Research Paper

A karyological study of some Dianthus L. species (Caryophyllaceae) in Northeast of Iran

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Abstract

This study focuses on karyological investigation of four taxa of Dianthus (Caryophyllaceae) including D. crossopetalus, D. orientalis subsp. stenocalyx, D. orientalis subsp. gorganicus, and Dianthus sp. distributed in Northeast of Iran. The karyotype asymmetry/symmetry was evaluated using two methods: 1) \( CV_{CL} \) (coefficient of variation of chromosome length) and \( CV_{CI} \) (heterogeneity of the centromeric index); and 2) \( MCA \) (mean centromeric asymmetry). The karyotype asymmetry was also used to investigate the relationships among the taxa. Results obtained from the current study revealed that there are two different ploidy levels (\( 2n = 2x = 30 \) and \( 2n = 4x = 60 \)) among the investigated taxa. The indices \( CV_{CL} \) and \( MCA \) described karyotype asymmetry correctly based on variation in chromosome length. Diagram of \( CV_{CL} \) vs. \( MCA \) seems to be appropriate for karyological delimitation and taxonomic relationships among the Dianthus taxa under study.

Introduction

Dianthus L. (Caryophyllaceae) comprises a group of annual or perennial species worldwide.

The genus consists of 30 species in Iran. Dianthus with high intra-populational and interspecific variation of morphological and genetic characters has been recognized as a complex genus (BEHROOZIAN 2010). Therefore, morphological characters alone do not provide effective clues for delimitation of Dianthus species (BEHROOZIAN 2010). Additional studies such as cytology and molecular approaches could be useful for resolving taxonomic problems in plants (SEN 1975; LARSON et al. 2010; MARCUSSEN & BORGEN 2011; GAO et al. 2012). The last decades, karyological investigations provided fundamental characters for plant systematics and evolutionary analysis (STACE 2000). Recently, scientists have developed a variety of methods for karyotype asymmetry analysis (reviewed in ZUO & YUAN 2011; PERUZZI & EROĞLU 2013).

Dianthus with seven ploidy levels (2x, 3x, 4x, 5x, 6x, 8x, and 12x; reviewed in WEISS et al. 2002 and BALAO et al. 2009), is one of the genera with the most extensive ploidy series known in the subfamily Caryophylloideae. BALAO et al. (2010) suggested that different ploidy levels within and between populations...
of the genus are related to highly morphological variations. They declared that the evolution of cytological features within the genus has taken place in conjunction with morphological diversification. Furthermore, different ploidy levels have been reported for populations at the subspecific level (Weiiss et al. 2002). Karyological studies on Dianthus are often restricted to just reporting of chromosome numbers, whereas karyological analyses were rarely reported. The aims of the present study are: 1) to report somatic chromosome numbers; 2) to assess karyotype asymmetry/symmetry; and 3) to reveal the karyotype relationships among some Dianthus species distributed in Northeast of Iran.

Material and methods

In order to study the karyological characteristics of Dianthus species, seeds were collected from various regions of Northeast of Iran. A minimum of five specimens were investigated for each species included in the current study (except for D. sp., see below). Depending on the number of populations per species, these individuals belong to one or more populations. At least five chromosome plates were analyzed per specimen of species. No intraspecific karyological variability has been found. Therefore, one voucher specimen per taxon was presented in Table 1. Voucher specimens are deposited in Ferdowsi University of Mashhad Herbarium (FUMH) (Table 1).

This study focuses on four taxa including D. orientalis subsp. stenocalyx (Boiss.) Rech.f., D. orientalis subsp. gorganicus Rech.f., the new record D. crossoptetalus (Fenzl ex Boiss.) Grossh (Ghaieiremaninejad et al. 2012), and an unknown Dianthus taxon. The last taxon was collected from an isolated and small population during field sampling (Table 1). The individuals of this taxon seem to be morphologically intermediate between D. crinitus subsp. turcomanicus (Schischk.) Rech.f. and D. orientalis subsp. stenocalyx. Their vegetative habit is similar to the first subspecies while their floral characters tend more to the second. Karyological data of D. crinitus subsp. turcomanicus, which is published in previous study (Jaffari & Beirooziyan 2010), are included in the current study to reveal its karyological relationship with the unknown taxon. One individual of this unknown taxon, however, is sampled to investigate its karyological characteristics. This taxon is named here after as Dianthus sp. in this study.

The seeds were germinated at 25 °C. Five to ten root tips of each taxon were pretreated with 8-hydroxyquinoline (0.002 M) for three hours at room temperature. Then, they were fixed in Carnoy’s solution (3:1 absolute ethanol – glacial acetic acid) for 24 h at 4 °C. The fixed root tips were hydrolyzed in 1 N HCl at 60 °C for 5–7 min followed by washing them in distilled water. Then they were stained with aceto-orcein (1% w/v) for 2 h. Finally, the root tips were squashed in acetic acid 45%. The metaphase chromosomes were counted for at least 10 cells of each seedling root. The examined metaphase plates were photographed using Olympus BX52 microscope with automatic camera.

Chromosome measurements including long and short arms, total length of chromosome set, arm ratio index and relative chromosome length were performed for four taxa using the Nutepe software. Chromosomes were identified according to the Levan method (Levan et al. 1964). The homologous chromosomes of diploid taxa were paired by their similarity in size and shape. A number of parameters were used to determine the karyotype asymmetry and symmetry according to Paszkó (2006): $CV_{CI} = \frac{(S_{CI} \times x_{CI}) \times 100}{S_{CI} = \text{the standard deviation of the centromeric index; } x_{CI} = \text{the mean centromeric index}}$, $CV_{CL} = A_2 \times 100$ ($A_2$ is proposed by Zarco.

Table 1
Voucher specimens included in the karyological study of four taxa of Dianthus

<table>
<thead>
<tr>
<th>Voucher No.</th>
<th>Species</th>
<th>Location</th>
<th>Longitude (E)</th>
<th>Latitude (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35410</td>
<td>D. crossoptetalus</td>
<td>Khorassan shomali province, E. Jajarm, between Khorashah and Jorbat</td>
<td>56°45′3.6″</td>
<td>37°06′32.4″</td>
</tr>
<tr>
<td>44075</td>
<td>D. orientalis subsp. stenocalyx</td>
<td>W. Bojnourd, Qorkhod protected area,</td>
<td>56°12′6.9″</td>
<td>37°24′2.7″</td>
</tr>
<tr>
<td>44111</td>
<td>D. orientalis subsp. gorganicus</td>
<td>Golestan province, NW of Islamabad</td>
<td>56°02′19.3″</td>
<td>37°43′0.2″</td>
</tr>
<tr>
<td>44119</td>
<td>Dianthus sp.</td>
<td>SW. Bojnourd, between Hessar-Hosseini and Rakhtian</td>
<td>57°09′24.2″</td>
<td>37°18′12.4″</td>
</tr>
</tbody>
</table>
Table 2
Karyotype formula according to LEVAN et al. (1964) used in the present work: SC – the shortest chromosome length; LC – the longest chromosome length; CL – mean length of chromosome; CI – mean centromeric index; SD – standard deviation; K.F. – karyotype formula. Karyological data of D. crinitus subsp. turcomanicus were used with permission from JAFFARI and BEHROOZIAN 2010.

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>2n</th>
<th>Range SC-LS</th>
<th>Ratio LC/SC</th>
<th>CL (µm) Mean (±SD)</th>
<th>CI (µm) Mean (±SD)</th>
<th>K.F.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>D. crossopetalus</td>
<td>30</td>
<td>1.94–3.45</td>
<td>1.78</td>
<td>2.61 (±0.37)</td>
<td>35.63 (±7.54)</td>
<td>10m + 20sm</td>
</tr>
<tr>
<td>2</td>
<td>D. orientalis subsp. stenocalyx</td>
<td>30</td>
<td>2.01–3.62</td>
<td>1.80</td>
<td>2.74 (±0.38)</td>
<td>40.30 (±6.57)</td>
<td>24m + 4sm + 2st</td>
</tr>
<tr>
<td>3</td>
<td>D. orientalis subsp. gorganicus</td>
<td>60</td>
<td>1.81–3.84</td>
<td>2.12</td>
<td>2.73 (±0.44)</td>
<td>37.38 (±9.84)</td>
<td>24m + 28sm + 8st</td>
</tr>
<tr>
<td>4</td>
<td>Dianthus sp.</td>
<td>60</td>
<td>2.16–3.35</td>
<td>2.17</td>
<td>2.67 (±0.29)</td>
<td>34.80 (±7.65)</td>
<td>24m + 32sm + 4st</td>
</tr>
<tr>
<td>5</td>
<td>D. crinitus subsp. turcomanicus</td>
<td>60</td>
<td>1.92–3.84</td>
<td>2.1</td>
<td>2.73 (±0.43)</td>
<td>47.11 (±5.54)</td>
<td>46m + 12sm + 2st</td>
</tr>
</tbody>
</table>

1986), and PERUZZI and EROĞLU (2013); MC = A × 100 (A is proposed by WATANABE et al. 1999). CV_{CI} and MC_{CI} indices provide the most suitable methods for estimating karyotype asymmetry, while the CV_{CI} did not describe karyotype asymmetry correctly (Zuo & Yuan 2011; Peruzzi & Ergülu 2013). Therefore, a bidimensional scatter plot was drawn with the couple of parameters CV_{CI} and MC_{CI} to reveal karyotype relationships among the organisms included in the current study. In addition, an idiogram was constructed for each taxa based on the average of the mean values calculated for the karyotypes using data taken from Table 2.

Table 3
Asymmetry indices used for the classification of Dianthus taxa: CV_{CL} – coefficient of variation of chromosome length; CV_{CI} – heterogeneity of the centromeric index; MC_{CI} – mean centromeric asymmetry. Karyological data of D. crinitus subsp. turcomanicus were used with permission from JAFFARI and BEHROOZIAN (2010).

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>CV_{CL}</th>
<th>CV_{CI}</th>
<th>MC_{CI}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>D. crossopetalus</td>
<td>14.23</td>
<td>21.16</td>
<td>29</td>
</tr>
<tr>
<td>2</td>
<td>D. orientalis subsp. stenocalyx</td>
<td>14.01</td>
<td>16.31</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>D. orientalis subsp. gorganicus</td>
<td>16.22</td>
<td>25.91</td>
<td>33</td>
</tr>
<tr>
<td>4</td>
<td>Dianthus sp.</td>
<td>12.98</td>
<td>21.99</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>D. crinitus subsp. turcomanicus</td>
<td>13.86</td>
<td>11.76</td>
<td>5.8</td>
</tr>
</tbody>
</table>
Fig. 1
Somatic chromosomes of four taxa of *Dianthus*: A) *D. crossopetalus*; B) *D. orientalis* subsp. *stenocalyx*; C) *D. orientalis* subsp. *gorganicus*; and D) *Dianthus* sp. Karyotypes of the two first species are illustrated below each metaphasic plate.
followed by submetacentric chromosomes (36.32%), whereas subtelocentric chromosomes are rare (6.25%). The detailed information of karyotype formulae is shown in Table 2.

Asymmetry indices estimated on the basis of statistical data resolved the Dianthus karyotypes into the range of symmetrical to lowest asymmetrical values. Karyotype asymmetry also depends on both the relative variation in chromosome length (CVCL) and heterogeneity of the centromeric index (CVCI). Dianthus orientalis subsp. gorganicus is characterized by the highest value of both CVCL and CVCI, and the remaining species were characterized by lower values of both CVCL and CVC (Table 3). In addition, the results indicate that the most asymmetrical karyotype belongs to D. orientalis subsp. gorganicus with the highest karyotype asymmetry of CVCL = 16.22 and MCA = 33.

According to the scatter plot (Fig. 3) obtained from the parameters CVCL vs. MCA, D. crinitus subsp. turcomanicus and D. orientalis subsp. gorganicus show the lowest and highest MCA, respectively. Furthermore, D. crossopetalus and D. sp. exhibit relatively high MCA. Among all taxa investigated in the present study, D. sp. and D. orientalis subsp. gorganicus demonstrate the lowest and highest CVCL, respectively.

Discussion

The results obtained from the present study indicate that both subspecies of D. orientalis are identified with two different ploidy levels. Dianthus orientalis subsp. stenocalyx (2n = 2x = 30) has a widespread distributional range from east to west of Iran, whereas D. orientalis subsp. gorganicus (2n = 4x = 60) is distributed in narrow range in Northeastern Iran. JANAKI AMMAL and SELIGMAN (1956) reported co-occurrence of diploid and tetraploid individuals...
Fig. 3
Scatter diagram of *Dianthus* taxa based on the karyotype parameters $CV_{cl}$ vs. $M_{cx}$. *D. crospetalus* (plus sign), *Dianthus* sp. (cross sign), *D. orientalis* subsp. *gorganicus* (white circle), *D. orientalis* subsp. *stenocalyx* (dark circle), *D. crinitus* subsp. *turcomanicus* (triangle). Karyological data of *D. crinitus* subsp. *turcomanicus* were used with permission from JAFFARI and BEHROOZIAN (2010).
within a population of Dianthus monspessulanus L. (2n = 2x = 30 and 2n = 4x = 60). Likewise, WEISS et al. (2002) reported occurrence of tetraploid, pentaploid and hexaploid cytotypes in two subspecies including Dianthus plumarius subsp. blandus (RCHB.) HAYEK (2n = 4x = 60, 2n = 5x = 75, and 2n = 6x = 90) and D. plumarius subsp. hoppeii (Port.) Hegi (2n = 6x = 90). They also expressed that an autopolyploidization model agrees with the occurrence of polyploids within diploid species such as D. monspessulanus, D. superbus L., D. acicularis FISCH. ex LEDEB., D. petraeus WALDST. & KIT., and putatively diploid species D. arenarius L., and D. gratianopolitanus Vill. Moreover, the report of GATT et al. (1998) on significant production of unreduced pollen grains in diploid species D. knappii (PANT.) ASCH. & KANITZ ex BORBÁS further strengthens this hypothesis. However, the occurrence of polyploidy and genome duplication as two major evolutionary phenomena seem to play prominent roles in speciation within Dianthus (CAROLINE 1957; WEISS et al. 2002; BALAO et al. 2010). Results obtained from the scatter diagrams (Fig. 3) showed that D. orientalis subsp. gorganicus could be identified by its highly asymmetric karyotypes while D. orientalis subsp. stenocalyx separated from the other taxa with the lowest asymmetry karyotype.

Karyotypes and systematics

The CVL vs. MVE diagram is rather preferable than scatter diagrams based on other indices because it is better suited to demonstrate karyotype relationships among taxa, especially when chromosome size variation is negligible (PE-RUZZI & EROGLU 2013). Dianthus orientalis subsp. gorganicus and Dianthus sp. displayed a high asymmetry index value with a high level of karyotype asymmetry (Table 3). In the other hand, D. orientalis subsp. stenocalyx and D. crospotetalus showed a low asymmetry index value indicating greater karyotype symmetry and stabilized genome (SHAMURAILAT-PAM et al. 2012). Two subspecies of D. orientalis were not grouped together in the scatter plot (Fig. 3). Dianthus orientalis subsp. stenocalyx with the lowest karyotype asymmetry (among the taxa included in the present study) is separated from the other taxa (Fig. 3). Conversely, D. orientalis subsp. gorganicus with the highest karyotype asymmetry shows obviously difference in morphological features of chromosomes as compared to those of the subspecies D. orientalis subsp. stenocalyx. It was also reported that increasing karyotype asymmetry could occur via a shift of the centromere position from median to subterminal or terminal chromosome and differences in the size of individual chromosomes (LIU et al. 2006). According to the karyotype difference between D. orientalis subsp. stenocalyx and D. orientalis subsp. gorganicus, it seems, however, that the taxonomic position of D. orientalis subsp. gorganicus could be considered as an independent species. Nonetheless, the exactly taxonomic position of this taxon should be investigated by further data.

There has been some uncertainty regarding the taxonomic status of D. crospotetalus. In Flora Kavkaza (GROSSHEIM 1930) this species is regarded as synonymous with D. crinitus Sm. In Flora of Turkey (DAVIS 1967) this species is recognized as a variety of D. crinitus, while in Flora Orientalis (BOISSIER 1975), Flora of USSR (SHISHKIN 1936), Flora Iranica (RECHINGER 1986), and ASSADI (1985), it is considered as an autonomous species. Dianthus crinitus is a tetraploid species (2n = 2x = 60) with symmetrical karyotype (JAFFARI and BEHROOZIAN 2010), while results of the present study demonstrated that D. crospotetalus (2n = 2x = 30) could be assessed as an intermediate karyotype asymmetry when compared to the other taxa (Table 3). In addition, the karyological position of D. crospotetalus in scatter diagram (Fig. 3) is obviously discriminated from that of D. crinitus. As a result, based on the karyotype asymmetry analyses (JAFFARI & BEHROOZIAN 2010 and the results of the current study) and morphometric investigation (unpublished results), our karyological interpretation on the taxonomic position of D. crospotetalus is that this taxon is probably an autonomous species rather than a variety or synonym of D. crinitus.

Dianthus sp. with an unknown taxonomic position is morphologically intermediate between D. crinitus subsp. turcomanicus and D. orientalis subsp. stenocalyx. Results of this study show that this species has its own karyotype characteristics (Fig. 3). Although karyo-
type features comparably provide valuable insights into taxonomic relationship (Silijak-Yakovlev & Peruzzi 2012) but we suggest that more investigations are necessary to clarify its exactly taxonomic position.

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