The effects of fresh and rapid desiccated tissue on estimates of Ophiopogoneae genome size

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ABSTRACT

Fresh plant material is usually used for genome size estimation by flow cytometry (FCM). Lack of fresh material is cited as one of the main reasons for the dearth of studies on plants from remote locations. Genome sizes in fresh versus desiccated tissue of 16 Ophiopogoneae species and five model plant species were estimated. Our results indicated that desiccated tissue was suitable for genome size estimation; this method enables broader geographic sampling of plants when fresh tissue collection is not feasible. To be useful, after dessication the Ophiopogoneae sample should be green without brown or yellow markings; it should be stored in deep freezer at −80 °C, and the storage time should be no more than 6 months.

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1. Introduction

Genome size is the amount of DNA in a non-replicated, basic, gametic chromosome set (Soltis et al., 2003). It has been estimated in several thousand plant species since 1950 (Hanson et al., 2001), including at least 152 gymnosperms species (Leith et al., 2001) and 7542 angiosperms species (Bennett and Leitch, 2001), including at least 152 gymnosperms species (Leith et al., 2001) and 7542 angiosperms species (Bennett and Leitch, 2011). Genome size variation in angiosperms is especially impressive, ranging from 0.06 pg (Genlisea tuberosa Rivadavia) to 152.23 pg (Paris japonica (Franch. & Sav.) Franch.) (Pellicer et al., 2005; Lattier and Ranney, 2014). Best practices for FCM or seedlings in a very early ontogenetic stage; and (5) low operating costs (Dolezel, 1991).

The tribe Ophiopogoneae (Asparagaceae) has three genera: Ophiopogon Ker Gawl., Liriope Lour., and Peliosanthes Andr., which are mainly distributed in tropical, subtropical, and temperate regions of East and Southeast Asia. DNA content has been reported for only a few species (Bennett, 1972; Bharathan et al., 1994; Zonneveld et al., 2005; Lattier and Ranney, 2014). Best practices for FCM generally recommend that DNA content is measured using fresh tissue. Lack of fresh material is cited as one of the main reasons for the dearth of studies on plants from remote locations. Therefore, analyzing dehydrated tissue might be an attractive alternative. Tissue dessiccation, using either herbarium presses or silica gel, is a rapid and undemanding approach that has been used traditionally for sample preservation in field botany (Suda and Travniciˇek, 2006a). Vogtmayr (2000) was the first who estimated genome size in herbarium voucher specimens of mosses. The number of FCM studies that successfully used desiccated plant material has increased considerably, e.g., in Juncus biglumis (Schonswetter et al., 2007), Lychnis spp. (Popp et al., 2008), and Senecio carniolicus (Suda et al., 2007). Bainard et al. (2011) showed that rapidly dried tissue

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with silica gel can be efficiently used in genome size studies. Bai et al. (2012) also successfully used rapidly desiccated tissue for estimating genome size in 37 taxa.

Here, we compare the use of fresh and desiccated tissue for estimating genome size in the Ophiopogoneae and five model plant species (Arabidopsis thaliana (L.) Heynh., Oryza sativa L., Zea mays L., Glycine max (L.) Merr., and Lycopersicon esculentum Mill.).

### 2. Materials and methods

#### 2.1. Plant material

We sampled 22 accessions representing 16 species of Ophiopogon, Liriope, and Peliosanthes in the Ophiopogoneae, and collected five model plant species from different families spanning a nearly

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**Table 1**

Analysis of genome size variation between fresh and desiccated tissue in Ophiopogoneae and 5 model plant species.

| Study species | Herbarium voucher number | Locality in China (Province, City) | 2C-Value ± SE (fresh tissue) | 2C-value ± SE (desiccated tissue) | Paired t-test |
|---------------|--------------------------|-----------------------------------|-----------------------------|----------------------------------|--------------|
| O. bodinieri Lévl. | 20081615 | Yunnan, KIB | 11.22±0.11 | 11.73±0.58 | t = 0.987, P = N.S. |
| O. bockianus Diels | HGWZ506 | Hunan, Yongshun | 18.40±0.42 | 16.68±0.68 | t = 1.993, P = N.S. |
| O. chinii Wang et Tang | nie2350 | Guangxi, Longzhou | 17.49±0.29 | 17.39±0.90 | t = 0.140, P = N.S. |
| O. chinii var. glaucifolius F. T. Wang & L. K. Dai | nie3739 | Yunnan, Malipo | 11.98±0.29 | 12.26±0.18 | t = -0.216, P = N.S. |
| O. dracaenoides (Baker) Hook. f. | HGWZ557 | Yunnan, Mengla | 13.31±1.07 | 14.53±0.34 | t = -0.581, P = N.S. |
| O. mairei Lévl. | B673 | Hunan, Zhangjiajie | 12.31±0.11 | 12.91±0.67 | t = -1.060, P = N.S. |
| O. marmortus Pierre ex Rodrig. | HGWZ625 | Yunnan, Puer | 11.22±0.08 | 12.05±0.19 | t = -3.609, P = N.S. |
| O. peliosanthisoides Wang et Tang | HGWZ570 | Yunnan, Ninglang | 8.84±0.10 | 8.90±0.20 | t = -0.203, P = N.S. |
| O. pingbienensis Wang et Dai | nie3535 | Yunnan, Lijiang | 13.97±0.11 | 15.18±0.44 | t = -3.476, P = N.S. |
| O. platyphyllus Merr. et Chun | HGWZ655 | Hainan, Baoting | 18.50±0.14 | 19.45±1.61 | t = -0.647, P = N.S. |
| O. revolutus Wang et Dai | nie2340 | Guangxi, Longzhou | 14.73±0.15 | 14.29±0.81 | t = 0.616, P = N.S. |
| O. umbraticola Hance | HGWZ00791 | Yunnan, KIB | 12.58±0.16 | 12.76±1.24 | t = -0.144, P = N.S. |
| L. spicata (Thunb.) Lour. | nie3830 | Yunnan, Maguan | 9.57±0.10 | 9.77±0.28 | t = -0.560, P = N.S. |
| P. ophiopogonioides F. T. Wang & Tang | HGWZ536 | Yunnan, Pingbian | 11.33±0.17 | 11.82±0.28 | t = -1.611, P = N.S. |
| P. sinica Wang et Tang | HGWZ598 | Yunnan, Jinghong | 11.07±0.13 | 10.21±0.33 | t = 3.983, P = N.S. |
| P. yunnanensis Wang et Tang | HGWZ593 | Yunnan, Jinghong | 13.38±0.19 | 13.70±0.23 | t = -1.273, P = N.S. |

**Model species**

| Taxa | Herbarium voucher number | Locality in China (Province, City) | 2C-Value ± SE (fresh tissue) | 2C-Value ± SE (desiccated tissue) | Paired t-test |
|------|--------------------------|-----------------------------------|-----------------------------|----------------------------------|--------------|
| Arabidopsis thaliana (L.) Heynh. | 0.3±0.01 | 0.34±0.01 | t = -3.463, P = N.S. |
| Oryza sativa L. spp. japonica | 0.98±0.03 | 1.05±0.01 | t = -2.945, P = N.S. |
| Lycopersicon esculentum Mill. | 1.98±0.04 | 1.89±0.08 | t = 1.096, P = N.S. |
| Glycine max (L.) Merr. | 2.30±0.08 | 2.27±0.05 | t = 0.319, P = N.S. |
| Zea mays L. | 4.97±0.25 | 4.65±0.08 | t = 1.364, P = N.S. |
16-fold range of genome sizes (from 0.32 pg/2C to 5.43 pg/2C). The genome sizes of the model species according to the literature are: A. thaliana 0.32 pg (Bennett et al., 2003), O. sativa spp. japonica 0.86 pg (Goff et al., 2002), L. esculentum 2.05 pg (Bennett and Smith, 1976), G. max 2.25 pg (Greilhuber and Obermayer, 1997), Z. mays (B73) 5.43 pg. Living plants were cultivated in a greenhouse of Kunming Institute of Botany (KUN) in Yunnan, China. Leaf samples were taken from three individual plants per accession. Fresh leaf material was stored in a hermetically sealed box in a deep freezer (at –80°C, for no more than 3 d until it could be processed. The same leaf material was dried in silica gel. Desiccated tissues were stored in a hermetically sealed box in a deep freezer (at –80°C, for no more than 6 months). Voucher specimens were deposited at KUN.

2.2. Determining 2C-values

About 0.5 cm² leaf material was chopped up using a new razor blade in a Petri dish containing 1500 mL of Galbraith nuclear solution buffer (45 mmol/L MgCl₂, 30 mmol/L C₆H₅O₇Na₃·2H₂O, 20 mmol/L MOPS, 0.1% (v/v) Triton X-100, pH 7.0 (Galbraith et al., 1983)). The nuclear suspension was then filtered through disposable filters (30 μm) to remove the cell debris, and then stained with 150 μL propidium iodide (PI) (50 μg/mL; included RNase (500 μg/mL)) for 10 min. Samples were analyzed on a CyFlow Space (Partec, Münster, Germany) flow cytometer equipped with a blue laser operating at 488 nm. For all samples, at least 5000 nuclei were measured. FlowMax 2.82 analyzed the resulting histograms. Z. mays (B73) (2C = 5.43 pg) was chosen as an internal standard. The 2C-value of a sample was calculated as follows: (mean of sample peak/mean of standard peak) * 2C-value (pg) of the standard species (Table 1). These values per sample were used to obtain the accession averages.

2.3. Statistical analysis

Pearson correlation was used to analyze the relationships between genome sizes estimated from fresh and desiccated tissue using the whole data set, and Paired t-test was applied to FCM data obtained using desiccated vs. fresh tissues for each accession separately; SPSS 16.0 (SPSS Inc., Chicago, IL, USA) was used to perform the statistical analyses.

3. Results

The 2C-value for the fresh and desiccated tissue samples of 16 study species and five model species are presented in Table 1. A highly significant correlation between values obtained measuring fresh and desiccated tissues was observed combining data for all studied accessions (r = 0.986, P < 0.001; Fig. 1). For every accession, the 2C-values of fresh and desiccated tissue also showed no significant difference (Table 1).

Similarly, a significant correlation was observed for the five model plant species (r = 0.959, P < 0.001; Fig. 2) with no difference between the 2C-values of fresh and desiccated tissue (Table 1).

4. Discussion

Fresh, young leaf tissues are the usual source material for FCM analysis (Suda and Travnicek, 2006a). However, need in fresh material has been repeatedly stressed as a crucial limitation for FCM application in botany (especially for expeditions to remote areas when rapid dispatch or maintenance of fresh samples is difficult) (Suda and Travnicek, 2006b). Desiccated tissue has been proved to be suitable for FCM in some but not all cases (Kolar et al., 2012). It was firstly used in mosses (Voglmayr, 2000), and the desiccated moss tissue contained intact nuclei after drying. Two-year-old silica gel-dried samples of J. biglumis L. (Juncaceae) used in FCM showed variation in nuclear DNA content within instrumental error (Schonswetter et al., 2007). Air desiccation had no negative effects on nuclei integrity and subsequent DAPI fluorescence in Vaccinium subg. Oxycoccus (Suda and Travnicek, 2006b). In general, variation in genome size detected by FCM using desiccated tissue falls within an acceptable range of error (Bai et al., 2012), but some authors cast doubt on wide applicability of desiccated tissues. Bainard et al. (2011) thought that tissue treatment caused larger differences than other methodological factors, and Suda and Travnicek (2006a, b) proposed that desiccated tissue in FCM analysis should be restricted to determine ploidy, but not the applied to absolute genome size quantification. The previous studies comparing usage of fresh vs. desiccated tissue for estimation of genome sizes produced mixed results. Little et al. (2007), Suda et al. (2007), and Balao et al. (2009) reported the genome size difference between fresh and desiccated tissues for Selaginella, S. carniolicus, and Dianthus broteri to be less than 1.2%. Bainard et al. (2011) showed that desiccated tissue can alleviate the constraint of fresh tissue;
about 75% plant species were able to yield adequate histograms after 1 year storage at room temperature, although the remaining 25% produced no fluorescence signal.

In the present study, all studied species produced high quality histograms with highly significant correlation of the 2C-values obtained from fresh versus desiccated tissues. There was comparably low level of variation in 2C-values obtained with fresh and desiccated tissues with no significant difference between the later for any studied species. Thus, the observed intraspecific variation in genome size, which is a common phenomenon (Moscone et al., 2003), should be attributed to instrumental errors (Greilhuber and Obermayer, 1997; Greilhuber, 1998, 2005; Wang et al., 2015).

The 2C-values of the tested Ophiopogoneae species were similar to those reported earlier in the literature, but with some noticeable differences. For example, for L. spicata the detected 1Cx-value was 5.95 pg while it was earlier reported to be 3.99 pg (Lattier and Ranney, 2014). A reason for the observed discrepancy can be that Lattier and Ranney (2014) used Psamn sativum L. 'Cirard' (2C = 8.76 pg) as an internal standard, while we used Z. mays (B73) (2C = 5.43 pg). Usage of different reference standards may result in different estimates for the same material (Dolezel et al., 1998), with other potential causes being instrumental and methodological differences.

To conclude, our results show suitability of the desiccated tissues for reliable estimation of genome size if the issue after desiccation remains green without brown or yellow spots and is stored at ~80 °C for no more than 6 months. All the samples stored in this way should give FCM results of acceptable quality with good histogram resolution and little loss of fluorescence intensity.

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