INVESTIGATION OF NUCLEAR DNA CONTENTS OF LYCORIS SPECIES (AMARYLLIDACEAE) WITH DIFFERENT CHROMOSOME NUMBER BY FLOW CYTOMETRY

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Abstract

The chromosome number and karyotype of Lycoris genus display great variability. Flow cytometry was used to estimate 1Cx-values with the aim to analyze the genome size of Lycoris species with different basic chromosome numbers. Three Lycoris species with x=11, three Lycoris species with x=8 and two hybridization origination species, L. straminea (2n=19) and L. haywardii (2n=22) were quantified by flow cytometry in this study. The results demonstrated that: (1) the 1Cx-values of Lycoris lines with x=11 ranged from 20.22 pg to 25.46 pg, among which, L. radiata var. pumila and L. radiata (triploid) are markedly smaller than the other species with x=11. (2) The 1Cx-values of L. aurea, L. chinensis and L. longituba with x=8 were close, which were 30.40 pg, 32.42 pg and 31.40 pg respectively, much larger than those with x=11, suggesting different origin between Lycoris species with x=11 and x=8. (3) The 1C DNA contents of L. straminea and L. haywardii were 26.97 pg and 23.96 pg respectively, which were close to the averages of their hypothetic parental lines, well proving their hybridization origination. To our knowledge, the data may be helpful for the evolution studies of Lycoris genus.

Key words: Lycoris, Genome size, Flow cytometry.

Introduction

Knowledge of genome size is helpful for plant scientists working in the area of genome analysis, biotechnology, plant breeding, and physiology. In addition, genome size (Cx-value) could be applied to investigate the relationships within genera, and valuable information could be obtained from such work for species under studies on biodiversity (Bennett & Leitch, 2005). It has been successfully used to investigate the origin of plants with different basic number (Lavia & Fernández, 2008) and different karyotype (Poggio et al., 2007).

Flow cytometry (FCM) is accepted as the method of choice for the measurements of genome size, which is accurate, dynamical, low cost, and also with the advantages of high throughput and general applicability (Kurita & Hsu 1998; Lavia & Fernández, 2008). Nowadays, a large number of angiosperm taxa belonging to several genera had been estimated by FCM, such as Miscanthus (Li et al., 2013), Pongamia pinnata (Ramesh et al., 2014), Turnera ulmifolia (López et al., 2011), Hepatica (Mabuchi et al., 2005), Narcissus (Zonneveld, 2008), Galanthus (Zonneveld et al., 2003). In consideration of an easy reference, scientists have worked together to produce pooled lists of plant DNA C-value in electronic form (Bennett & Leitch, 2005; Bennett & Leitch, 1995; Bennett & Leitch, 1997; Zonneveld et al., 2005). Now researchers can easily search the plant DNA C-value at http://www.kew.org/genomessize/homepage.

The genus Lycoris, a member of the family Amaryllidaceae, consists of 30 species around the world, of which 15 species are native to China. Polyploidization and hybridization have been considered as important modes of speciation within Lycoris (Bose & Flory, 1963; Kurita 1988a, b; Kurita & Hsu 1996). To date, triploid and tetraploid of L. radiata and triploid L. sprengeri have been reported (Kurita 1987a, Zhou et al., 2007; Zhang et al., 1999). Some species are originated from inter-specific hybridization, such as, L. haywardii and L. straminea, which were deduced as the hybrid of L. radiata var. pumila and L. sprengeri and the hybrid of L. radiata var. pumila and L. chinensis respectively (Bose & Flory 1963; Kurita 1987b, c). The most important feature of Lycoris genus is that the basic chromosome number displays great variability, including x=6, x=7, x=8, and x=11. And among those, x=11 and x=8 are common, and x=11 was considered as the most primitive basic chromosome number within Lycoris (Hsu et al., 1994). Speciation and phylogenetic relationships in Lycoris genus have been extensively studied by approaches of morphology, cytology, and molecular biology (Hsu et al., 1994; Bose & Flory, 1963; Kurita 1988a, b, Kurita & Hsu, 1996; Kurita 1987a, b, c; Shi et al., 2006; Chung, 1999; Hayashi et al., 2005). And genome size is a new criterion to investigate the relationships within genera. Investigation of genome size in Lycoris genus may help to uncover the relationships of Lycoris species with different basic chromosome number. Till now, only the genome size of L. aurea was estimated by FCM, which is 23961 Mb/1C (Zonneveld et al., 2005).

In order to investigate whether Lycoris species with different basic chromosome number have different genome size, three Lycoris species with x=11, three Lycoris species with x=8 and two species of hybrid origin with 2n=19 and 2n=22 were analyzed by FCM in this study. The results will facilitate the investigation of the origin of relationships among Lycoris species.

Materials and Methods

Plant materials: Plant materials were obtained from the nursery of Nanjing Botanical Garden Mem.Sun Yat-Sen. They were L. sprengeri Comes ex Baker, (2n=22), L. radiata (L’ Hérétier) Herbert, (two diploid lines (2n=22),
one triploid lines (2n=33), L. haywardii Traub, (2n=22), L. radiata var. pumila Gery, (2n=22), L. aurea (L’Héritier) Herbert, (2n=16), L. chinensis Traub, (2n=16), L. longituba Y. Xu & G. J. Fan, (2n=16) and L. straminea Lindley, (2n=19). Seeds of Triticum aestivum L. cv. Chinese Spring were kindly supplied by Jizhong Wu (Jiangsu Academy of Agriculture Sciences, Nanjing, China), which was used as standard (2C = 34.90 pg) (Zonneveld et al., 2005).

Each Lycoris species, composed of stems from three individuals, were collected in the summer 2015, when they were flowering. And leaves of Triticum aestivum L. cv. Chinese Spring were collected when the seedlings were tapped in 45% acetic acid and pressed by a microscope slide (Zhou et al., 2007). The chromosome numbers were counted by a photomicroscope (Nikon Eclipse 50i, Japan). The chromosome counts were determined using at least 10 well-spread metaphase cells for each species.

Chromosome preparation: The root tips were used for cytogenetic analysis. Firstly, root tips about 1-2 cm long were gathered and soaked in 0.002 M 8-hydroxyquinoline for 6 h at 4 °C. These tips were fixed in the solution of absolute ethanol and glacial acetic acid at the ratio of 3:1 for 24 h at 4°C. Subsequently, the root tips were washed with tap water and then treated with 1 M hydrochloric acid at 60 °C for 6 min. After that, the root tips were stained with phenol-fuchsin for 12 h. Finally, the stained root tips were tapped in 45% acetic acid and pressed by a microscope slide (Zhou et al., 2007). The chromosome numbers were counted by a photomicroscope (Nikon Eclipse 50i, Japan). The chromosome counts were determined using at least 10 well-spread metaphase cells for each species.

FCM analysis: All samples were investigated by flow cytometry (BD. AccuriC6) at a laser wave length of 488 nm. The G’s buffer (Galbraith et al., 1983) was used for nuclear isolation. Samples were chopped on ice with 1 ml G’s buffer. Immediately, the nuclear suspensions were filtered through a 33 µm Nylon filter, then RNase A (50 µg/ml) was added into the suspensions. The mixed-nuclear suspensions were incubated at 37°C for 15 min, and stained with PI stock at 50 µg/ml working concentration. They were kept at 4°C until analysis.

Each sample was assayed 3 times and each time 5,000 nuclei were analyzed. Meanwhile, the coefficient of variation (CV) values was under 5%. And the following formula: 1C nuclear DNA content of test sample (pg) = 2C peak mean of test sample x 1C nuclear DNA content of Triticum aestivum L. cv. Chinese Spring (17.5 pg) / 2C peak mean of Triticum aestivum L. cv. Chinese Spring was used to calculate the genome size of the Lycoris species. The results were subjected to SPSS 17.0. One-way ANOVA was performed for all the data. A significance level of 1% was selected for 1Cx value of species with x=11.

Results and Discussion

Lycoris genus has large chromosome and large genome (Kurita 1986; Kurita 1987b; Go et al., 2012), while only the 1C DNA content of L. aurea was reported (Zonneveld et al., 2005). In this study, the genome sizes of six diploid species (L. aurea, L. chinensis, L. longituba, L. sprenger, L. radiata and L. radiata var. pumila), one triploid species and two species of hybridization origin (L. haywardii, and L. straminea) were measured by FCM. The species, ploidy, chromosome number, DNA contents and 1Cx-values are listed in Table 1. The results demonstrated that each Lycoris species surveyed had a large genome, larger than that of Triticum aestivum L. cv. Chinese Spring. Interestingly, the 1C DNA contents of all diploid species varied from 20.22 pg to 32.42 pg, demonstrating great differences in genome size.

<table>
<thead>
<tr>
<th>Species</th>
<th>Ploidy</th>
<th>Chromosome number</th>
<th>Mean genome size( Mbp/1C)</th>
<th>1C DNA (pg) ± SE</th>
<th>1Cx-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. radiata (diploid 1)</td>
<td>2x</td>
<td>2n=22</td>
<td>22960</td>
<td>23.50 ± 1.62</td>
<td>23.50ab</td>
</tr>
<tr>
<td>L. radiata (diploid 2)</td>
<td>2x</td>
<td>2n=22</td>
<td>24884</td>
<td>25.46 ± 0.59</td>
<td>25.46a</td>
</tr>
<tr>
<td>L. radiata (triploid)</td>
<td>3x</td>
<td>2n=33</td>
<td>32594</td>
<td>33.35 ± 0.41</td>
<td>22.24b</td>
</tr>
<tr>
<td>L. radiata var. pumila</td>
<td>2x</td>
<td>2n=22</td>
<td>19755</td>
<td>20.22 ± 0.17</td>
<td>20.22c</td>
</tr>
<tr>
<td>L. sprenger</td>
<td>2x</td>
<td>2n=22</td>
<td>23800</td>
<td>24.36 ± 0.56</td>
<td>24.36a</td>
</tr>
<tr>
<td>L. aurea</td>
<td>2x</td>
<td>2n=16</td>
<td>29718</td>
<td>30.41 ± 0.60</td>
<td>30.40</td>
</tr>
<tr>
<td>L. chinensis</td>
<td>2x</td>
<td>2n=16</td>
<td>31682</td>
<td>32.42 ± 0.33</td>
<td>32.42</td>
</tr>
<tr>
<td>L. longituba</td>
<td>2x</td>
<td>2n=16</td>
<td>30765</td>
<td>31.39 ± 0.55</td>
<td>31.39</td>
</tr>
<tr>
<td>L. haywardii</td>
<td>2x</td>
<td>2n=22</td>
<td>23416</td>
<td>23.96 ± 1.22</td>
<td>23.96</td>
</tr>
<tr>
<td>L. straminea</td>
<td>2x</td>
<td>2n=19</td>
<td>26354</td>
<td>26.97 ± 0.11</td>
<td>26.97</td>
</tr>
</tbody>
</table>

*ab-c: Means following the same letter in a column are not significantly different. Tukey test (P = 0.01)
As shown in Table 1, the 1C DNA contents of species with x=8, close to that of triploid L. radiata (2n=33), are much larger than those of species with x=11. L. aurea, L. chinensis and L. longituba, with x=8. Former researches demonstrated that L. aurea, L. chinensis and L. longituba are very similar in morphology, cytology, and pollen characters (Zhou et al., 2007). And the 1C DNA contents of these species are 30.40 pg, 32.42 pg and 31.39 pg respectively, also indicating a close relationship among them. In this study, L. radiata (diploid and triploid), L. sprengeri and L. radiata var. pumila were selected as representative species with x=11 for genome size analysis. The results demonstrated that 1C DNA content of diploid L. radiata and L. sprengeri are close. And L. radiata var. pumila is the smallest among those with 2n=22 (Table 1), consistent with its morphological characters, which is smaller in bulbs, narrower and shorter in leaves than other species (Fig. 1). Leaves of L. sprengeri appear in spring, while leaves of L. radiata var. pumila and L. radiata appear in autumn, the plant morphology and leaves of species with x=11, except L. sprengeri, were shown in Fig. 1. 1C DNA content of triploid L. radiata is 33.35 pg, which increased with the chromosome number, but not in the expected proportion with that of L. radiata in diploid or that of L. radiata var. pumila. The result is acceptable for that triploid species of L. radiata is not a simple autotriploid (Kurita 1987a; Hayashi et al., 2005).

1C values of two diploid L. radiata are 23.50 pg and 25.46 pg respectively, demonstrating a little differences. For there are some differences in karyotypes of L. radiata (Bose & Flory 1963; Kurita 1988a, b; Zhou et al., 2007; Kurita 1987a; Mookerjea 1955; Shao et al., 1994; Qin et al., 2004a, b; Zhou et al., 2004; Liu et al., 2016), we deduced that differences in 1C DNA content between two diploid L. radiata may result from material differences. And another phenomenon in this study may also result from the differences in materials, which is that the 1C value of L. aurea here is different from that listed by Zonneveld (Zonneveld et al., 2005). And also differences of karyotypes in L. aurea have been reported (Bose & Flory 1963; Kurita 1987c).

In this study, 2 species of hybrid origin, L. haywardii and L. straminea, were analyzed. L. haywardii with 2n=22, was deduced as the hybrid of L. radiata var. pumila and L. sprengeri, and L. straminea with 2n=19, was deduced as the hybrid of L. radiata var. pumila and L. chinensis (Bose & Flory 1963; Kurita 1987b, c). The 1C DNA contents of L. straminea and L. haywardii are 26.97 pg and 23.96 pg respectively, close to the average of the deduced parent lines, confirming the hybrid origin hypothesis, which is consistent with the results of Shi’s ITS analysis (Shi et al., 2006).

1Cx-value refers to the amount of DNA in the unreplicated monoploid (x) chromosome set. The 1Cx-values of species with x=11 and x=8 were analyzed (Fig. 2). As shown in Fig. 2, the 1Cx-values of species with x=8 clustered together, markedly larger than those of species with x=11, indicating that different originations between Lycoris species with x=11 and species with x=8, 1Cx-values of species with x=11 have a little difference, the 1Cx-value of L. radiata var. pumila is smaller than diploid L. radiata and L. sprengeri with x=11, indicating that some evolution any events may take place in the origin of L. radiata var. pumila. Further investigation using molecular and in situ hybridization methods may uncover the evolution events in origin of L. radiata var. pumila, as well as the origin of triploid L. radiata.

![Fig. 1](image1.jpg)

Fig. 1. The whole plants of L. radiata and L. radiata var. pumila, including leaves, bulbs and roots, leaves (A); Leaves cut from L. radiata and L. radiata var. pumila (B) (1 for triploid L. radiata, 2 for L. radiata P1, 3 for L. radiata P2 and 4 for L. radiata var. pumila).

![Fig. 2](image2.jpg)

Fig. 2. Scatter plot between 1Cx-value and basic chromosome number. Circles denote groups with close 1Cx values.

In conclusion, DNA content is an important aspect for estimating the phylogenetic relationships of plants, especially for analyzing the relationships among plants belonging to the same genus (Mabuchi et al., 2005; Lysak et al., 2009). Our results revealed that 1) genome size varies greatly in Lycoris genus, and genome sizes of Lycoris species with x=8 are much larger than those with x=11. 2) 1C DNA contents of Lycoris species with x=8 are very close, while 1C DNA contents of Lycoris species with x=11 are also close except that of L. radiata var. pumila, which is much smaller than...
other species with x=11. To our knowledge, this is the first time to quantify the DNA contents of Lycoris species. The data may be helpful for the evolution any studies of Lycoris genus, the relationship within the Lycoris genus, and also for scientists working in the areas of biodiversity, genome analysis and plant breeding.

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