Somatic metaphase chromosomes of *Papaver dubium* stained with aceto-iron-hematoxilin.

Table 2. Karyotype characteristics of seven mitotic chromosomes in five diverse populations of *Papaver dubium*.

Chr.	Pop.	type	Short arm (µm)	Long arm (µm)	Length of chromosom (µm)	Relative length	Arm Ratio index
1	G1	sm	1.60 ± 0.06	3.72 ± 0.24	5.32 ± 0.29	15.67 ± 0.22	2.32 ± 0.12
	G2	sm	1.34 ± 0.07	3.00 ± 0.16	4.34 ± 0.22	14.99 ± 0.49	2.27 ± 0.09
	G3	sm	1.65 ± 0.12	3.75 ± 0.25	5.39 ± 0.45	16.14 ± 0.35	2.27 ± 0.11
	G4	sm	1.53 ± 0.05	3.54 ± 0.16	5.07 ± 0.19	16.86 ± 0.34	2.32 ± 0.09
	G5	sm	1.75 ± 0.03	3.70 ± 0.24	5.45 ± 0.25	16.95 ± 0.37	2.12 ± 0.13
	mean		1.57 ± 0.07	3.54 ± 0.14	5.12 ± 0.20	16.12 ± 0.41	2.27 ± 0.05
2	G1	sm	1.91 ± 0.16	3.29 ± 0.20	5.20 ± 0.33	15.32 ± 0.54	1.77 ± 0.12
	G2	sm	1.64 ± 0.06	3.04 ± 0.11	4.67 ± 0.15	16.18 ± 0.36	1.87 ± 0.06
	G3	sm	2.08 ± 0.33	3.77 ± 0.54	5.85 ± 0.86	16.80 ± 0.89	1.85 ± 0.13
	G4	sm	1.61 ± 0.05	3.02 ± 0.10	4.63 ± 0.13	15.42 ± 0.14	1.89 ± 0.07
	G5	sm	1.78 ± 0.07	3.05 ± 0.22	4.83 ± 0.28	14.99 ± 0.46	1.70 ± 0.07
	mean		1.80 ± 0.09	3.23 ± 0.14	5.04 ± 0.23	15.74 ± 0.33	1.81 ± 0.03

Table 2. (Continue)

3	G1	sm	1.51 ± 0.10	3.66 ± 0.25	5.17 ± 0.34	15.15 ± 0.37	2.42 ± 0.05
	G2	sm	1.36 ± 0.05	2.94 ± 0.12	4.31 ± 0.15	14.87 ± 0.17	2.17 ± 0.08
	G3	sm	1.73 ± 0.11	3.30 ± 0.31	5.03 ± 0.37	15.17 ± 0.38	1.93 ± 0.17
	G4	sm	1.42 ± 0.04	3.07 ± 0.12	4.48 ± 0.12	14.93 ± 0.13	2.18 ± 0.12
	G5	sm	1.38 ± 0.07	3.27 ± 0.13	4.64 ± 0.20	14.45 ± 0.22	2.39 ± 0.08
	mean		1.48 ± 0.07	3.25 ± 0.13	4.73 ± 0.18	14.91 ± 0.20	2.22 ± 0.08
4	G1	sm	1.61 ± 0.09	3.28 ± 0.27	4.89 ± 0.34	14.36 ± 0.46	2.03 ± 0.12
	G2	sm	1.38 ± 0.07	2.91 ± 0.08	4.29 ± 0.14	14.84 ± 0.23	2.14 ± 0.08
	G3	sm	1.34 ± 0.10	3.44 ± 0.32	4.78 ± 0.42	14.26 ± 0.21	2.54 ± 0.11
	G4	sm	1.40 ± 0.05	2.97 ± 0.06	4.37 ± 0.10	14.56 ± 0.19	2.14 ± 0.06
	G5	sm	1.43 ± 0.05	3.22 ± 0.12	4.65 ± 0.16	14.50 ± 0.27	2.25 ± 0.05
	mean		1.43 ± 0.05	3.16 ± 0.10	4.60 ± 0.12	14.50 ± 0.10	2.22 ± 0.09
5	G1	sm	1.38 ± 0.06	3.16 ± 0.17	4.54 ± 0.21	13.44 ± 0.31	2.30 ± 0.10
	G2	sm	1.25 ± 0.05	2.69 ± 0.08	3.94 ± 0.12	13.64 ± 0.11	2.18 ± 0.09
	G3	sm	1.47 ± 0.17	3.27 ± 0.32	4.74 ± 0.47	14.00 ± 0.18	2.28 ± 0.16
	G4	sm	1.32 ± 0.06	2.75 ± 0.10	4.06 ± 0.09	13.54 ± 0.18	2.15 ± 0.18
	G5	sm	1.27 ± 0.04	3.09 ± 0.12	4.36 ± 0.14	13.60 ± 0.21	2.43 ± 0.08
	mean		1.34 ± 0.04	2.99 ± 0.11	4.33 ± 0.15	13.65 ± 0.10	2.27 ± 0.05
6	G1	sm	1.50 ± 0.09	3.00 ± 0.18	4.50 ± 0.25	13.25 ± 0.09	2.01 ± 0.09
	G2	sm	1.20 ± 0.07	2.60 ± 0.10	3.80 ± 0.13	13.13 ± 0.10	2.22 ± 0.11
	G3	sm	1.17 ± 0.11	2.94 ± 0.28	4.11 ± 0.39	12.25 ± 0.37	2.51 ± 0.09
	G4	sm	1.33 ± 0.04	2.55 ± 0.10	3.88 ± 0.11	12.92 ± 0.15	1.93 ± 0.10
	G5	sm	1.34 ± 0.03	2.86 ± 0.07	4.20 ± 0.10	13.14 ± 0.25	2.13 ± 0.05
	mean		1.31 ± 0.06	2.79 ± 0.09	4.10 ± 0.12	12.94 ± 0.18	2.16 ± 0.10
7	G1	sm	1.35 ± 0.06	3.00 ± 0.17	4.35 ± 0.22	12.82 ± 0.18	2.23 ± 0.09
	G2	sm	1.28 ± 0.08	2.29 ± 0.06	3.57 ± 0.12	12.35 ± 0.17	1.83 ± 0.09
	G3	sm	1.08 ± 0.10	2.69 ± 0.27	3.78 ± 0.37	11.39 ± 0.70	2.48 ± 0.08
	G4	sm	1.28 ± 0.05	2.26 ± 0.09	3.54 ± 0.12	11.77 ± 0.18	1.78 ± 0.09
	G5	sm	1.31 ± 0.04	2.67 ± 0.12	3.98 ± 0.16	12.38 ± 0.17	2.03 ± 0.04
	mean		1.26 ± 0.05	2.58 ± 0.14	3.84 ± 0.15	12.14 ± 0.25	2.07 ± 0.13

±: mean ± standard error; sm = submetacentric

Table 3. Karyotype formulae (KF), Difference between relative length of the longest and the shortest chromosomes (DRL), Total form percentage (TF), Ratio of the longest to the shortest chromosome (R), the intra-chromosomal asymmetry index (A1), the inter-chromosomal asymmetry index (A2), and Stebbin's asymmetry category (ST) in five populations of *Papaver dubium* .

Pop.	KF	TF	DRL	R	A1	A2	ST
G1	7sm	31.97	2.85	1.22	0.53	0.08	3A
G2	7sm	32.65	3.83	1.31	0.51	0.09	3A
G3	7sm	31.24	5.41	1.55	0.55	0.15	3A
G4	7sm	32.93	5.09	1.43	0.50	0.12	3A
G5	7sm	31.97	4.57	1.37	0.53	0.10	3A
mean		32.15	4.35	1.38	0.52	0.11	3A

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