INTRODUCTION

The species of the genus Clypeola L. are annual plants belonging to the tribe Alysseae of Brassicaceae. Distribution of this genus is limited to northern hemisphere. Rechinger (1968) noted five species for this genus in Iran including C. aspera Turrill, C. lappacea Boiss, C. dichotoma Boiss, C. jonthlaspi L. and C. microcarpa Boiss.

Al-Shehbaz et al. (2006) and Warwick et al. (2008) found the chromosome basic number x = 8 for Alysseae. They also confirmed the probability of presence of aneuploidy series below or more than eight in this tribe. Basic chromosome number in Clypeola was found x = 7, 8 by Warwick et al. (2006 & 2008). They found n = 7, 8, 14 & 16 in haploid and 2n = 14, 16 & 32 in diploid individuals. Warwick et al. (2008) found that C. aspera and C. lappacea (both taxa with n = 7) formed a well-supported clade (98% bootstrap support), separate from C. jonthlaspi (an n = 8 species).
MATERIALS AND METHODS

Boiss. C. dichotoma L. C. lappacea Turrill C. aspera Boiss. C. jonthlaspi Turill

For meiotic studies, young flower buds were collected from 10 randomly selected plants of each population and fixed in glacial acetic acid and ethanol (1:3) for 24 hrs. Flower buds were washed and preserved in 70% ethanol at 4°C until used (Sheidai et al. 1999). Cytological preparations used squash technique and 2% aceto-orcein as the stain. The best time for fixing is 11-13.

For karyotype study the seeds of studied species after sterilization, were kept in 4°C for about 7 days. Freshly grown root tips were collected from the germinated seeds of at least ten randomly selected plants in each population. They were pretreated with 0.2 M 8-hydroxyquinolin for about 3-5 hours or with Ice water for about 2 hours and fixed in ethanol and acetic acid (3:1) for 24 hrs. The fixed tips were then washed thoroughly in distilled water and macerated in 60°C in HCL for about 3-5 min. Squash technique was used for cytological studies with 2% aqueous aceto-orcein as the stain. The somatic chromosome number and karyotype details were studied in at least five well-prepared metaphase plates. The chromosomes were photographed by digital camera and measured by Image Tools3 software (Sheidai et al. 2000; Borgen 1987). The Chromosomes were identified according to Levan et al. (1964). Karyotype symmetry was determined according to Stebbins (1971), while other karyotype parameters like haploid total chromosome length, total form percentage (TF% = Sum of short arms of the chromosomes/Total chromosome length) and coefficient of variation (CV; Verma 1980) of the chromosome size, Stebbins two ways system of karyotype symmetry (Stebbins 1971) as well as A1 and A2 indices of Romero-Zarco (1986) were determined (Sheidai & Jalilian 2008).
Table 2. Karyotypic features of the Clypeola species and populations studied:

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>T.L (µm)</th>
<th>L (µm)</th>
<th>S (µm)</th>
<th>L/S</th>
<th>TF%</th>
<th>X</th>
<th>S%</th>
<th>A1</th>
<th>A2</th>
<th>C.V</th>
<th>D.R.L</th>
<th>St</th>
<th>KF</th>
<th>2n</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. aspera</em></td>
<td>Neyzar village</td>
<td>34.79</td>
<td>5.05</td>
<td>1.9</td>
<td>2.65</td>
<td>38.28%</td>
<td>2.89</td>
<td>37.62%</td>
<td>0.36</td>
<td>0.3</td>
<td>33.32%</td>
<td>0.09</td>
<td>1B</td>
<td>9m+3Sm</td>
<td>24</td>
</tr>
<tr>
<td><em>C. aspera</em></td>
<td>Neyzar village</td>
<td>40.547</td>
<td>5.49</td>
<td>2.15</td>
<td>2.55</td>
<td>37.28%</td>
<td>3.12</td>
<td>39.16%</td>
<td>0.39</td>
<td>0.28</td>
<td>35.72%</td>
<td>0.11</td>
<td>2B</td>
<td>7 m+6 Sm</td>
<td>26</td>
</tr>
<tr>
<td><em>C. aspera</em></td>
<td>Ozbak kuh</td>
<td>35.21</td>
<td>5.35</td>
<td>1.41</td>
<td>2.79</td>
<td>39.32%</td>
<td>2.7</td>
<td>26.35%</td>
<td>0.34</td>
<td>0.35</td>
<td>37.09%</td>
<td>0.11</td>
<td>1B</td>
<td>10 m+3 Sm</td>
<td>26</td>
</tr>
<tr>
<td><em>C. aspera</em></td>
<td>Ozbak kuh</td>
<td>39.34</td>
<td>5.95</td>
<td>2.05</td>
<td>2.9</td>
<td>41.03%</td>
<td>3.27</td>
<td>34.40%</td>
<td>0.31</td>
<td>0.31</td>
<td>31.34%</td>
<td>0.09</td>
<td>1B</td>
<td>11 m+1 Sm</td>
<td>24</td>
</tr>
<tr>
<td><em>C. aspera</em></td>
<td>Kalishane village</td>
<td>29.41</td>
<td>4.72</td>
<td>1.47</td>
<td>3.21</td>
<td>40.87%</td>
<td>2.45</td>
<td>31.14%</td>
<td>0.31</td>
<td>0.37</td>
<td>30.09%</td>
<td>0.11</td>
<td>1B</td>
<td>12 m</td>
<td>24</td>
</tr>
<tr>
<td><em>C. aspera</em></td>
<td>Yaz-Deyhuk</td>
<td>43.52</td>
<td>5.48</td>
<td>2.08</td>
<td>2.63</td>
<td>39.24%</td>
<td>3.34</td>
<td>37.95%</td>
<td>0.34</td>
<td>0.26</td>
<td>26.68%</td>
<td>0.07</td>
<td>2B</td>
<td>11 m+2 Sm</td>
<td>26</td>
</tr>
<tr>
<td><em>C. aspera</em></td>
<td>Yaz-Deyhuk</td>
<td>38.42</td>
<td>5.75</td>
<td>1.84</td>
<td>3.12</td>
<td>40.13%</td>
<td>3.2</td>
<td>32.32%</td>
<td>0.33</td>
<td>0.32</td>
<td>32.57%</td>
<td>0.1</td>
<td>1B</td>
<td>11 m+1 Sm</td>
<td>24</td>
</tr>
<tr>
<td><em>C. aspera</em></td>
<td>Fars- 35 km Shiraz</td>
<td>36.76</td>
<td>5.81</td>
<td>1.4</td>
<td>4.15</td>
<td>40.70%</td>
<td>3.06</td>
<td>24.09%</td>
<td>0.31</td>
<td>0.37</td>
<td>37.44%</td>
<td>0.11</td>
<td>1C</td>
<td>10 m+2 Sm</td>
<td>24</td>
</tr>
<tr>
<td><em>C. aspera</em></td>
<td>Fars- 35 km Shiraz</td>
<td>49.03</td>
<td>6.18</td>
<td>2.58</td>
<td>2.39</td>
<td>37.93%</td>
<td>3.77</td>
<td>41.74%</td>
<td>0.38</td>
<td>0.31</td>
<td>25.56%</td>
<td>0.07</td>
<td>2B</td>
<td>7 m+6 Sm</td>
<td>26</td>
</tr>
<tr>
<td><em>C. aspera</em></td>
<td>Qazvin- Abvek</td>
<td>47.87</td>
<td>6.48</td>
<td>2.48</td>
<td>2.61</td>
<td>36.89%</td>
<td>3.68</td>
<td>38.27%</td>
<td>0.37</td>
<td>0.28</td>
<td>28.29%</td>
<td>0.08</td>
<td>2B</td>
<td>7 m+6 Sm</td>
<td>26</td>
</tr>
<tr>
<td><em>C. aspera</em></td>
<td>Neyganan village</td>
<td>35.79</td>
<td>5.34</td>
<td>1.67</td>
<td>3.19</td>
<td>39.82%</td>
<td>2.75</td>
<td>31.27%</td>
<td>0.33</td>
<td>0.34</td>
<td>34.80%</td>
<td>0.1</td>
<td>1B</td>
<td>11 m+2 Sm</td>
<td>26</td>
</tr>
<tr>
<td><em>C. lappacea</em></td>
<td>Malayer-Borujerd</td>
<td>16.09</td>
<td>3.27</td>
<td>2.85</td>
<td>1.14</td>
<td>37.69%</td>
<td>2.29</td>
<td>87.15%</td>
<td>0.38</td>
<td>0.28</td>
<td>28.68%</td>
<td>0.02</td>
<td>1B</td>
<td>5 m+2 Sm</td>
<td>14</td>
</tr>
<tr>
<td><em>C. lappacea</em></td>
<td>Bid Sorkh- gauht</td>
<td>21.47</td>
<td>4.13</td>
<td>2.26</td>
<td>1.82</td>
<td>37.21%</td>
<td>3.06</td>
<td>54.72%</td>
<td>0.4</td>
<td>0.21</td>
<td>21.07%</td>
<td>0.08</td>
<td>2A</td>
<td>4 m+ 3 Sm</td>
<td>14</td>
</tr>
</tbody>
</table>

Table 3. Meiotic characters of the studied Clypeola species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>N</th>
<th>Voucher No</th>
<th>ROD</th>
<th>RB</th>
<th>I</th>
<th>IV</th>
<th>IX</th>
<th>TX</th>
<th>TOX</th>
<th>B</th>
<th>ROD N</th>
<th>RBN</th>
<th>IN</th>
<th>IVN</th>
<th>IXN</th>
<th>TXN</th>
<th>TOXN</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. jounthlaspi</em></td>
<td>Bumehen</td>
<td>16</td>
<td>1903</td>
<td>13.25</td>
<td>1.91</td>
<td>0.13</td>
<td>0</td>
<td>13.25</td>
<td>3.83</td>
<td>17.08</td>
<td>0</td>
<td>0.82</td>
<td>0.11</td>
<td>0.01</td>
<td>0.00</td>
<td>0.82</td>
<td>0.23</td>
<td>1.06</td>
</tr>
<tr>
<td><em>C. aspera</em></td>
<td>Abid</td>
<td>12</td>
<td>1904</td>
<td>9.33</td>
<td>1.83</td>
<td>0.5</td>
<td>0.16</td>
<td>9.33</td>
<td>4.33</td>
<td>13.66</td>
<td>0</td>
<td>0.77</td>
<td>0.15</td>
<td>0.04</td>
<td>0.01</td>
<td>0.77</td>
<td>0.36</td>
<td>1.13</td>
</tr>
<tr>
<td><em>C. aspera</em></td>
<td>Abid</td>
<td>13</td>
<td>1904</td>
<td>9.36</td>
<td>0.33</td>
<td>0</td>
<td>0.9</td>
<td>7.44</td>
<td>16.44</td>
<td>0</td>
<td>0.70</td>
<td>0.28</td>
<td>0.02</td>
<td>0.00</td>
<td>0.06</td>
<td>0.57</td>
<td>1.26</td>
<td></td>
</tr>
<tr>
<td><em>C. lappacea</em></td>
<td>brugerd-kermanshah</td>
<td>7</td>
<td>1901</td>
<td>5.7</td>
<td>0.52</td>
<td>0.88</td>
<td>0</td>
<td>5.7</td>
<td>1.05</td>
<td>6.76</td>
<td>0.05</td>
<td>0.81</td>
<td>0.07</td>
<td>0.13</td>
<td>0.00</td>
<td>0.81</td>
<td>0.15</td>
<td>0.97</td>
</tr>
<tr>
<td><em>C. lappacea</em></td>
<td>brugerd-kermanshah</td>
<td>8</td>
<td>1901</td>
<td>5.58</td>
<td>1.58</td>
<td>0.58</td>
<td>0.16</td>
<td>5.58</td>
<td>3.66</td>
<td>8.83</td>
<td>0.08</td>
<td>0.70</td>
<td>0.20</td>
<td>0.07</td>
<td>0.02</td>
<td>0.70</td>
<td>0.46</td>
<td>1.10</td>
</tr>
</tbody>
</table>
RESULTS

Karyotypic features

Details of karyotypic features of studied Clypeola species are provided in table 2 and figs. 1 & 2. The karyotype for C. jonthalspi was not prepared due to the small size of chromosomes with inconspicuous centromer.

Clypeola jonthlaspi. In this study five populations of C. jonthlaspi (Neyzar, Neyriz, Modiriat bridge, Taq-Bostan and Marand) were studied. C. jonthlaspi showed 2n=4x=32 which agrees with former publications (Aryavand 1975; Warwick et al. 2006 & 2008; Al-Shehbaz 2006).

Clypeola aspera. In this study 7 populations were studied. Polysomatic was observed in this species which were two chromosome numbers 2n=24 and 2n = 26 . The number of 2n=26 agrees with former studies (Aryavand 1975), the number of 2n=24 is new for this species.

Clypeola lappacea. In this study 2 populations were studied, 2n=2x=14 which agrees with previous literatures with reported n=7 (Maassoumi 1980; Al-Shehbaz & Al-Omar 1982 & 1983; Warwick et al 2008).

Clypeola dichotoma. One population for this species was studied and the number of 2n=2x=14 was obtained. This report is according to previous studies with n=7 (Aryavand 1975; Maassoumi 1980).

The Clypeola species were placed in 2A, 1B, 2B and 1C classes of Stebbins Karyotype symmetry, which are considered relatively primitive in this system except for C. aspera, population of Fars, 35 Km to Shiraz with 2n=24 that is stated in 1C class. Therefore, it seems that the studied Clypeola species have symmetrical karyotypes.

Conclusion

Clypeola microcarpa has been variously treated as distinct subspecies or variety of C. jonthalspi (Chaytor & Turill 1935; Breistroffer 1936; Runemark 2002; Bush 1939). In Flora Iranica (Rechinger 1981) it was considered as a distinct species and recorded from Khorassan province. Runemark (2002) recorded 2n=16 for this taxon, but the specimens fitted to C. microcarpa from Khorassan province showed 2n=4x=32. Therefore, it may be that this species do not occur in Iran.

Mandakova and Lysak (2008) pointed that simultaneous morphological variation in Brassicaceae is not necessarily along with modifications in Karyotype. It means that morphologically distinct taxa in Brassicaceae may have similar karyotypes. This is in accordance with the C. jonthalspi subspecies in Iran. Observed chromosome number is consistence with basic chromosome number for this genus (Warwick et al. 2006 & 2008). The polysomy and mixoploidy (mixoploidy is a condition in which the tissue is composed of cells with different ploidy levels) is observed in C. aspera. In similar studies, Borgen (1987) pointed to mixoploidy in Lobularia (previously considered as an Alyssese element). Bolourian (2009) recorded two chromosome set in some cells of Alyssum, beside polysomatic phenomenon in most of Alyssum populations.

Meiotic studies

The meiotic behavior of chromosomes in chiasmata formation, frequency and chromosomes different conjunctions and genetic abnormalities are recorded. The meiotic configurations are listed in table 3.

Clypeola jonthlaspi. The number n=16 was obtained for C. jonthlaspi (fig. 3). This is in concordance with its previously recorded sporophyte number (Aryavand 1975; Ancev and Goranova 1997; Warwick et al. 2006 & 2008; AL-Shehbaz 2006). In C. jonthlaspi abnormalities as synozytic node, unreduced and sterile pollen grains were observed. In this population most cells were in diakinesis-metaphase I and there were only few cells in telophase II.

Clypeola aspera. Two different chromosome numbers n=12 and n=13 (fig. 3) were observed in different individuals of C. aspera from Abid village population (Disploidy). In meiotic study of this species, abnormalities such as, anaphase bridge (fig. 4 B), disordered divisions in anaphase II (fig. 4 A), chromosome stickiness, laggard chromosome, and unreduced pollen grains (fig. 4 C) were observed. There were unreduced and sterile pollen grains (fig. 4 K). Presence of B chromosome (fig. 4 L) and polyploid cell (fig. 3 I) is recorded here for the first time in C. lappacea.

Clypeola lappacea. Obtained chromosome number in this study for C. lappacea were n=8 and n=7 in different individuals of Borujerd-Kermanshah populations (Disploidy). Variation in meiosis steps in this species as synozitic knot and diffuse (fig. 4 G) were observed. Laggard chromosome in anaphase II, telophase II (fig. 4 E), desynaptic (fig. 4 D), anaphase bridge in anaphase I (fig. 4 F), multipolar and tripolar cells (fig. 4 I-J), micromucleus (fig. 4 H) were observed. There were unreduced and sterile pollen grains (fig. 4 K).
Fig. 1. Representative ideograms of two species of Clypeola. A-L, C. aspera, A-B: P-1, Tabas-Neyzar village, C-D: P-3, Yazd-Eshqabad road-Ozbak kuh, E-F: P-5 Yazd, 40 Km Deyhuk, G: P-4, Tabas-Eshqabad road Kalshane villag, H-I: P-6 Fars, 35 Km Shiraz, K: P-7 Qazvin- Abyek, L: P-9, South Khorasan, Boshrouyeh, Neyganan village; M-N, C. lappacea, M: P-11, Malayer-borujerd road, 60 km Borujerd, N: P-12, Kermanshah, Bid Sorkh. Scale = μm.
Conclusion
Desynapsis which was observed in *C. lappacea* could result in meiotic abnormalities which reduce the fertility of species. Desynapsis formation is effective in micronucleus formation and effect pollen fertility (Enss and Larter 1960). Pagliarini (2000) pointed that presence of univalent chromosomes (due to low chiasmata numbers or presence of asynapsis or desynapsis genes) is capable of causing irregularity in cell division in metaphase I or laggard in anaphase I, both of which are effective in micronucleus formation and pollen fertility. Due to the high presence of sterile pollen grains and desynapsis and micronucleus formation in *C. lappacea*, the results of present study confirm the Pagliarini (2000) and Enss and Larter (1960) results. The most observed meiotic abnormalities in *C. lappacea* were desynapsis, laggared chromosome and stickiness. Sheidai et al. (2008) pointed to multi-polar as an effective factor in unreduced pollen formation through irregularity in chromosome separation in anaphase I in *Silene* species. It seems that it could be the case in *C. aspera* too. According to the chromosome number of *C. lappacea* from western parts of Iran with 2n=14, we will find that this diploid species is distributed in eastern parts, but *C. jonathaslsp* and *C. aspera* which are tetraploids have vast distribution area.
Fig. 4. Meiosis in *Clypeola lappacea* and *C. aspera*. A-C. *C. aspera*, A, disordered division in anaphase II, B, anaphasic bridge, C, unreduced normal and sterile pollen; D-K, *C. lappacea*, D, desynapsis, E, laggard chromosome in telophase II, F, anaphase I bridge, G, diffuse, H, micronucleus.
Fig. 4. continued. I, disordered chromosome, multi-polar cell; J, three polar cell; K, unreduced, normal and sterile pollen grain; L, arrow shows quadrivalent and B chromosome. Scale= 10 µm.
REFERENCES


Breistroffer, M. 1936: Revision systematique des variations Du Clypeola jonthlaspi L. –Candollea 7: 140-166.


