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Polygonatum Kingianum Genome Size Estimation by Flow Cytometry

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Abstract: *Polygonatum kingianum*, an herb of *Polygonatum* in Liliaceae, is a kind of Chinese herbal medicine with homology of medicine and food. *Polygonatum kingianum* has extensive scientific research, but its genome size is seldom studied. In order to enrich the genome data of *P. kingianum*, this study was based on flow cytometry to detect *P. kingianum* collected from different areas and estimate its genome size. We use corn as the internal reference plant and rice as the internal reference plant to correct the data, and control the CV value below 5.0% to ensure the accuracy of the results. The results showed that the genome size of *P. kingianum* was about 6.90±0.41 pg. Moreover, high-quality results have CV values of approximately 1.0%–2.0%, and the conventional results have CV values of approximately 3.0%. The average coefficient of variation in this study is 2.5%-3.5%; therefore, the genome size of the three populations in this study has high reliability. In a word, this study provides the first estimate of the genome size of *P. kingianum*. In the future, these results can be used to establish sequence data and provide a basis for determining the whole genome of *P. kingianum*. Moreover, these results provide a basis for selecting research materials for future work on P. kingianum genetic evolution and molecular breeding.

Keywords: Flow Cytometry (FCM), Genome Size, *Polygonatum Kingianum*

1. Introduction

Polygonati rhizoma is a famous traditional Chinese medicinal drug that is also called “Huang Jing” [1]. In the Chinese Pharmacopoeia, *Polygonatum kingianum* Coll. et Hemsl., *P. sibiricum* Red., and *P. cyrtonema* Hua. were specified as the original polygonati rhizoma plants. *Polygonatum kingianum* is mainly distributed in the center of southwest Yunnan Province. Compared with *P. sibiricum*, *P. kingianum* rhizome undergoes succulent hypertrophy and produces a higher yield. Alternatively, the *P. cyrtonema* rhizome is similar to that of *P. kingianum* (Figure 1). The main medicinal ingredients of *P. kingianum* are polysaccharides [2] and steroidal saponins [3]. Animal and cell experiments have shown that polygonati rhizoma improve and prevent osteoporosis [4]; have anti-inflammatory [5], bacteriostasis [6], anti-virus [7], anti-depression [8], anti-tumor [9], cardiotonic [10], anti-aging [11], and anti-oxidation functions; improve learning and memory [12]; serve as hypoglycemics [13] and hypolipidemics [14]; and improve immunologic functions [15]. Consequently, *P. kingianum* is an easily acquired resource with extremely high medicinal value and great developmental potential.

Figure 1. *The rhizomes of the three Polygonatum species in the Chinese Pharmacopoeia. A. Polygonatum kingianum, B. P. sibiricum, and C. P. cyrtonema.*

With the rapid development of new sequencing technology, there are a lot of medicinal plants whose whole genome sequences have been deciphered. The majority of medicinal plants are non-model plants, and next-generation sequencing generated entire genome sequences that provided molecular markers linked to potential genetic traits and candidate gene identification. For example, the *Dendrobium officinale* [16] genome was sequenced in 2015, and the *Panax notoginseng* [1] and *Erigeron breviscapus* [17] genomes were sequenced in 2017. Elucidating the whole genome greatly influences molecular-assisted selection [18] and our understanding of the genetic evolution of medicinal plants. Genome size prediction is necessary for understanding the genome content and read depth in next-generation sequencing, especially for species that have no reference genome.

Flow cytometry (FCM) is used to predict genome size because of its high efficiency and accuracy, and has been widely used in plants and animals. For example, FCM determined the genome sizes of coconut [19] and fern and lycophyte spores [20] in 2016, brackish water fishes and penaeid shrimps [21] in 2018, and the medicinal plant *Morinda officinalis* in 2018 [22]. Using FCM to estimate genome size is important for genome characterization, which is valuable for genetic improvement and evolutionary studies [23, 24]. To date, no studies have estimated the *P. kingianum* genome size by FCM, to our knowledge. Therefore, in this study, we used FCM to detect DNA content in nine samples to estimate *P. kingianum* genome size. Our results may provide a foundation for future studies on *P. kingianum* genetic evolution and molecular breeding.

2. Materials and Methods

2.1. Plant Material

We analyzed the genome size of nine *P. kingianum* samples. We collected fresh *P. kingianum* leaves in China at the same time and divided them into three groups based on the region from which they were obtained (HG1 from Baise City, Guangxi Province; HG2 from Wenshan City, Yunnan Province; and HG3 from Puer City, Yunnan Province; Figure 2). We collected three biological replicates per group. The samples were preserved with liquid nitrogen and stored at −80°C for FCM analysis.

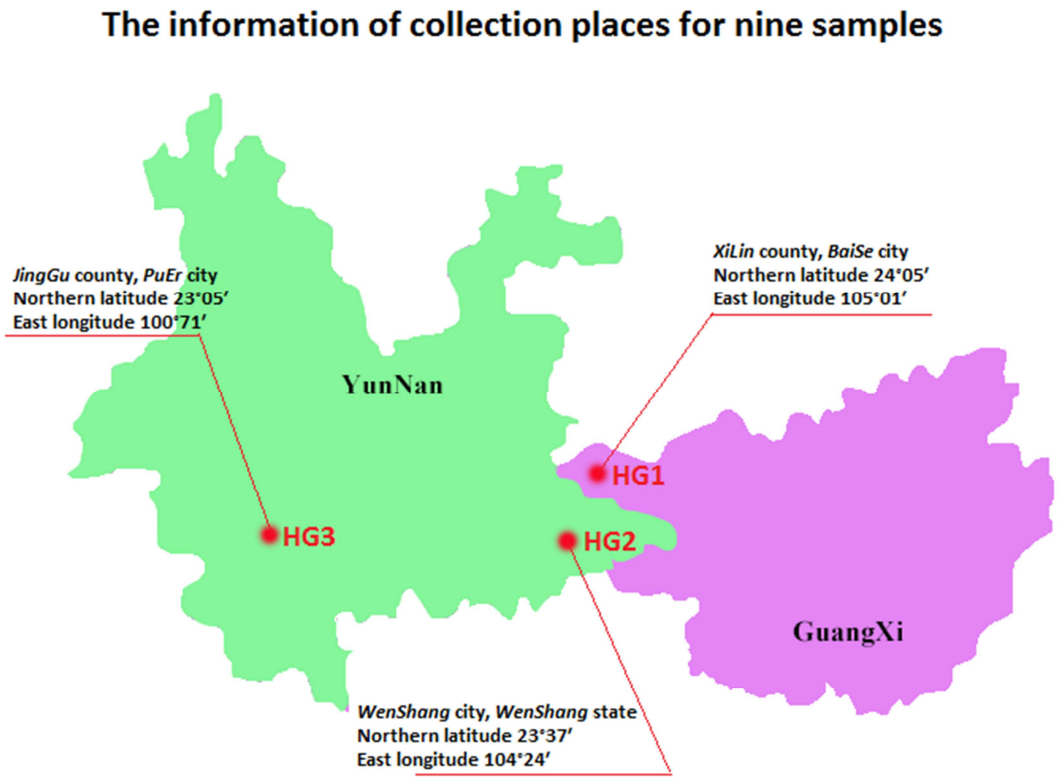


Figure 2. *Detailed collection locality information for the nine Polygonatum kingianum samples. Green represents Yunnan Province and purple represents Guangxi Province.*

2.2. Nuclear Suspension Preparation

First, we placed a small amount of fresh *P. kingianum* leaves (20 mg) in a Petri dish and added 1 ml ice-cold WPB buffer [200 mM Tris-HCl, 4 mM $MgCl_2$, 2 mM Na_2EDTA, 86 mM NaCl, 10 mM sodium metabisulfite, 1% (g/v), PVP, 1% (v/v) Tritonx-100, pH 7.5, 4°C]. Then, we immediately chopped the buffer-soaked leaves with a sterile scalpel blade, rapidly mixed the homogenate, and filtered the suspended solids through a 42-mm nylon mesh. Then, we added a DNA fluorochrome stock solution swing lightly, 50 mg/ml PI fluorescence dye, and 50 mg/ml RNase. We kept the samples on ice with occasional shaking during the experiments. The relative fluorescence of the stained nuclei was measured by FCM.

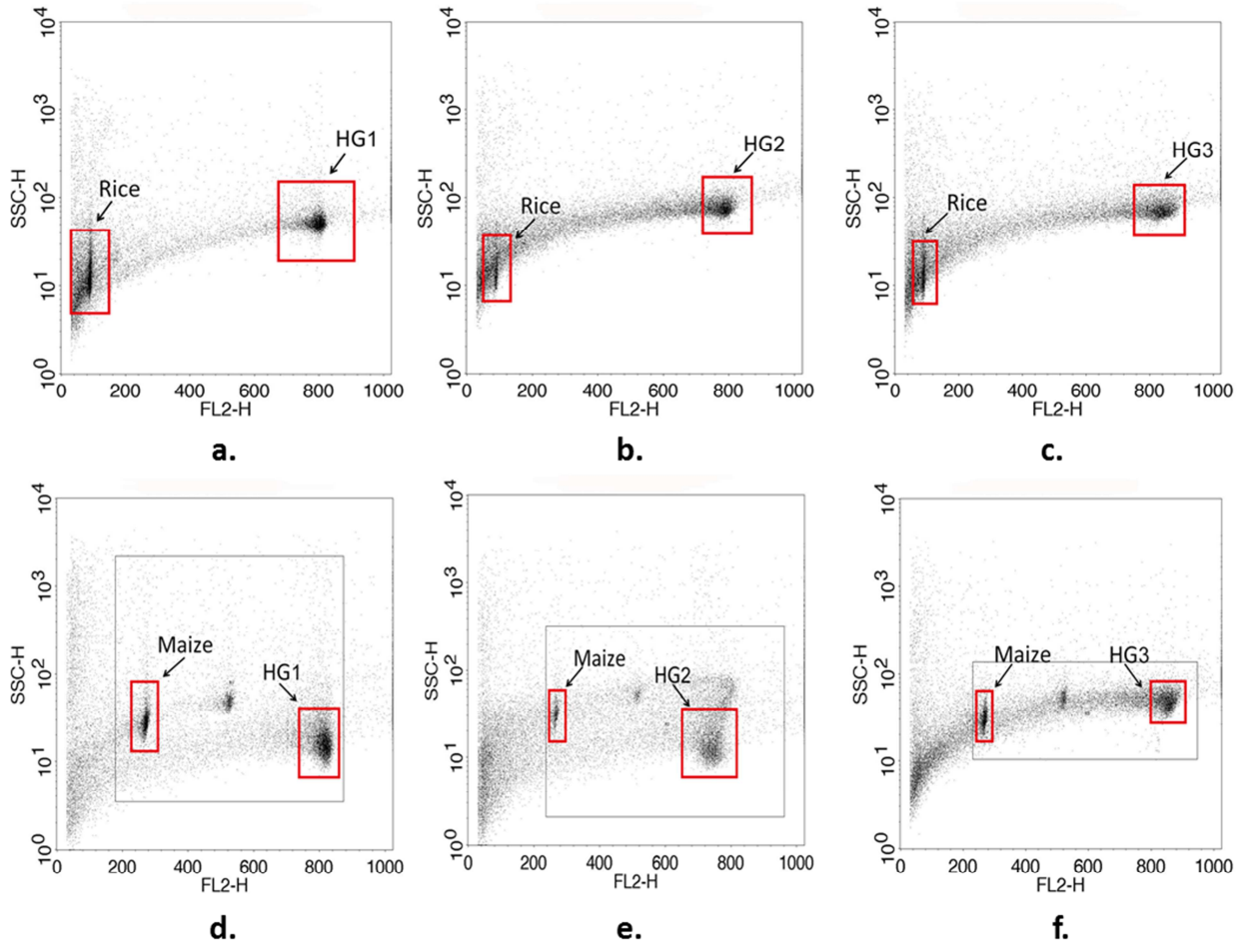
2.3. FCM Analysis

We used the BD FACScalibur flow cytometer for analysis. FCM analysis was excited at 488 nm to estimate the *P. kingianum* genome size and collected at least 10, 000 events (cells) per sample. The Maize B73 [25] and Nipponbare [26] genomes, which have known genome sizes (2.35 and 0.45 pg, respectively), served as the internal reference plant genomes. We used the Nipponbare genome for correction. On the basis of the fluorescence peak intensity of the internal reference plant and measured plant, we adjusted the mixture ratio to make the nuclear concentrations consistent; then, we tested the co-sample. The experimental results were generally considered reliable when the mean coefficient of variation (CV) value was ≤ 5%. The standard formula of genome size (in pg) = (Sample fluorescence channel number FL/Maize B73 or Nipponbare fluorescence channel number FL) × 2.35 (0.45) pg. We calculated and expressed the genome size based on a haploid DNA nuclear content 1 pg = 978 Mbp [27]. We obtained two groups of data, which we named Maize-HG and Rice-HG.

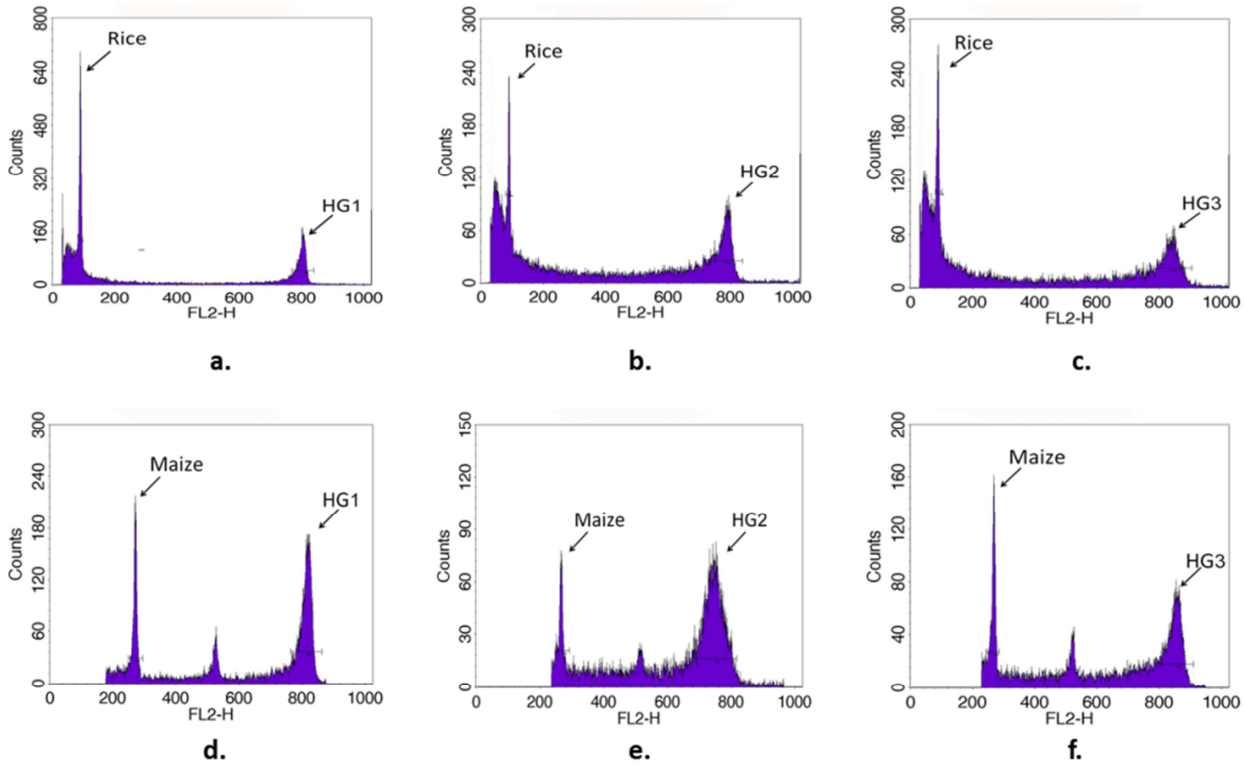
3. Results

3.1. Polygonatum Kingianum FCM Analysis

We estimated the *P. kingianum* genome size using two kinds of internal reference plant genomes. Rice-HG had no biological replications, whereas Maize-HG had three biological replicates per set. We obtained scatter diagrams and histograms of the *P. kingianum* samples and the details are shown in Figure 3A and B, respectively. The test results of all samples are shown in Tables 1 and 2.



A



B

Figure 3. *Rice-HG and Maize-HG scatter diagrams (A) and histograms (B). a., b., and c. Rice-HG, for which rice was the internal reference plant. d., e., and f. Maize-HG, for which maize was the internal reference plant; Maize-HG scatter diagrams and histograms represent one biological replicate.*

Table 1. *Polygonatum kingianum flow cytometry results with maize as the internal reference plant.*

Sample groups	Biological replications	FD1: maize	FD2: *P. kingianum*	Ratio: FD2/FD1
	1	265.44	808.82	3.05
HG1 (Baise city)	2	291.80	825.98	2.83
	3	269.87	808.92	3.00
	1	264.32	742.44	2.81
HG2 (Wenshan city)	2	291.80	761.76	2.61
	3	263.11	741.68	2.82
	1	264.51	843.85	3.19
HG3 (Puer city)	2	291.80	847.84	2.91
	3	264.51	843.66	3.19

FD: Fluorescence density; the collection localities of all samples are in the parentheses.

Table 2. *Polygonatum kingianum flow cytometry results with rice as the internal reference plant.*

Sample groups	FD1: rice	FD2: *P. kingianum*	Ratio: FD2/FD1
HG1	51.93	796.53	15.34
HG2	53.49	784.60	14.67
HG3	51.20	837.65	16.36

3.2. Polygonatum Kingianum Genome Size Estimation

Table 3. *Estimated genome sizes of P. kingianum from three regions.*

Sample groups	Average C-value (mean±SD; pg)	Average CV (%)
HG1	6.95±0.26	2.53
HG2	6.46±0.27	3.46
HG3	7.28±0.38	3.07
Average	6.90±0.41	3.02

SD: Standard deviation; CV: Coefficient of variation.

Using maize as the internal reference plant, the average estimated *P. kingianum* genome size was 6.90±0.41 pg (Table 3). HG3 from Puer City had the largest genome (7.28±0.38 pg), followed by HG1 from Baise City 6.95±0.26 pg, and HG2

from Wenshan City had the smallest genome size (6.46±0.27 pg). The average CVs of HG1, HG2, and HG3 were 2.53, 3.46, and 3.07, respectively. There were obvious genome size differences between the three groups. The highest relative difference was between HG2 and HG3 (12.69%), followed by HG1 and HG2 (7.59%), and then HG1 and HG3 (4.75%).

4. Discussion

Polygonatum kingianum is a traditional Chinese medicine that has extremely high medicinal value. However, no information was available on *P. kingianum* genome size. To our knowledge, this is the first report of *P. kingianum* genome size, and we determined genome size using FCM. Previous research revealed that the genome sizes of Liliaceae plants were relatively large; for example, *Allium cepa* L. has a genome size of 19.01 pg (18.16 Gb) and *Hemeorcallis citrina* Baroni has a genome size of 7.83 pg (7.48 Gb) [28]. We analyzed the genome sizes of three *P. kingianum* groups from different regions: HG1 (6.95±0.26 pg), HG2 (6.46±0.27 pg), and HG3 (7.28±0.38 pg). All of these results represent the mean values of biological replicates; we used maize as the internal reference plant and corrected the data with rice as the internal reference plant. To ensure the accuracy of the results, the CV value was controlled below 5.0% [29]. Moreover, high-quality results have CV values of approximately 1.0%–2.0%, and the conventional results have CV values of approximately 3.0% [30]. The mean CV value in this study varied from 2.5%–3.5%; therefore, the genome sizes of the three *P. kingianum* groups in our study had high reliability.

Different *P. kingianum* populations were collected from different regions in China: HG1 was from Baise City, Guangxi Province; HG2 was from Wenshan City, Yunnan Province; and HG3 was from Puer City, Yunnan Province. There were obvious genome size differences between the three groups. The relative differences were 12.69% between HG2 and HG3, 7.59% between HG1 and HG2, and 4.75% between HG1 and HG3. These differences may be caused by species diversity, environmental differences, and planting patterns. Wenshan City is a major producer of *P. kingianum*; high planting density and concentrated planting patterns may result in a lower genetic diversity of *P. kingianum*, which could explain why HG2 has a smaller genome than HG1 and HG3. Additionally, according to the Flora of China, there is considerable variation in *P. kingianum*; the variable genomic size determined in this study could verify this variability at the gene level to some extent.

5. Conclusion

In conclusion, we provided the first estimate of *P. kingianum* genome size to date. In the future, these results may establish sequence data that provide a foundation for determining the whole genome of *P. kingianum*. The relative differences between the three groups (HG1, HG2, and HG3) were significant; this, to some degree, supported the known considerable variation in *P. kingianum* at the gene level. Moreover, these results provide a basis for selecting research materials for future work on *P. kingianum* genetic evolution and molecular breeding.

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