Study of Karyological Characteristics in *Papaver bracteatum* and *Papaver somniferum*

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Summary In this study the variation of various karyotype characteristics in different populations of *Papaver somniferum* (opium poppy) and *Papaver bracteatum* (Iranian poppy) was evaluated. All populations were diploid, as the basic chromosome number of *P. bracteatum* and *P. somniferum* was 7 and 11, respectively. In *P. bracteatum*, the 14202, 16986 and P1007107 populations represented 2 metacentric and 5 submetacentric chromosomes, while the 23157 and P1006859 populations represented 1 metacentric, 5 submetacentric and 1 subtelocentric chromosomes. In *P. somniferum* the 13912 population had 2 metacentric, 5 submetacentric, 4 subtelocentric chromosomes, and the 13913 population comprised 2 metacentric, 7 submetacentric and 2 subtelocentric chromosomes, while the rest of the populations had 2 metacentric, 6 submetacentric and 3 subtelocentric chromosomes. All of the populations indicated 3A category, except for P1007107 whose category was 2A. In *P. bracteatum* the maximum and minimum chromosomal total length were 28.70 and 22.90 in the 23157 and 14202 populations, respectively, while in *P. somniferum* they were 32.97 and 29.05 in the 15093 and 13911 populations, respectively. Variance analysis indicated that there was no significant difference among the karyological traits in both species and thus, populations with different ecological origin were karyologically similar. The depicted scatter plot based on extracted components from the Principal Component Analysis (PCA) showed that *P. somniferum* populations separated from *P. bracteatum* populations.

Key words Karyotype, *P. bracteatum*, *P. somniferum*, Karyotype asymmetry.

The family Papaveraceae comprises six genus, including *Roemeria*, *Glaucium*, *Meconopsis*, *Papaver*, *Chelidonium* and *Hypecoum*, among which the genus *Papaver* L., comprising about 100 species, is the largest, most advanced and has the greatest importance (Mihalik 1999, Goldblatt 1974). Not only is this genus distributed in the temperate regions of the northern hemisphere, especially Europe and Asia, it also has representative species in North Africa, the west coast of North America, South Africa and South Australia, showing the ancient origin of the genus (Gill and Caine 1988, Goldblatt 1974). The genus, which was subdivided by Fedde (1909) and later by Kadereit (1988) into 9 and 11 sections, respectively, contains several annual, biennial and perennial species (Mihalik 1999, Goldblatt 1974). Despite many of its annual species being major weeds, the notability of the genus relies on the production of medically and economically important benzylisoquinoline alkaloids (BIAs), many of which have potent pharmacological activities. BIAs include the narcotic analgesics morphine and codeine, the cough suppressant and potential anticancer drug noscapine (Barken *et al.* 2008), the antimicrobial agents sanguinarine and berberine, and the vasodilator papaverine (Beaudoin and Facchini 2013). The basic chromosome numbers in

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the genus are $x=6$, $x=7$ and $x=11$, for which the order of prevalence is 7, 11 and 6; the $x=7$ has been found in diploid, tetraploid, hexaploid, octoploid and decaploid levels, $x=11$ in diploid and tetraploid levels and $x=6$ only in the diploid level (Gill and Caine 1988). Two of the most important *Papaver* species based on medical uses are *P. somniferum* (opium poppy) and *P. bracteatum* (Iranian poppy).

Karyotype asymmetry is one of the most important karyotype characters that can reflect the general morphology of the genome. Stebbins (1971) proposed a categorized system for the measurement of karyotype asymmetry and classification of genotypes. Typically, a symmetrical karyotype comprises metacentric and submetacentric chromosomes whose lengths are almost equal. Two events can increase karyotype asymmetry: 1) movement of the centromere position from median/sub median to terminal or subterminal, and 2) accumulation of differences in the relative size between the chromosomes of the complement (Zuo and Yang 2011).

In this study, five populations of each species, *P. somniferum* and *P. bracteatum*, were collected, and the intra- and inter-specific changes in chromosome number and size were assessed.

Materials and methods

Plant materials

Seeds of 10 accessions belonging to two species, *P. somniferum* (14202, 23157, 16986, P1006859 and P1007107 accessions) and *P. bracteatum* (15093, 15122, 13911, 13912 and 13913 accessions), were provided by the Research Institute of Forests and Rangelands and Iranian Biological Resource Center. All accessions, except 13913 whose origin was Afghanistan, were collected from different geographical regions of Iran. Table 1 shows the geographical origin and accession numbers of the studied accessions.

Sample preparation

The surface of the seeds was disinfected using 70% ethanol (60s) followed by sodium hypochlorite (10min) under sterilized conditions. The seeds were then germinated on filtration paper in Petri plates using distilled water under darkness at room temperature (20–25°C) in a completely randomized design. When the length of the roots reached 0.5 to 1.5 cm, they were soaked in 0.002M 8-hydroxy quinoline for 4h at 18°C. After washing, the pretreated roots were subjected to a fixation procedure for 24h using Carnoys-1 solution (glacial acid acetic: absolute ethanol=1:3). The roots were then washed for 1min and soaked in 70% ethanol for long-term conservation. Before staining, samples were hydrolyzed for 5min in 1N chloridric acid at 60°C. The hydrolyzed roots were washed quickly and stained by aceto orcein 2% for 2h. The root tips were separated on microscopic slides and squashed using the acetic squash method. The microscopic slides were

Table 1. Accession number and origin of *P. bracteatum* and *P. somniferum* populations.

Species	Accession number	Origin
<i>P. bracteatum</i>	14202	Iran, West Azarbaijan, Salmas
	23157	Iran, Zanzan, Abhar
	16986	Iran, Kurdistan, Sanandaj
	P1006859	Iran, Mazandaran, Yoush
	P1007107	Iran, Tehran, Lar Dam road
<i>P. somniferum</i>	15093	Iran, Golestan
	15122	Iran, Markazi
	13911	Iran, Lorestan, Kohdasht
	13912	Iran, Fars, Komareh sorkhi
	13913	Afghanistan, Kandahar

observed under an OLYMPUS BH-2 microscope to find desirably separated cells and chromosomes, and three selected cells from different root tips were photographed by a SONY 1080 camera.

Data analysis

The chromosome length in combination with the r -value was used as criteria to pick out homologous chromosomes. Subsequent measurements were performed on haploid data sets. The following traits in each karyotype were measured: TLC (total length of chromosomes), MTLC (mean of total length of chromosomes), MAX (maximum length of chromosome), MIN (minimum length of chromosome), MLA (mean of long arms), MSA (mean of short arms), MrV (mean of r value), MdV (mean of d value), MAR (mean of arm ratio), MCI (mean of chromosome index), MRLC (mean of relative length of chromosomes), DRL% (difference of range of relative length percentage), TF% (total form percentage), S% (relative length of shortest chromosome), A_1 (intrachromosomal asymmetry index) and A_2 (interchromosomal asymmetry index). All karyotype formulas and categories were determined based on Levan *et al.* (1964) and Stebbins (1971), respectively. The formulas are as follows.

$$r \text{ value} = \frac{L}{S}$$

$$d \text{ value} = L - S$$

$$\text{arm ratio} = \frac{S}{L}$$

$$\text{C.I.} = \frac{S}{L + S}$$

$$\text{RLC\%} = \frac{\text{total length of each chromosome}}{\text{total length of chromosomes}} \times 100$$

$$\text{DRL\%} = (\text{maximum relative length}) - (\text{minimum relative length}) \times 100$$

$$\text{TF\%} = \frac{\text{total length of short arms}}{\text{total length of chromosomes}} \times 100$$

$$\text{S\%} = \frac{\text{length of shortest chromosome}}{\text{length of longest chromosome}} \times 100$$

$$A_1 = 1 - \frac{\sum_{i=1}^n \frac{b_i}{B_i}}{n} \quad (\text{Romero Zarco 1986})$$

n =number of homologous chromosome pairs, b_i =the average length of short arms in every homologous chromosome pair, B_i =the average length of long arms in every homologous chromosome pair, $A_2 = S/\bar{x}$ (Romero Zarco 1986), S =standard deviation of chromosome lengths, $\bar{x}$ =mean of chromosome lengths.

Data were analyzed by Micromasure, Microsoft Office Excel 2007, SPSS 15 and SAS 9.3 software. The magnification of the Micromasure software was calibrated utilizing a 10 μm ruler.

Results and discussion

Tables 2 and 3 show the karyotype characteristics of both species, and Figs. 1 and 2 represent the ideograms and some of the metaphase cells for all populations, respectively. All populations of

Table 2. Karyotype characteristics of *P. bracteatum* populations.

Accession code	14202	23157	16986	P1006859	P1007107
<i>2n</i>	14	14	14	14	14
Karyotype formula	1M+1m+5sm	1m+5sm+1st	1M+1m+5sm	1m+5sm+1st	1M+1m+5sm
Karyotype category	3A	3A	3A	3A	2A
TLC	22.90	28.70	25.94	27.66	27.09
MTLC	3.27	4.09	3.70	3.95	4.16
Max	3.41	4.50	4.04	4.50	4.60
Min	3.18	3.61	3.47	3.7	3.46
MLA	2.16	2.76	1.95	2.67	2.47
MSA	1.11	1.34	1.26	1.27	1.39
MrV	2.05	2.15	2.01	2.21	1.84
MdV	1.05	1.41	1.17	1.40	1.07
MAR	0.53	0.49	0.53	0.49	0.58
MCI	0.34	0.32	0.34	0.32	0.36
MRLC	14.28	14.28	14.28	14.28	14.28
DRL	0.98	3.10	2.19	2.87	4.20
TF	33.95	32.69	34.18	32.29	36.10
S	93.36	80.23	85.90	82.30	75.23
A ₁	0.46	0.50	0.46	0.50	0.41
A ₂	0.02	0.07	0.04	0.07	0.10

Table 3. Karyotype characteristics of *P. somniferum* populations.

Accession code	15093	15122	13911	13912	13913
<i>2n</i>	22	22	22	22	22
Karyotype formula	2m+6sm+3st	2m+6sm+3st	2m+6sm+3st	2m+5sm+4st	2m+7sm+2st
Karyotype category	3A	3A	3A	3A	3A
TLC	32.97	32.41	29.05	29.71	30.69
MTLC	3.02	2.94	2.64	2.70	2.79
Max	3.67	4.08	3.53	3.77	3.42
Min	2.54	2.41	1.98	2.07	2.33
MLA	2.12	2.07	1.89	1.88	1.22
MSA	0.87	0.87	0.76	0.81	0.85
MrV	2.53	2.47	2.60	2.41	2.35
MdV	1.24	1.20	1.13	1.07	1.08
MAR	0.44	0.45	0.44	0.46	0.47
MCI	0.29	0.30	0.29	0.30	0.31
MRLC	9.09	9.09	9.09	9.09	9.09
DRL	3.41	5.15	5.33	5.73	3.57
TF	29.18	29.50	28.80	30.15	30.60
S	69.31	59.08	56.09	54.87	67.96
A ₁	0.56	0.54	0.55	0.54	0.52
A ₂	0.13	0.19	0.17	0.18	0.19

both species were diploid, as the basic chromosome number of *P. bracteatum* and *P. somniferum* was 7 and 11, respectively. In *P. bracteatum*, the 14202, 16986 and P1007107 populations represented 2 metacentric and 5 submetacentric chromosomes, while the 23157 and P1006859 populations represented 1 metacentric, 5 submetacentric and 1 subtelocentric chromosomes. In *P. somniferum*, the 13912 population had 2 metacentric, 5 submetacentric, 4 subtelocentric chromosomes, and the 13913 population comprised 2 metacentric, 7 submetacentric and 2 subtelocentric chromosomes, while the rest of populations had 2 metacentric, 6 submetacentric and 3 subtelocentric chromosomes. All of the populations indicated 3A category, except for P1007107 whose category was 2A. In *P. bracteatum* the maximum and minimum of chromosomal total length was 28.70 and 22.90 in the 23157 and 14202 populations, respectively, while in *P. somniferum* it

Fig. 1. Ideograms of different populations. 15093, 15122, 13911, 13912 and 13913 represent *P. somniferum* populations. 14202, 23157, 16986, P1006859 and P1007107 represent *P. bracteatum* populations.

was 32.97 and 29.05 in the 15093 and 13911 populations, respectively. The range of chromosome size in *P. bracteatum* was determined to be between 3.18 to 4.60, while in *P. somniferum* it was 1.98 to 4.08, which is consistent with Hrishi (1960). However, Kaul *et al.* (1979) reported that the range of chromosome size in *P. somniferum* is 6.3 to 11.4. The methods utilized for chromosome measurements were optic and user dependent, and some of the observed variations might be related to user errors. Interestingly, the average relative length of chromosomes in all populations of each species was equal, demonstrating the capability of this trait to elucidate the similarity among the various karyotypes. In both species, the intrachromosomal asymmetry index (A_1) and karyotype categories illustrated that the obtained karyotypes are semi-symmetric, and therefore both species might be evolutionally relatively advanced. Moreover, the interchromosomal asymmetry index (A_2) in both species demonstrated low differences among chromosome lengths in each karyotype. Farjaminejad *et al.* (2011) assessed the karyological characteristics in several populations of *P. bracteatum* Lindl in Iran. Consistent with our study, they reported that the studied populations had 2 metacentric and 5 submetacentric chromosomes, and the karyotype categorizes as 2A and 3A.

Fig. 2. Some of the metaphase cells in *P. somniferum* and *P. bracteatum*. 15093, 15122, 13911, 13912 and 13913 represent *P. somniferum* populations. 14202, 23157, 16986, P1006859 and P1007107 represent *P. bracteatum* populations.

Variance analysis based on completely randomized design with three replications indicated that there was no significant difference among the karyological traits in the populations of both species. Thus, in each species, the populations with different ecological origins are karyologically similar, and the different environmental conditions probably had no obvious effect on chromosomal characters. Although it is evident that three karyograms for each population do not allow attaining a general inference on accurate karyotype characteristics, they allow for an approximate evaluation. Farjaminejad *et al.* (2011) reported that there were no significant differences among the karyological asymmetry indices in various populations of *P. bracteatum*. Madadi *et al.* (2009) studied the karyotype and morphology of mitotic chromosomes in five populations of the medicinal plant *Papaver dubium* collected from various geographical locations of northwestern Iran, and reported that 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.

Fig. 3. Scatter plot of populations of both species, *P. somniferum* and *P. bracteatum*, based on PCA1 and PCA2.

In their study, all of the populations were placed in the 3A class of Stebbins asymmetry categories. Moreover, in the karyological study of the medicinal plant *Papaver rhoeas* from northwestern Iran, Asghari-Zakaria *et al.* (2011) showed that karyological characteristics of all the materials studied were similar, and that all of the populations are placed in the 4A class of Stebbins asymmetry categories.

To survey whether the characteristics could efficiently segregate the two species' populations, principle component analysis (PCA) was applied to our data. In total, two components were extracted; the first and second components explained 70.44 and 24.41 of the total variance, respectively. Based on the two components (PCA 1 and PCA 2), a scatter plot (Fig. 3) was produced. In this plot, *P. somniferum* populations were located in the third zone, but *P. bracteatum* populations were dispersed throughout the other zones of the plot. Thus, the populations were segregated successfully.

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