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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, 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., 2010; Fleischmann et al., 2014). Such large-scale analyses have been enabled in part by using flow cytometry (FCM), a high-throughput method of estimating DNA content from isolated nuclei that are stained with a DNA-selective fluorochrome (Bainard et al., 2011). The popularity of FCM lies in its numerous advantages: (1) easy and convenient sample preparation; (2) high accuracy that permits the detection of minute variations in nuclear DNA amount; (3) rapid detection of mixed samples or endopolyploidy; (4) its non-destructive nature, which permits the comprehensive investigation of rare and endangered species 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 desiccation, 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 Travnicek, 2006a). Voglmayr (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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Table 1
Analysis of genome size variation between fresh and desiccated tissue in Ophiopogoneae and 5 model plant species.

Taxa	Herbarium voucher number	Locality in China (Province, City)	2C-Value ± SE (fresh tissue)	2C-value ± SE (desiccated tissue)	Paired t-test
Study species					
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.
	nie2350	Guangxi, Longzhou	17.49±0.29	17.39±0.90	t = 0.140, *P* = N.S.
O. chingii Wang et Tang	nie3739	Yunnan, Malipo	11.98±0.29	12.26±0.18	t = -0.216, *P* = N.S.
O. chingii var. *glaucifolious* F. T. Wang & L. K. Dai	nie2325	Guangxi, Fangcheng	15.16±2.57	14.50±0.85	t = 0.153, *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. peliosanthoides 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.
	nie2340	Guangxi, Longzhou	14.73±0.15	14.29±0.81	t = 0.616, *P* = N.S.
O. revolutus Wang et Dai	HGWZ556	Yunnan, Mengla	12.58±0.16	12.76±1.24	t = -0.144, *P* = N.S.
	HGWZ636	Yunnan, Puer	9.57±0.10	9.77±0.28	t = -0.560, *P* = N.S.
	HGWZ598	Yunnan, Jinghong	11.33±0.17	11.82±0.28	t = -1.611, *P* = N.S.
O. szechuanensis Wang et Dai	HGWZ593	Yunnan, Jinghong	11.07±0.13	10.21±0.33	t = 3.983, *P* = N.S.
O. umbraticola Hance	HGWZ00791	Yunnan, KIB	13.38±0.19	13.70±0.23	t = -1.273, *P* = N.S.
L. spicata (Thunb.) Lour.	nie3830	Yunnan, Maguan	11.90±0.10	12.81±0.40	t = -3.268, *P* = N.S.
P. ophiopogoniodes F. T. Wang & Tang	HGWZ536	Yunnan, Pingbian	21.75±0.10	23.73±0.84	t = -2.404, *P* = N.S.
P. sinica Wang et Tang	HGWZ587	Yunnan, Puer	29.36±1.05	30.38±0.60	t = -0.624, *P* = N.S.
	nie2364	Guangxi, Longzhou	25.75±0.56	26.27±1.12	t = -0.279, *P* = N.S.
P. yunnanensis Wang et Tang	nie3724	Yunnan, Malipo	24.55±0.89	22.72±0.27	t = 1.250, *P* = N.S.
Model species					
Arabidopsis thaliana (L.) Heynh.			0.32±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.

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., *Zea mays* L., *Oryza sativa* 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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