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A Cytotaxonomic Study On *Dianthus* L. Species In North Eastern Iran

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Abstract: In order to investigate the karyotype of *Dianthus*, a study was carried out on nine populations of *Dianthus crinitus*, *D. binaludensis* (an endemic species in Iran) and *D. polylepis* in the North-East of Iran. For present research, 8- Hydroxy quinolein 0.0002 M and aceto-orcein were used as pretreatment solution and staining. The basic chromosome number of *Dianthus* was X= 15. *D. binaludensis* and *D. polylepis* were diploid, while *D. crinitus* was found to be tetraploid. In addition, for the first time the karyotypes of the three species of *Dianthus* were prepared and compared with their morphological characters. *D. binaludensis* and *D. polylepis* were found to be morphologically close, with the same ploidy level but *D. crinitus* differs from the other species by in that it is tetraploid suffruticous in habit, has white petals and a membranous herbaceous stigma with nerves.

Key words: *Dianthus*, Caryophyllaceae, karyology, diploid, tetraploid

INTRODUCTION

Dianthus L. belongs to the Silenoideae tribe of the Caryophyllaceae family (Engler, 1887). Over 300 *Dianthus* species have been identified in Europe, Asia, North America, north Africa (Gorshkova, 1970; Davis, 1965-85; Punt and Hoen, 1995), On the basis of Flora Iranica report, *Dianthus* has six sections, 30 and 12 species and subspecies respectively. Just, four species belong to sect. *Fimbriati* grow in the province of Khorasan-Razavi (northeastern of Iran). These species are: *D. crinitus* Sm. p.p, *D. binaludensis* Rech. f, *D. polylepis* Bienert ex Boiss. and *D. orientalis* Adams. p.p. (Rechinger, 1988). This genus is important by having medicinal characters, aromatic materials and polymorphism in morphology, genetic and hybridization (McGeorge and Hammett, 2002; Facciola, 1990; Hughes, 1993; Lee *et al*., 2005; Su Yeons, 2002). The differences of morphology among the populations prevent to identify species easily. On the other hand, the recognizing of distinct species is difficult because of similarity among the species. Previous morphological studies of this genus confirmed the variation in the amount of indument on the stem and leaves and in the size, shape and color of the leaves, petals and the calyx (Carine and Shykoff, 2003). The aim of present research was to identify chromosome number and find relationship between variation of karyotype and morphology. According to previous karyological reports, this genus was diploid and seldom triploid in some hybrid like *D. caryophyllus* x *D. isensis* (Nimura *et al*., 2004, 2006; Nimura, 2008; Goralski *et al*., 2009). There also have been some karyological studies on *Dianthus* sect. *Plumaria*, *Dianthus nardiformis* Joe ka. and *Dianthus spiculifolius* Schur (Weiss *et al*., 2002; Ioni *et al*., 2003). Karyological reports cited by Weiss *et al*. (2002) were shown in Table 1. Likewise, for the first time in Iran, this study investigated the karyological characteristics of *Dianthus* growing in northeastern Iran for identifying any relationship between their morphological characters and the cytological data.

MATERIALS AND METHODS

The materials were collected from Northeastern Iran (Table 2) during May to June 2008. Voucher specimens were deposited in the herbariums of IAUM and FUMH. For karyotype preparing, a pretreatment was performed at room temperature for three hours before root tip fixation of the three species of *Dianthus* in 0.002 M 8-hydroxyquinoline. Then following fixation in a cold mixture of ethanol and acetic acid (3:1), the next steps were maceration in 1N HCl at 60°C for 5-8 min, washing in water, cutting off the meristems and squashing them in a drop of 45% acetic acid (Krahulkova, 2003). Next, the chromosomes were described according to Levan's terminology (Levan *et al*., 1964). Then, the karyotypes were compared using total form percentage (Forni-Martins *et al*. 1994). Finally, the ratios of the longest to the shortest chromosomes were calculated

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Table 1: Previous reports of somatic chromosomes number in *Dianthus*

Species	2n	References
D. acicularis	30, 90	Spasskaja and Plaksina (1995), Rohweder (1934)
D. arenarius subsp. *bohemicus*	60	Kovanda (1984)
D. arenarius subsp. *borussicus*	60	Carolin (1957), Baksay (1972), Kovanda (1984)
D. broteri	60	Carolin (1957), Coy *et al.* (1997)
D. gallicus	45, 60, 90	Rohweder (1934), Blackburn and Morton (1957), Carolin (1957)
D. hungaricus subsp. *hungaricus*	30, 60, 90	Rohweder (1934), Kovanda (1984), Baksay (1972), Kovanda (1984), Kmetova (1985)
D. lumnitzeri	30, 60	Puch (1941), Gencev (1937)
D. lumnitzeri var. *lumnitzeri*	90	Baksay (1972), Kovanda (1984), Kmetova (1985)
D. lumnitzeri var. *palaviensis*	60, 90	Kovanda (1982), Kmetova (1985), Weiss *et al.* (2002)
D. moravicus	60, 90	Kovanda (1982, 1984), Weiss *et al.* (2002)
D. petraeus s. l.	30, 60, 90	Rohweder (1934), Gencev (1937), Carolin (1957), Grif (1965)
D. plumarius subsp. *blandus*	60, 75, 90	Carolin (1957)
D. plumarius subsp. *hoppei*	90	Kmetova (1985), Carolin (1957)
D. plumarius subsp. *neilreichii*	60, 90	Weiss *et al.* (2002)
D. regis-stephani	60, 90	Rohweder (1934), Gencev (1937), Carolin (1957), Baksay (1972)
D. spiculifolius	60, 90	Rohweder (1934), Gencev (1937)

Table 2: The locations of the studied *Dianthus* populations

Population number	Species	Location	Alt
1	*D. binaludensis*	S. Moghan Mts	2150
2	*D. binaludensis*	Boujan Mts. northern slopes	1800
3	*D. binaludensis*	Mountains between Kang and Zoshk	2000
4	*D. polylepis*	Kardeh Mts.	1450
5	*D. polylepis*	N Torbat Heydariyeh, Khomari pass	1650
6	*D. polylepis*	Fariman, Dam Mts.	1700
7	*D. crinitus* subsp. *turcamanicus*	NW Kalat-e Naderi, Sarrud Mts.	1360
8	*D. crinitus* subsp. *turcamanicus*	S. Moghan Mts	1900
9	*D. crinitus* subsp. *turcamanicus*	NW Mashhad, Ardok Mts.	1450

(Verma, 1980). Symmetry karyotypes were determined using Stebbins' two-way system that is based on percent of r-value (Stebbins, 1971). Present study was conducted in plant research lab of Islamic Azad university, Mashhad branch.

RESULTS

The number of somatic chromosomes along with the details of the karyotypes of the studied species of *Dianthus* is shown in Table 3. The results indicated that *D. binaludensis* (Moghan population) is diploid (2n = 30) and has the karyotype formula (11M+1m+2sm+1st) with 76.65% metacenteric chromosomes (Fig. 1a, 4a). The Boujan population of this species had the same ploidy level (2n = 30) but with the karyotype formula (12M+1m+1sm+1st) and 50% metacenteric chromosomes (Figs. 1b, 4b). Also, the Zoshk population was diploid with the karyotype formula (12M+1m+2sm) and 80% metacenteric chromosomes that were found to be more symmetric than the population in Boujan (Fig. 1c, 4c). *Dianthus polylepis* (Kardeh population) was found to be diploid with the karyotype formula (10M+1m+4sm) and 66% metacenteric chromosomes (Fig. 2a, 5a) that were more asymmetrical than the other populations of this species. The Torbat-e Heydariye population was also found to be diploid with the karyotype formula (11M+2m+2sm) and 73% metacenteric chromosomes

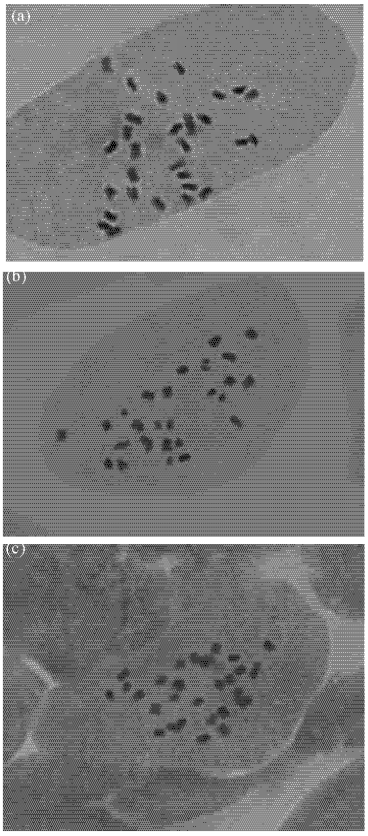

Fig. 1: *D. binaludensis* (a) Moghan population, (b) Boujan population and (c) Zoshk population

Table 3: Karyotypic details of the studied *Dianthus* populations

Population	Class	2n	TF (%)	S (%)	D.R.L.	T.V	T (L/S)	L (μ)	S (μ)	TL	K.F.
1	2A	30	43.69	60	1.67	670.85	1.66	3.20	1.92	76.16	(11M+1m+2sm+1st)
2	2A	30	40.16	60	1.67	400.65	1.66	3.20	1.92	76.48	(12M+1m+1sm+1st)
3	2A	30	45.79	60	1.67	760.77	1.66	3.20	1.92	76.16	(12M+1m+2sm)
4	2A	30	43.98	60	1.67	561.58	1.66	3.20	1.92	69.12	(10M+1m+4sm)
5	2A	30	44.96	50	4.02	854.52	1.66	3.20	1.92	95.36	(11M+2m+2sm)
6	2A	30	45.08	75	0.92	662.56	1.33	1.56	1.92	71.68	(11M+1m+3sm)
7	2A	50	46.92	50	0.82	888.57	1.66	3.20	1.92	144.64	(25M+3m+1sm+1st)
8	2B	60	46.60	50	0.73	1044.46	1.66	3.20	1.92	164.80	(23M+6m+1sm)
9	2B	60	45.46	33	1.39	1454.79	1.50	3.84	2.56	183.72	(20M+8m+2sm)

TL: Total chromosome length, S: Shortest chromosome, L: Longest chromosome, L/S: Longest/shortest, TF%: Total karyotype form percentage, T.V: Total volume, D.R.L: Difference of range of relative length, S%: Bazzichelli index *D. binaludensis*: (1) (Moghan population). 2) Boujan population. 3) Zoshk population *D. polylepis*: 4) Kardeh population. 5) Torbat Heidariye population. 6) Fariman population *D. crinitus* subsp. *Turcominicus*: 7) Sarrud population. 8) Moghan population. 9) Ardak population

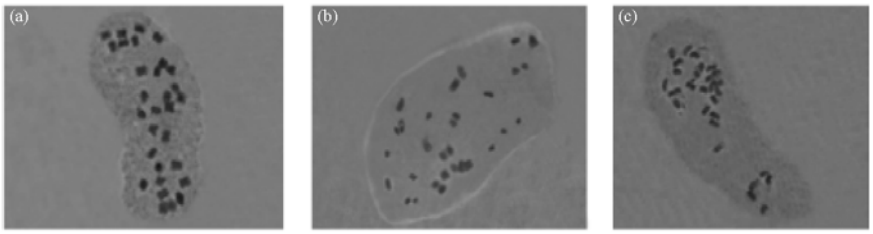

Fig. 2: *D. polylepis* (a) Karde population (b) Torbat Heydariye population and (c) Fariman

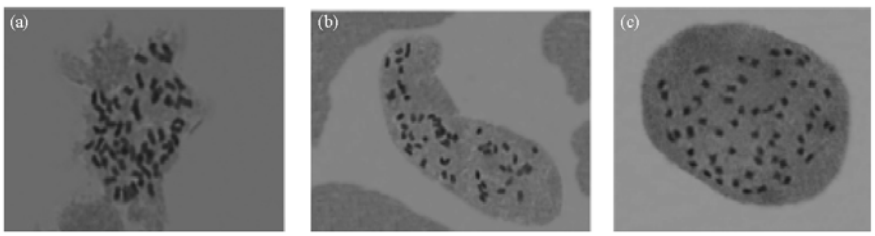

Fig. 3: *D. crinitus* (a) Sarrud population (b) Moghan population and (c) Ardak population

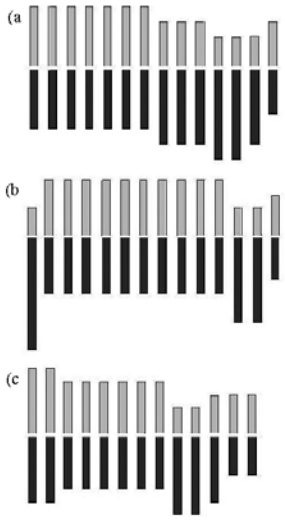

Fig. 4: Ideogram of *D. binaludensis* (a) Moghan population (b) Boujan opulation and (c) Zoshk population

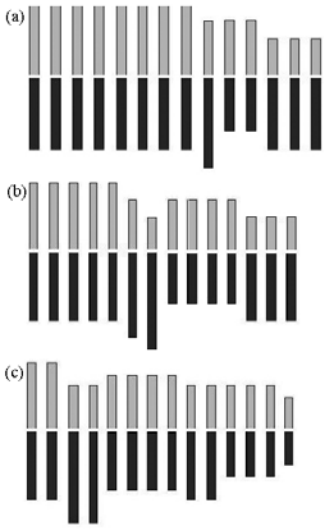

Fig. 5: Ideogram of *D. polylepis* (a) Kardeh population (b) Torbate Heydariyeh population and (c) Fariman population

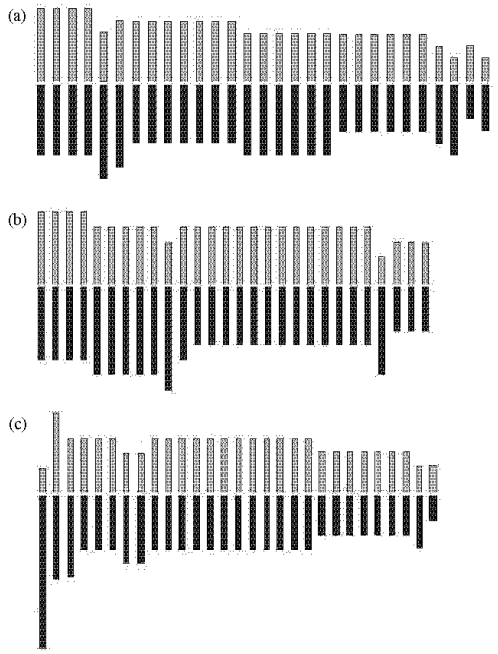

Fig. 6: Ideogram of *D. crinitus* (a) Ortokand population (b) Moghan population and (c) Ardak population

(Fig. 2b, 5b). The Fariman population diploid with the karyotype formula (11M+1m+3sm) and 73% metacenteric chromosomes (Fig. 2c, 5c). All populations of *D. crinitus* were found to be tetraploid (2n = 60). In the Sarrud population the karyotype formula was determined as (25M+3m+1sm+1st). Of all the chromosomes identified, 83% were metacenteric (Fig. 3a, 6a). The karyotype formula of the populations of Moghan and Ardak were (23M+6m+1sm) with 76% etacenteric chromosomes and (20M+8m+2sm) with 66% metacenteric chromosomes, respectively (Fig. 3b, c, 6b, c). All populations fell into class 2B. Also, the findings with regard to total chromatin length and the size of the longest and shortest chromosomes are shown in Table 2. In this regard, the Ardak population of *D. crinitus* had the highest chromatin length, with a total chromatin length of 183.72 μM, while the Fariman population of *D. polylepis* had the lowest with 71.68 μM.

DISCUSSION

The basic number of chromosomes in *Dianthus* is X = 15. Among the previous studies on *Dianthus* sect. *Plumaria,* Weiss identified tetraploid and hexaploid cytotypes for *D. nardiformis* Jan ka (Weiss *et al*., 2002). Also Ioni reported diploid and tetraploid cytotypes for *D. spiculifolius*, Schur (Ioni *et al*., 2003). Diploid was reported in *D. cyprius* by Yildiz and Gucel (2006). According to interesting report, different cytotype of *D. broteri* had 2n = 45, 90 and 180 (Balao *et al*., 2009). But usually diploid were identified (Carolin, 1957). According to the karyological results in the present study, the whole populations of *D. binaludensis* and *D. polylepis* were found to be diploid, while the populations of *D. crinitus* were identified as tetraploid. Also, the karyotypes of the above mentioned populations was relatively symmetric. Thus, on the basis of Stebbins' two- way table, all of the populations in this study fall into class 2B. The populations of *D. polylepis* in Fariman and Torbat-e Heydarieh were the most asymmetric ones. On the other hand, previous morphological studies of this genus confirmed the variation in the amount of indument on the stem and leaves and in the size, shape and color of the leaves, petals and the calyx. In other words, the characteristics of this species vary from one population to another (Carine and Shykoff, 2003). In the present study, however, *D. binaludensis* and *D. polylepis* were found to be morphologically close, with the same ploidy level, habit and flowers of similar color, size and shape. They are caespitose-suffruticous and have similarities like white to purple petals, scaly stigma without nerves and scaly bracts. However, they differ in that *D. polylepis* has lanceolate bracts while *D. binaludensis* has ovate bracts. Both of them grow on northern slopes of stony mountains and at the same altitude. *D. crinitus* differs from the other species by in that it is tetraploid suffruticous in habit, has white petals and a membranous herbaceous stigma with nerves. This species is divided into varieties. In spite of the variation in morphological characteristics, the types of chromosomes are almost homogeneous. There was no relationship between the type of chromosomes and the morphological finding.

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