Genome size, morphological and palynological variations, and heterostyly in some species of the genus Linum L. (Linaceae) in Iran

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INTRODUCTION

The occurrence of flowers with different sexual organ arrangements in different plants of a single species is called heterostyly (Darwin, 1888). It is a sexual polymorphism in which populations are composed of two (distyly) or three (tristyly) floral morphs with reciprocal arrangements of anthers and stigmas (reciprocal herkogamy) (Ganders, 1979). Plants in distylos species produce either all long-styled (LS or Pin) or all short-styled (SS or Thrum) flowers. Flowers with the LS morphology have stigma(s) positioned above anthers,
whereas flowers with the SS morphology have anthers above stigma(s). Heterostyly has been documented in 28 angiosperm families (Barrett et al., 2000).

Family Linaceae is geographically widespread with about 300 species worldwide distributed (Hickey, 1988). Several species are shrubs and occur in tropical areas, while perennial and annual species are found in temperate areas. About 22 species, subspecies or varieties are reported from Iran (Sharifnia and Assadi, 2001) which are classified into five sections (Rechinger, 1974). Due to wide range of diversity within the genus Linum, it has received considerable attention from botanists (Diederichsen and Richards, 2003). Distyly is widespread and very common in Linum and about 40% of these species are distylos occurring in four out of five sections of the genus, namely Linum, Syllinum, Dasylinum and Linasstrum (Rogers, 1979; Sharifnia and Assadi, 2001).

Various differences have been reported in the long-styled and short-styled plants in different species, for example, Armbruster et al. (2006) found variation in distyly of Linum suffructicosum L., with bent styles and stamens, achieving a three-dimensional arrangement. In heterostylos species, some morphological and micro-morphological characters were different in long-styled and short-styled plants including the number and size of pollen grains, stamens shape, shape and color of stigma and its surface papillae (Richards and Barrett, 1992). In some heterostylos species of Linum such as; Linum perenne, Linum grandiflorum and Linum alpinum, exine sculpturing structure differed in the long-styled and short-styled plants (Dulberger, 1981).

C-value (genome size = size of the monoploid chromosome set) data is considered as useful characteristics for infrageneric classification, species delimitation or hybrid identification (Keller et al., 1996; Buitendijk et al., 1997; Bare et al., 1998, 2004). Moreover, correlation between DNA content and plant life-histories, plant phenology, environmental factors, climatic variation and geographical plant distribution has been determined in various studies performed on plant species (Bennett, 1976; Poggio et al., 1998; Baranyi and Greilhuber, 1995; Bureš et al., 2004). Evans (1968) found that varieties of Linum usitatissimum which grew in different ecological condition, such as; high nitrogen concentrations and high temperatures increased 10% in DNA content. In the present study, difference in morphological and palynological characters as well as genome size (C-value content) was investigated in the long-styled and short-styled plants of three Linum species namely Linum austriacum L., Linum album Ky.ex Boiss, and Linum glaucum Boiss., for the first time.

MATERIALS AND METHODS

Plant samples

Three heterostylos species including L. austriacum, L. album and L. glaucum Boiss. & Nöe were studied. Plant specimens were collected from both short-styled and long-styled population of these species during the growing season in spring 2010 and 2011 (Table 1). From each species three populations were studied and in each population four plant specimens were used for detailed investigation.

Morphology

15 qualitative and quantitative morphological characters from both vegetative and reproductive organs of these plants were studied. Morphological characters used include: the length and diameter of stems, number and status of stem branches, size, shape and diameter of the basal leaves and inflorescence leaves, dimensions of calyx and corolla and pedicle length. T-test analysis was performed to show morphological differences between long-styled and short-styled plants, while unweighted paired group using average method (UPGMA) and neighbor joining (NJ) trees as well as ordination plot based on principal coordinate analyses (PCoA) were used for grouping the species and also long-styled and short-styled plants in each species.

For multivariate analyses the mean of quantitative characters were used, while qualitative characters were coded as binary/multistate characters. Standardized variables (mean = 0, variance = 1) were used for statistical analyses. The average taxonomic distance and Manhattan distance were used as dissimilarity coefficient in cluster analysis of morphological data (Podani, 2000). UPGMA and NJ trees as well as ordination plot based on PCoA were used for grouping the species and also long-styled and short-styled plants in each species.

Palynology

The pollen was obtained from the mature buds and used for light microscopy (LM) and scanning electron microscopy (SEM) by the prolonged acetylation method of Erdtman (1960). For LM, the pollen was mounted in glycerin jelly and sealed with paraffin. The polar (P), equatorial (E) and colpus lengths, and sizes of pollen grains from six populations in three species were measured under the light microscope and P/E ratios were calculated. For SEM, the pollen grains were transferred directly to double-sided tape affixed stubs and were vacuum-coated with gold in Biorad E5200 auto sputter coater and photographed with a Camscan MV2300 scanning electron microscope. The terminology in this paper is based on Moore et al. (1991) work. T-test analysis was performed to show pollen characteristic difference between long-styled and short-styled plants.

Flow cytometry

Three species of the genus Linum were analyzed with flow cytometry. For each taxon, three to five populations were collected and their 2C-value DNA content was determined. The nuclei suspensions were prepared from small amount of mature fresh leaf tissue together with an equal weight of mature leaf tissue of the external standard. The external standard used in for L. austriacum was Parsley (Petroselinum crispum cv. Champion Moss Curled) which had a 2C DNA value of 4.46 pg (Yokoya et al., 2000), for L. album, Rosa wichurana Crep was used with 2C value of 1.13 pg (Yokoya et al., 2000) and for L. glaucum Boiss. & Nöe was Allium cepa which had a 2C DNA value of 33.5 pg (Greilhuber and Ebert, 1994). One-step protocol was used for preparation of the nuclear suspension. The leaves were chopped with a single-used sharp
and short-styled plant populations in *L. glaucum*, showed a higher mean value of the plant height, size of the basal leaves width, flower leaves width, calyx width, sepal length and petal length in the long- styled plants, while the mean value of branch number, basal leaves length, flower leaves width, calyx width, pedicel length and sepal length was higher in the short- styled plant populations. T-test analysis of morphological characters showed significant difference (p<0.05) for basal leaf length, calyx width and length.

Similar analysis in *L. austriacum* showed a higher mean value of the plant height and branch number in the long- styled plants, while the mean value of the other characters was higher in the short- styled plant populations. In *L. album*, a higher mean value of the basal leaves length, flower leaves length, flower leaves width, calyx width and sepal width occurred in the long-styled plants, while, the short- styled plant populations had a higher mean value for the other characters studied. T-test analysis of morpho-logical characters showed significant difference (p<0.05) for some of the characters studied. PCoA plot of long- styled and short- styled plant populations based on all morphological characters also separated these two kinds of plants in the three species studied (Figures 1 and 2).

The three species studied also differed in morphological characters studied and were separated in
Table 2. Morphology characteristics in long styled and short styled plant populations studied.

<table>
<thead>
<tr>
<th>Plant population</th>
<th>Statistical parameter</th>
<th>Stem length</th>
<th>Branch length</th>
<th>Base length</th>
<th>Base width</th>
<th>Flower length</th>
<th>Flower width</th>
<th>Calyx length</th>
<th>Calyx width</th>
<th>Pedicel length</th>
<th>Sepal length</th>
<th>Sepal width</th>
<th>Petal length</th>
<th>Petal width</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Linum album</strong></td>
<td>Mean</td>
<td>19.87</td>
<td>5.00</td>
<td>1.35</td>
<td>0.37</td>
<td>1.32</td>
<td>0.27</td>
<td>0.72</td>
<td>0.36</td>
<td>0.08</td>
<td>0.42</td>
<td>0.17</td>
<td>2.12</td>
<td>1.75</td>
</tr>
<tr>
<td></td>
<td>Std. deviation</td>
<td>5.53</td>
<td>5.09</td>
<td>0.31</td>
<td>0.15</td>
<td>0.40</td>
<td>0.09</td>
<td>0.09</td>
<td>0.04</td>
<td>0.01</td>
<td>0.09</td>
<td>0.02</td>
<td>0.09</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>L. album</strong></td>
<td>Mean</td>
<td>28.47</td>
<td>5.25</td>
<td>1.60</td>
<td>0.31</td>
<td>1.30</td>
<td>0.20</td>
<td>0.82</td>
<td>0.35</td>
<td>0.09</td>
<td>0.5</td>
<td>0.15</td>
<td>2.47</td>
<td>2.30</td>
</tr>
<tr>
<td></td>
<td>Std. deviation</td>
<td>7.23</td>
<td>3.59</td>
<td>0.42</td>
<td>0.08</td>
<td>0.33</td>
<td>0.01</td>
<td>0.12</td>
<td>0.05</td>
<td>0.02</td>
<td>0.08</td>
<td>0.05</td>
<td>0.49</td>
<td>0.53</td>
</tr>
<tr>
<td><strong>L. austriacum</strong></td>
<td>Mean</td>
<td>58.87</td>
<td>8.75</td>
<td>1.37</td>
<td>0.15</td>
<td>0.87</td>
<td>0.10</td>
<td>0.42</td>
<td>0.35</td>
<td>0.10</td>
<td>0.20</td>
<td>0.10</td>
<td>1.75</td>
<td>1.37</td>
</tr>
<tr>
<td></td>
<td>Std. deviation</td>
<td>3.72</td>
<td>0.95</td>
<td>0.29</td>
<td>0.04</td>
<td>0.15</td>
<td>0.00</td>
<td>0.05</td>
<td>0.05</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.26</td>
<td>0.18</td>
</tr>
<tr>
<td><strong>L. austriacum</strong></td>
<td>Mean</td>
<td>49.37</td>
<td>4.50</td>
<td>1.67</td>
<td>0.18</td>
<td>1.12</td>
<td>0.12</td>
<td>0.46</td>
<td>0.4775</td>
<td>0.10</td>
<td>0.20</td>
<td>0.13</td>
<td>2.15</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>Std. deviation</td>
<td>5.02</td>
<td>1.29</td>
<td>0.30</td>
<td>0.08</td>
<td>0.35</td>
<td>0.04</td>
<td>0.04</td>
<td>0.045</td>
<td>0.00</td>
<td>0.01</td>
<td>0.04</td>
<td>0.12</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>L. glaucum Boiss. &amp; Nöe</strong></td>
<td>Mean</td>
<td>59.00</td>
<td>4.50</td>
<td>1.60</td>
<td>0.23</td>
<td>1.02</td>
<td>0.24</td>
<td>0.52</td>
<td>0.36</td>
<td>0.10</td>
<td>0.23</td>
<td>0.10</td>
<td>5.00</td>
<td>5.35</td>
</tr>
<tr>
<td></td>
<td>Std. deviation</td>
<td>8.30</td>
<td>0.57</td>
<td>0.14</td>
<td>0.02</td>
<td>0.12</td>
<td>0.24</td>
<td>0.05</td>
<td>0.04</td>
<td>0.03</td>
<td>0.04</td>
<td>0.01</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>L. glaucum Boiss. &amp; Nöe</strong></td>
<td>Mean</td>
<td>53.875</td>
<td>15</td>
<td>1.875</td>
<td>0.2</td>
<td>1.375</td>
<td>0.1775</td>
<td>0.475</td>
<td>0.45</td>
<td>0.1</td>
<td>0.225</td>
<td>0.125</td>
<td>1.975</td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td>Std. deviation</td>
<td>7.375</td>
<td>6.782</td>
<td>0.263</td>
<td>0</td>
<td>0.126</td>
<td>0.052</td>
<td>0.05</td>
<td>0.058</td>
<td>0</td>
<td>0.029</td>
<td>0.05</td>
<td>0.171</td>
<td>0.17</td>
</tr>
</tbody>
</table>

All values are in cm.

Palynology

In general, the pollen shape was circular in the three species studied, but its shape varied in the equatorial view in these species that is, circular in L. austriacum but elliptic in the other two species. Moreover, it was elliptic-obtuse and elliptic-truncate in L. album (Table 3). The polar and equatorial views of the pollen grains were similar in Ls and Ss plants of the species studied, while the aperture shape varied in these populations. For example, it was polygonal gemmate in L. austriacum but gemmate shape in the short-styled plants of this species. The aperture shape was pilate and baculate in the long-styled plant populations of L. album while, it was pilate shape in the short-styled plant populations. Similarly in the long-styled plant populations of L. glaucum the aperture shape was gemmate and pilate to clavate but it was clavate in the short-styled plant populations. (Figure 4).

Comparison of pollen characteristics (Table 3) between long-styled and short-styled plant populations in L. glaucum, showed a higher mean
value of the aperture width and length, distance between apertures, polar length and colpi width as well as colpi length, in the long-styled plants, while the mean value of mesocolpi and equatorial length was higher in the short-styled plant populations. T-test analysis of morphological characters showed significant difference ($P < 0.05$) for distance between apertures, aperture width and length.

Similar analysis in *L. austriacum* showed a higher mean value of almost all pollen characteristics higher in the short-styled plant populations compared to those in the long-styled plant populations. In *L. album*, a higher mean value of the aperture length and distance between apertures were observed in the long-styled plants, while, the short-styled plant populations had a higher mean value for the other characters studied. T-test analysis of morphological characters showed significant difference ($P < 0.05$) for these characters.

PCoA plot of long- styled and short- styled plant populations based on all palynological characters also separated these two kinds of plants in all studied three species (Figures 5 and 6).

**Flow cytometry**

In order to compare C-value (genome size), difference
between long-styled and short-styled plants, three to five populations of each type were studied with flow cytometer in L. austriacum, L. album and L. glaucum Boiss. & Nöe (Table 4 and Figure 7). Each histogram has three peaks. The right peak refers to the known reference standards (the external standards for L. album and L. austriacum and L. glaucum Boiss. & Nöe were Parsley and Rosa wichurana and Allium cepa respectively) and the left peak belongs to the Linum species studied.

C-values obtained differed between long-styled and short-styled plants of the species studied. For example, in L. austriacum, the mean 2C-value of long-styled plants was 2.65 ± 0.30 pg but in the short-styled plants it was 1.99 ± 0.12 pg. However, this was not a significant difference (P = 0.11). In case of L. album, long-styled plants had the mean 2C-value of 4.61 ± 0.71 while short-styled plants had the mean 2C-value of 3.61 ± 0.07. Difference of 2C-values between long-styled and short-styled plants in this species was also not significant (P = 0.23). The same holds true (P= 0.17) for L. glaucum Boiss. & Nöe with 2C-value of 1.98 ±0.01 in Ls plants and 1.78 ± 0.11 in Ss plants. However, the ANOVA test performed among the three Linum species showed a significant difference in 2C-value content (F = 11.88, P<0.001).

A negative significant correlation (r = -0.85, P < 0.05), was observed between 2C-value and the stem length, but a positive significant correlation was observed between 2C-value and sepal length (r = 0.83, P < 0.05). Similarly, a positive significant correlation was observed between 2C-value and northern distribution of the Linum species studied (r = 0.54, P < 0.05), while a negative significant correlation (r = -0.57, P < 0.05) occurred with eastern distribution. No correlation was noticed between 2C-value and altitude of the species studied (r = 0.2., P >0.05).

**DISCUSSION**

Heterostyly has been characterized by the presence of two or three discrete morphs that differ in their sex organ position within popiations. This polymorphism is widely distributed among the angiosperms, but detailed studies are limited to few taxonomic groups and it is suggested that, when precise measurements of the sexual whorls are reported, an evolutionary meaningful variations of the heterostyly syndrome will be understood (Sánchez et al., 2010).

The evolution of heterostyly appears to occur within some general constrains on floral morphology (Ganders, 1979). Heterostyly flowers are generally moderate sized and have a floral tube, a limited number of stamens, and a syncarpous ovary with few carpels. For example, in the genus *Cordia* (Boraginaceae), smallest-flowered species showed dioecy but heterostyly occurred in medium to large size flowers (Opler et al., 1975), while in the genus *Melochia* (Sterculiaceae), the smallest-flowered plant lacks distyly (Martin, 1966). In *Hypericum aegypticum* and *Cratoxylum formosum* (Guttiferae) the heterostylous plants which have many stamens, are often grouped into three to five bundles with 30 to 125 stamens (Ornduff, 1975; Lewis, 1982).

Detailed comparison of flower characters between the long-styled and short-styled plants showed that in L. *glaucum*, the long-styled plants have significantly a
Table 3. Pollen characteristics in the Linum species studied.

<table>
<thead>
<tr>
<th>Plant population</th>
<th>Statistical parameter</th>
<th>Sculpture width</th>
<th>Sculpture length</th>
<th>Sculpture distance</th>
<th>Equator length</th>
<th>Polar length</th>
<th>Colpi width</th>
<th>Colpi length</th>
<th>P/E</th>
<th>Mesocolpi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alls</td>
<td>Mean</td>
<td>0.74</td>
<td>1.29</td>
<td>0.51</td>
<td>37.67</td>
<td>49.33</td>
<td>3.27</td>
<td>38.92</td>
<td>1.31</td>
<td>32.30</td>
</tr>
<tr>
<td></td>
<td>Std. Dev.</td>
<td>0.21</td>
<td>0.73</td>
<td>0.37</td>
<td>3.90</td>
<td>3.21</td>
<td>0.32</td>
<td>7.13</td>
<td></td>
<td>2.06</td>
</tr>
<tr>
<td>Alss</td>
<td>Mean</td>
<td>1.10</td>
<td>1.17</td>
<td>0.35</td>
<td>45.30</td>
<td>37.41</td>
<td>7.53</td>
<td>68.42</td>
<td>0.83</td>
<td>37.83</td>
</tr>
<tr>
<td></td>
<td>Std. Dev.</td>
<td>0.21</td>
<td>0.18</td>
<td>0.13</td>
<td>3.10</td>
<td>0.50</td>
<td>1.50</td>
<td>5.79</td>
<td></td>
<td>3.01</td>
</tr>
<tr>
<td>Auls</td>
<td>Mean</td>
<td>1.20</td>
<td>1.36</td>
<td>0.52</td>
<td>55.94</td>
<td>51.49</td>
<td>9.36</td>
<td>35.33</td>
<td>0.92</td>
<td>44.26</td>
</tr>
<tr>
<td></td>
<td>Std. Dev.</td>
<td>0.69</td>
<td>0.57</td>
<td>0.14</td>
<td>3.82</td>
<td>0.81</td>
<td>1.36</td>
<td>55.97</td>
<td></td>
<td>2.25</td>
</tr>
<tr>
<td>Auss</td>
<td>Mean</td>
<td>1.38</td>
<td>1.49</td>
<td>0.53</td>
<td>68.22</td>
<td>60.1</td>
<td>14.28</td>
<td>68.97</td>
<td>0.88</td>
<td>54.11</td>
</tr>
<tr>
<td></td>
<td>Std. Dev.</td>
<td>0.25</td>
<td>0.30</td>
<td>0.11</td>
<td>3.24</td>
<td>3.73</td>
<td>1.81</td>
<td>54.91</td>
<td></td>
<td>4.96</td>
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<tr>
<td>Glls</td>
<td>Mean</td>
<td>1.38</td>
<td>1.54</td>
<td>0.51</td>
<td>53.96</td>
<td>65.67</td>
<td>7.22</td>
<td>51.77</td>
<td>1.22</td>
<td>43.59</td>
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<tr>
<td></td>
<td>Std. Dev.</td>
<td>0.32</td>
<td>0.45</td>
<td>0.26</td>
<td>3.82</td>
<td>2.79</td>
<td>1.90</td>
<td>3.99</td>
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</tr>
<tr>
<td>Glss</td>
<td>Mean</td>
<td>1.04</td>
<td>1.13</td>
<td>0.31</td>
<td>55.49</td>
<td>53.04</td>
<td>6.03</td>
<td>48.74</td>
<td>0.96</td>
<td>47.29</td>
</tr>
<tr>
<td></td>
<td>Std. Dev.</td>
<td>0.16</td>
<td>0.14</td>
<td>0.07</td>
<td>3.18</td>
<td>3.55</td>
<td>4.26</td>
<td>3.05</td>
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<td>0.56</td>
</tr>
</tbody>
</table>


Higher mean value of the flower leaves length, and calyx width compared to those of the short-styled plants, while in *L. album*, the long-styled plants show a significant higher mean values in the calyx width, petal length and petal width. However, no significant difference in flower characters was observed in *L. austriachum* between the short-styled and long-styled plant populations. However these species showed distinct range of morphological variations as they were separated from each other in the UPGMA tree. The reproductive features such as; petal and sepal dimensions, pedicle length and some of vegetative characters such as basal and flower leaves dimensions were bigger in Ss samples rather than Ls samples.

Study of different aspects of floral morphology in heterostyly species has been widely used as a tool to understand the pollination process, as it significantly influences pollen transfer and reproduction (Turketti, 2010). Research shows that many of heterostylos species are self-compatible (Barrett and Cruzan, 1994) *Linum* species have distinct hermaphrodite flowers with attractive petals and nectars which absorbed different pollinator’s insect. Heterostyly facilitated cross-pollination in *Linum* and increases the insect mediated pollination (Darwin, 1864).

Rogers (1979) described *Linum suffruticosum* as heterostylos and intramorph-incompatible in eastern Spain showing that the pollen size is the same in the two morphs, but that the exine sculpturing differs. Three-dimensional heterostyly observed in *L. suffruticosum* is very effective in pollen distribution. When a pollinator insect visits this heterostylos species, the pollens of long-styled plants are placed on the underside of pollinator while, the pollens of short-styled plants are placed on the top of thorax and abdomen. The stigmas of long-styled plants contact the flies on the dorsum and pick up predominantly short-styled plants pollens.
Figure 4. Electronic micrograph of pollen exine sculpturing. (A) L. album Ss; B) L. album Ls; C) L. austriacum Ss; D) L. austriacum Ls; E) L. glaucum Boiss. & Nöe Ss; F) L. glaucum Boiss. & Nöe Ls.
Moreover, the stigmas of the short-styled plants contact the flies on the ventral surface, picking up predominantly long-styled pollens (Armbruster et al., 2006). Armbruster et al. (2006) also reported morphological variations in distylyous *L. suffruticosum* L., with bent styles and stamens, achieving a three-dimensional arrangement. Pin (L) and thrum (S) morphs were found to be of
nearly identical appearance, except for the length and orientation of the sexual parts. Style and stamen lengths differed significantly; however, the differences were small compared with those in many other species of *Linum* and other heterostylous species. There was no detectable difference in pollen size. However, stigma width differed significantly between morphs. The stamens differed between pin and thrum morphs. Pin stamens spread from near the base, appraised to the corolla wall, and extending one-third of the way up the petals; the openings of

Figure 6. PCoA plot of long- styled and short- styled plant populations in *L. austriacum* based on pollen characters. AuLs, long- styled plants; Auss, short- styled plants.
the dehiscing anthers face inwards. The thrum stamens were erect, forming a column in the center of the flower, and the anthers are rotated so that the openings of the dehiscing anthers face outwards.

Phylogenetic analysis and MACCLADE reconstruction of character states performed by Armbruster et al. (2006) also showed that heterostyly has originated several times, not only within Linum, but also within sect. Linastrum, including one to three reversions to monomorphism. They suggested that heterostyly has evolved at least twice in the two Linastrum clades, although equivocal character transitions and the limited sample of taxa preclude inference of the exact number of shifts. The least restrictive optimization of heterostyly is consistent with at least three independent origins of heterostyly in Linum.

Sánchez et al. (2010) also reported stigma height dimorphism, as opposed to distyly in the genus Nivenia as the only genus within the Iridaceae containing heterostylyous species. The presence of different types of polymorphism within the genus is consistent with hypotheses of the evolution of heterostyly. The role of the pollinators as the leading force of the transition seems to be apparent, since floral integration is related to reciprocity. Variation of sexual whorls was observed in different Nivenia species with Nivenia fruticosa as the only monomorphic species.

For the species with two floral morphs, stigma height was significantly different in all of them and the length of the stamens was statistically different between morphs in all cases, except for the population of Nivenia argentea at Aasvoëlkrans. Thus, those were considered as distylyous, and N. argentea at Aasvoëlkrans as stigma-height dimorphic since two style-length morphs were present but another heights remained indistinguishable between morphs.

Our palynological study revealed that the shape of pollen grains in the equatorial and polar views are uniform between Ss and Ls plant populations in the Linum species studied, but other pollen characteristics like the aperture shape and dimensions, polar and equatorial axis length differed between long- styled and short-styled plant populations. Previous reports indicate that in some heterostyled Linum species, the pollen surface in short-styled plants is composed of homo-sized apertures but the exine surface in long-styled plants contains small and big aperture with variable papillae shape and size (Dulberger, 1981). Nicholls (1986) in L. perenne found that short-styled plant was a better pollen-donor and the long-styled plant was a better pollen-receiver. The short-styled samples produced more pollen, but the long-styled samples matured more seeds. These observations suggest a degree of sexual dimorphism in L. perenne, which the short-styled plants behaving functionally more male and the long-styled plant functionally more female.

Richards and Barrett (1992) reported differences in the number and size of pollen grains (for example Pontederia cordata), dimorphic in stamens position (Primula vulgaris), (Darwin, 1877), pollen grain color (Linum pubescens), (Wolfé, 2001), stylar bending (in Linum grandiflorum), shape and colors of stigma and its surface papillae (Linum hirsutum, Unal and Yildirim-Fazla, 2007). In some heterostylyous species of Linum such as; L. perenne, L. grandiflorum and L. alpinum, exine sculpturing structure were differed between long-styled Heterostyly is usually associated with polymorphisms of pollen between samples (Barrett, 1992). Exine pattern of pollen interacts with biotic and abiotic pollination vectors and affected the surface area of the stigma interface and mediated stigma adhesions.

Aperture size, number and its complexity affected environmental vulnerability to desiccation, fungal invasion and mechanical stress, and serve as portals for pollen tube exit during germination (Edlund et al., 2004). Wang et al. (2009) found that in Pedicularis (Orobanchaceae) there was a significant association between pollen aperture types and corolla types, as well as between pollination syndromes and corolla. There was a distinct correlation between exine ornamentation, floral morphology and pollination in Bauhinia. (Ferguson and Pearce, 1986).

Nuclear DNA C-value varies in different taxonomic taxa (Yokoya et al., 2000) and is considered as a mean for adaptation, and is affected key parameters of plant growth such as; the duration of the cell size, cell cycle,

Table 4. Nuclear DNA amount and genome size in studied taxa of the genus Linum.

<table>
<thead>
<tr>
<th>Plant name</th>
<th>2C DNA amount (pg)</th>
<th>Genome size (Mbp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. austriacum long-styled</td>
<td>2.65</td>
<td>2591.7</td>
</tr>
<tr>
<td>L. austriacum short-styled</td>
<td>1.99</td>
<td>1946.22</td>
</tr>
<tr>
<td>L. album short-styled</td>
<td>3.61</td>
<td>3530.58</td>
</tr>
<tr>
<td>L. album long-styled</td>
<td>5.00</td>
<td>4890.0</td>
</tr>
<tr>
<td>L. glaucum Boiss. &amp; Nöe long-styled</td>
<td>1.98</td>
<td>1936.44</td>
</tr>
<tr>
<td>L. glaucum Boiss. &amp; Nöe short-styled</td>
<td>1.78</td>
<td>1740.84</td>
</tr>
</tbody>
</table>
Figure 7. Flow cytometric histograms showing the difference in 2C DNA content in studied heterostylos species of the genus Linum. (A) L. album short-styled; (B) L. album long-styled; (C) L. glaucum Boiss. & Née short-styled; (D) L. glaucum Boiss. & Née long-styled.
rate of cell division, sensitivity to radiation, ecological behavior in plant communities and life forms (Bennett et al., 2000). The 2C-values obtained here indicate genomic content difference between short-styled and long-styled plant populations and the possible role of quantitative genetic changes in heterostyly. Significant difference in
2C-values of the three *Linum* species studied indicates the possible role of DNA content change during species diversification. This may also suggest the use of 2C-value as supplementary data for species grouping in the genus *Linum*. Although not significant, negative relation between 2C-values and some morphological and palynological characters occurred in *L. austriacum* and *L. album*. The short-styled plants of *L. austriacum* and *L. album* have smaller genome size and nuclear DNA 2C-values compared to the long-styled plants. In the short-styled plants, the petal and sepal dimensions, pedicle length, the mean size of basal and flower leaves and polar and equatorial axis of pollen are relatively bigger than the long-styled plants.

Significant correlation obtained between 2C-values and some of the quantitative morphological characters may indicate the effect of genome size on this character and the possible adaptive nature of these characters (although not apparent to us by now). Such correlation has been reported in other plant species including *Cirsium* species (Nouroozi et al., 2011). Significant positive correlation occurred between 2C-values and ratio of pappus length/seed size and involucre length.

Positive significant correlation was observed between 2C-value and northern distribution of the *Linum* species studied, while a negative significant correlation occurred with eastern distribution. No correlation was noticed between 2C-value and altitude of the species studied. This may indicate some relation with ecological and population differentiations which should be investigated to be confirmed. Studies performed on *Cirsium* species (Nouroozi et al., 2011) showed significant negative correlation between 2C-value and latitude, and positive significant correlation between 2C-value and the mean annual rain fall, while Bureš et al. (2004) reported that the species with larger genomes prefer dry habitats in areas with more oceanic climates, and species with smaller genomes grow in more humid habitats (wetlands) in continental areas.

**Abbreviations**

- PCoA: Principal coordinate analysis
- LM: light microscopy
- SEM: scanning electron microscopy
- LS: long-styled
- Ss: short-styled

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