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Full Length Research Paper

Karyological study of the medicinal plant *Papaver rhoeas* from northwest of Iran

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Karyotype and morphology of mitotic chromosomes in six populations of the medicinal plant *Papaver rhoeas* collected from various geographical locations of the northwest of Iran were studied. Chromosome characteristics were measured from 10 complete metaphase cells using micromeasure software. The results showed that *P. rhoeas* is a diploid species with 2n=2x=14 chromosomes. The karyotype consisted of seven pairs of submetacentric chromosomes with one pairs of SAT chromosome (chromosome 2) having a secondary constriction at the end of its short arm. Karyological characteristics of all the materials studied were similar to each other; however, there were some variations on chromosome arm ratios and relative lengths among the different populations. All of the populations are placed on 4A class of Stebbin's asymmetry categories.

Key words: Biodiversity, chromosome analysis, medicinal plants, Papaveraceae.

INTRODUCTION

The Papaveraceae family polarizes the special attention for its pharmaceutical, ornamental and alimentary valences (Bara et al., 2007). The main trait of the family is their capacity to synthesize various and very complex alkaloids (Mihalik, 1998). According to Kadereit (1993) the ≈260 species of Papaveraceae are divided into four subfamilies including Chelidoniodeae, Eschcholzioideae, Platystemonoideae and Papaveriodeae involving 23 genera. Papaveriodeae sub family consists of eight genus including *Meconopsis*, *Papaver*, *Romeria*, *Steylomecon*, *Arctomecon*, *Argemon*, *Canbya* and *Romenya*.

Genus *Papaver* has the highest level of botanical and phytochemical variability, embracing many species with numerous subspecies and varieties yielding approximately 170 alkaloids from 13 alkaloid groups. Different authors have divided *Papaver* genus into five to eleven sections. The latest revision was made by Kadereit (1990) who divided the 11 sections of the genus into four groups based on morphological traits, primarily on the characteristics of the capsules. Chromosome numbers in the family are mainly multiples of x = 6, 7, 8, 9, 10, 11 (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. rhoeas* (2n= 2x= 14) belong to the section Rhoeadium in *Papaver* genus. There are a few reports on the chromosome number of *Papaver rhoeas* (Lavania and Srivastava, 1999; Vorniceanu et al., 2004). In this study, an attempt was made to develop detailed karyotype of this species.

MATERIALS AND METHODS

Seeds of six populations of *P. rhoeas,* collected from various geographical locations from north and northwest of Iran (Table 1), were soaked for germination in Petri plates on filtered paper moistened with distilled water in darkness at room temperature (22 to 24°C). The root tips were pretreated in 0.05% solution of colchicine for 2.5 h at room temperature before they were fixed in chromic acid -formalin (1:1 v: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 and chromosome lengths, arm ratio index and relative chromosome length were made from 10 enlarged well-spread metaphase cells for each population, using micromeasure software developed by the

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Table 1. Origin of *P. rhoeas* populations used in this study.

Population	Origin	Geographical position	Altitude (m)
G1	Population collected from Daryaman region, Germi, Ardabil province, northwest of Iran	N 39° 00' 18" E 48° 00' 44"	1154
G2	Population collected from Shiran region, Nir, Ardabil province, northwest of Iran	N 38° 05' 11" E 48° 04' 43"	1595
G3	Population collected from Sibly region, Astara, Guilan province, north of Iran	N 38° 25' 18" E 48° 51' 42"	5
G4	Population collected from Heiran region, Ardabil, Ardabil province, northwest of Iran	N 38° 25' 59" E 48° 34' 50"	1480
G5	Population collected from Shoendarasidag region, Germi, Ardabil province, northwest of Iran	N 38° 53' 04" E 47° 57' 29"	1790
G6	Population collected from Astara, Guilan province, north of Iran	N 38° 20' 59" E 48° 51' 36"	7

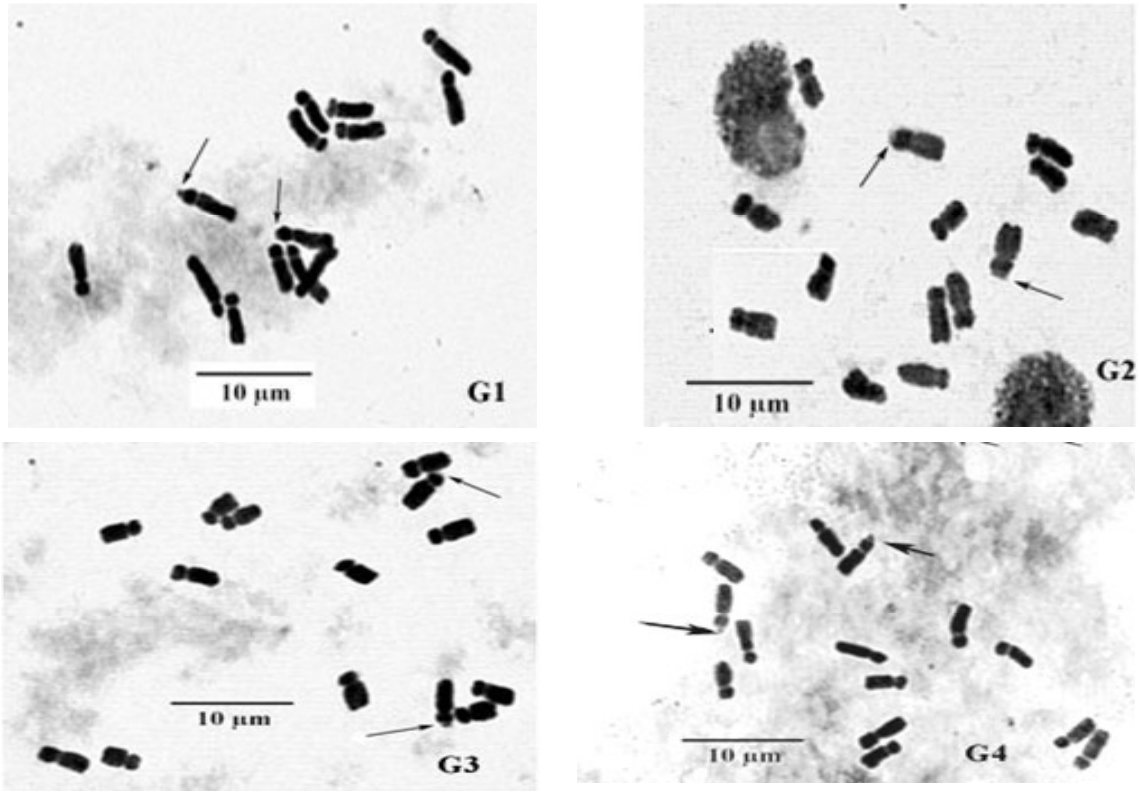

Figure 1. Somatic metaphase chromosomes (G1 to G6 populations) and general karyogram of *Papaver rhoeas* stained with aceto-iron-hematoxilin. Arrows show secondary constriction.

Biology department of Colorado State University, available on internet at http://www.colostate.edu.Depts.Biology. The nomenclature of chromosomes followed Levan et al. (1964) and chromosomes were named as 1 to 7 in descending order of length. 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), centromeric gradient (CG), coefficient of variation for chromosome length (CV), dispersion index (DI) (Lavania and Srivastava, 1992), Stebbins (1971) asymmetry category, the intra-chromosomal (A1) and the inter-chromosomal asymmetry index (A2) indices (Romero Zarco, 1986).

RESULTS AND DISCUSSION

Mitotic chromosomes of the six *P. rhoeas* populations are shown in Figure 1 and karyotypic characters of the seven mitotic chromosomes are shown in Table 2. The data on

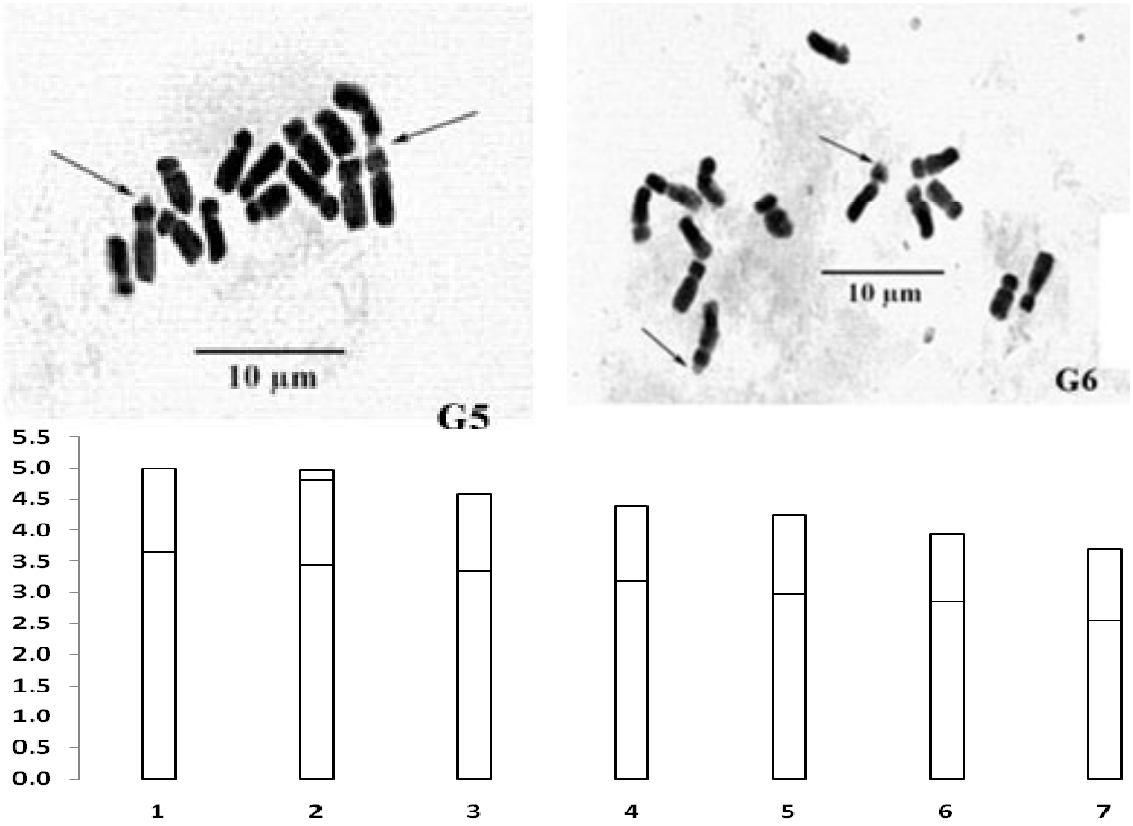

Figure 1 Contd

Table 2. The karyotype characteristics of seven mitotic chromosomes in six populations of *Papaver rhoeas*.

Chr	Pop	Type	Short arm (µm)	Long arm (µm)	Length of chr (µm)	Relative length (%)	Arm ratio index
1	G1	sm	1.41 ± 0.07	3.42 ± 0.21	4.83 ± 0.25	15.79 ± 0.14	2.44 ± 0.11
	G2	sm	1.35 ± 0.05	3.39 ± 0.09	4.74 ± 0.12	15.67 ± 0.11	2.53 ± 0.08
	G3	sm	1.26 ± 0.12	3.66 ± 0.11	4.92 ± 0.20	15.83 ± 0.11	2.91 ± 0.19
	G4	sa	1.05 ± 0.04	3.90 ± 0.11	4.94 ± 0.13	16.20 ± 0.07	3.70 ± 0.12
	G5	sm	1.38 ± 0.05	3.47 ± 0.17	4.85 ± 0.21	17.15 ± 0.11	2.53 ± 0.12
	G6	sm	1.65 ± 0.09	4.08 ± 0.23	5.73 ± 0.31	16.77 ± 0.16	2.46 ± 0.05
	Mean	sm	1.35 ± 0.08	3.65 ± 0.12	5.00 ± 0.15	16.23 ± 0.25	2.76 ± 0.20
2	G1	sm	1.50 ± 0.06	3.26 ± 0.16	4.76 ± 0.22	15.54 ± 0.11	2.17 ± 0.05
	G2	sm	1.50 ± 0.05	3.42 ± 0.13	4.92 ± 0.15	16.27 ± 0.09	2.30 ± 0.10
	G3	sm	1.65 ± 0.11	3.59 ± 0.12	5.24 ± 0.19	16.86 ± 0.09	2.17 ± 0.13
	G4	sm	1.28 ± 0.05	3.48 ± 0.06	4.75 ± 0.08	15.59 ± 0.05	2.72 ± 0.10
	G5	sm	1.49 ± 0.04	3.24 ± 0.13	4.73 ± 0.12	16.72 ± 0.08	2.20 ± 0.12
	G6	sm	1.70 ± 0.09	3.69 ± 0.25	5.40 ± 0.30	15.80 ± 0.17	2.18 ± 0.13
	Mean	sm	1.52 ± 0.06	3.44 ± 0.07	4.97 ± 0.12	16.13 ± 0.23	2.29 ± 0.09
3	G1	sm	1.29 ± 0.04	3.24 ± 0.16	4.53 ± 0.19	14.79 ± 0.10	2.52 ± 0.08
	G2	sa	1.13 ± 0.05	3.41 ± 0.10	4.54 ± 0.15	15.03 ± 0.08	3.04 ± 0.09
	G3	sm	1.34 ± 0.06	3.45 ± 0.16	4.79 ± 0.17	15.40 ± 0.11	2.58 ± 0.21
	G4	sa	1.04 ± 0.08	3.38 ± 0.11	4.42 ± 0.11	14.49 ± 0.09	3.26 ± 0.28
	G5	sm	1.17 ± 0.06	3.00 ± 0.18	4.16 ± 0.23	14.73 ± 0.12	2.57 ± 0.10
	G6	Sm	1.41 ± 0.09	3.63 ± 0.21	5.04 ± 0.29	14.74 ± 0.15	2.60 ± 0.08

Table 2 . Contd

	Mean	sm	1.23 ± 0.06	3.35 ± 0.09	4.58 ± 0.12	14.86 ± 0.13	2.76 ± 0.13
	G1	sm	1.33 ± 0.08	3.07 ± 0.14	4.41 ± 0.22	14.39 ± 0.11	2.33 ± 0.08
	G2	sm	1.31 ± 0.04	3.07 ± 0.07	4.38 ± 0.10	14.49 ± 0.05	2.34 ± 0.05
	G3	sm	1.23 ± 0.10	3.24 ± 0.13	4.47 ± 0.21	14.39 ± 0.11	2.64 ± 0.15
4	G4	sa	1.03 ± 0.10	3.34 ± 0.07	4.37 ± 0.09	14.33 ± 0.08	3.25 ± 0.27
	G5	sm	1.06 ± 0.05	2.87 ± 0.10	3.93 ± 0.15	13.91 ± 0.08	2.72 ± 0.09
	G6	sm	1.37 ± 0.06	3.41 ± 0.29	4.77 ± 0.33	13.96 ± 0.17	2.49 ± 0.16
	Mean	sm	1.22 ± 0.06	3.17 ± 0.08	4.39 ± 0.11	14.24 ± 0.10	2.63 ± 0.14
	G1	sm	1.24 ± 0.06	2.86 ± 0.14	4.10 ± 0.14	13.40 ± 0.10	2.35 ± 0.16
	G2	sm	1.21 ± 0.04	2.97 ± 0.05	4.18 ± 0.08	13.84 ± 0.05	2.47 ± 0.07
	G3	sm	1.18 ± 0.05	2.99 ± 0.10	4.18 ± 0.13	13.44 ± 0.07	2.53 ± 0.09
5	G4	sm	1.17 ± 0.03	3.04 ± 0.10	4.21 ± 0.09	13.81 ± 0.07	2.59 ± 0.12
	G5	sm	1.35 ± 0.05	2.73 ± 0.13	4.06 ± 0.16	14.38 ± 0.09	2.03 ± 0.09
	G6	sm	1.37 ± 0.07	3.29 ± 0.26	4.66 ± 0.32	13.62 ± 0.16	2.39 ± 0.11
	Mean	sm	1.26 ± 0.03	2.98 ± 0.08	4.23 ± 0.09	13.75 ± 0.15	2.39 ± 0.08
	G1	sm	1.25 ± 0.08	2.76 ± 0.15	4.01 ± 0.22	13.10 ± 0.12	2.23 ± 0.10
	G2	sm	1.10 ± 0.04	2.95 ± 0.09	4.05 ± 0.10	13.42 ± 0.06	2.71 ± 0.11
	G3	sm	1.19 ± 0.08	2.69 ± 0.12	3.87 ± 0.18	12.46 ± 0.10	2.26 ± 0.15
6	G4	sa	0.93 ± 0.02	3.12 ± 0.07	4.05 ± 0.08	13.28 ± 0.04	3.37 ± 0.07
	G5	sm	0.96 ± 0.01	2.35 ± 0.09	3.30 ± 0.09	11.69 ± 0.05	2.46 ± 0.10
	G6	sm	1.23 ± 0.04	3.19 ± 0.35	4.43 ± 0.37	12.95 ± 0.19	2.57 ± 0.23
	Mean	sm	1.11 ± 0.06	2.84 ± 0.13	3.95 ± 0.15	12.82 ± 0.26	2.60 ± 0.17
	G1	sm	1.23 ± 0.04	2.76 ± 0.18	3.98 ± 0.20	12.99 ± 0.11	2.24 ± 0.14
	G2	sm	1.08 ± 0.04	2.33 ± 0.07	3.41 ± 0.09	11.29 ± 0.05	2.17 ± 0.08
	G3	sm	1.18 ± 0.03	2.44 ± 0.10	3.61 ± 0.12	11.62 ± 0.07	2.07 ± 0.07
7	G4	sm	1.02 ± 0.07	2.73 ± 0.08	3.75 ± 0.12	12.30 ± 0.07	2.69 ± 0.23
	G5	sm	1.02 ± 0.04	2.21 ± 0.09	3.23 ± 0.12	11.43 ± 0.07	2.18 ± 0.08
	G6	sm	1.31 ± 0.09	2.85 ± 0.24	4.15 ± 0.33	12.15 ± 0.17	2.17 ± 0.07
	mean	sm	1.14 ± 0.05	2.55 ± 0.11	3.69 ± 0.14	11.96 ± 0.26	2.26 ± 0.09

Chr, Chromosome; pop, population. Data are mean ± standard error.

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 also present-ed in Table 3.

The results showed that *P. rhoeas* is a diploid species with 2n= 2x= 14 chromosomes. This is in agreement with the finding of Lavania and Srivastava (1999) and Vorniceanu et al. (2004). Arm ratio index of chromosomes ranged from 2.26 in chromosome 7 to 2.76 in chromosomes 1 and 3. Karyological characteristics of all the 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 G2 and chromosomes 1, 3, 4 and 6 in population G4 had arm ratio values greater than 3 and then according to Levan et al. (1964) were acrocentric chromosomes (Table 2). However, other chromosomes had arm ratio values smaller than 3.00 in all of the populations.

According to the relative length of chromosomes, there were some variations among populations. Chromosome 1 was the longest chromosome in all of the populations except for G2 and G3. However, chromosome 7 was the smallest one in all of the populations (Table 2). One of the chromosomes (chromosome 2) had a secondary constriction on the end of short arm. The SAT chromosome had major variation in size among cells and populations, probably due to differences in chromosome contraction.

In general, the karyotype of this species consisted of seven pairs of submetacentric chromosomes. Vorniceanu et al. (2004) showed that the chromosomes of *P. rhoeas*

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), centromeric gradient (CG), coefficient of variation for chromosome length (CV), dispersion index (DI), the intra-chromosomal asymmetry index (A1) and the inter-chromosomal asymmetry index (A2) and Stebbin's asymmetry category (ST) in six populations (Pop.) of *Papaver rhoeas*.

Pop.	KF	ST	DI	CV(%)	CG	A2	A1	R	DRL (%)	TF (%)
G1	7sm	4A	2.44	8.06	30.28	0.08	0.57	1.22	2.80	30.23
G2	6sm: 1sa	4A	3.47	11.57	29.96	0.12	0.59	1.44	4.98	28.70
G3	7sm	4A	3.63	13.22	27.48	0.13	0.59	1.45	5.24	28.93
G4	4sm: 3sa	4A	2.19	9.30	23.55	0.09	0.67	1.32	3.90	25.28
G5	7sm	4A	4.16	15.47	26.91	0.15	0.58	1.50	5.71	29.21
G6	7sm	4A	3.22	11.27	28.61	0.11	0.58	1.38	4.62	29.32
mean	7sm	4A	3.19	11.48	27.80	0.11	0.60	1.39	4.54	28.61

consisted of six pairs of submetacentric and one pairs of metacentric chromosomes and the arm ratio values was between 1.69 and 2.78. In addition, Lavania and Srivastava (1999) reported four pairs of submetacentric and three pairs of metacentric chromosomes in the karyotype of this species with the arm ratio values between 1.29 and 2.60. However, in this study, all of the chromosomes had arm ratio values greater than 2 and then were submetacentic (acrocentric in some populations).

According to asymmetry indices, the category of all the populations was the same and placed on 4A class of Stebbins (1971) asymmetry categories with minor variations among the populations (Table 3). This was dissimilar with previous reports (Lavania and Srivastava, 1992, 1999) in which they placed it on 2A category. Vorniceanu et al. (2004) also showed that the karyotype of this species is symmetric and less evolved. This considerable increase in asymmetry indicates that these populations experienced structural changes of the chromosomes or inter species mating. Hybridization often occurs among *P. rhoeas* and *Papaver carmeli* and spontaneous hybrids *P. rhoeas*× *Papaver dubium* occur quite often (Kadereit, 1990).

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