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RESEARCH NOTE

First report on DNA content of three species of *Amorphophallus*

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Abstract. The *Amorphophallus* genus is a perennial herb which belongs to the family Araceae. There are more than 170 species in this genus, which is widely distributed in tropical and subtropical areas. As a kind of food and medicine *Amorphophallus* has been used for more than 2000 years in China. Because of the high content of konjac glucomannan (KGM) and dietary fiber, it has attracted more attention worldwide. In this article, the DNA contents of *A. konjac*, *A. albus* and *A. bulbifer* in China, *A. albus*, *A. paeoniifolius* and *A. muelleri* in Indonesia were estimated by using flow cytometry. In the samples of China, the DNA contents were 12.95 ± 0.73 pg/2C in *A. konjac*, 10.51 ± 0.05 pg/2C in *A. albus* and 17.61 pg/2C in *A. bulbifer*, and for Indonesia, 14.16 ± 0.48 pg/2C in *A. albus* (flowering), 8.49 ± 0.2 pg/2C in *A. paeoniifolius* and 17.84 ± 1.46 pg/2C in *A. muelleri* were used. Interspecific variation was found significantly ($P < 0.01$), suggesting that DNA content might be a parameter that can be used to differentiate the species. Intraspecific variation has also been found significantly ($P < 0.01$), whether in the same region or between two regions. As far as we know, this is the first report on genome size estimation of the *A. konjac*, *A. albus* and *A. muelleri* using flow cytometry. Understanding the genome size of *Amorphophallus* species will help to sequence the genome and analyse the genetic diversity, evolutionary relationship and geographical variation pattern of *Amorphophallus* species.

Keywords. *Amorphophallus*; DNA content; flow cytometry; genome size variation.

Introduction

Amorphophallus belongs to the family Araceae, is a genus of perennial plants. According to the investigation, there are more than 170 kinds of *Amorphophallus* widely distributed in the tropics or subtropics of west Africa and south Asia (Wang and Zhang 2016). *Amorphophallus*, as a traditional crop, has been used as food and medicine for more than 2000 years in China. The edible part of plant is its bulb which needs further processing before using. In recent years, *Amorphophallus* has become more and more popular because of konjac glucomannan (KGM) in its bulbs, which is not only used in the industrial field, but also has good health care effect (Yi *et al.* 2005; Chua *et al.* 2010; Behera and Ray 2016; Devaraj *et al.* 2019; Tester and Al-Ghazzewi 2016), such as weight loss (Zalewski *et al.* 2015), intestinal health (Harmayani *et al.* 2014; Zhang *et al.* 2014), cancer treatment (Chen *et al.* 2017).

Amorphophallus includes diploid and triploid species (Chauhan and Brandham 1985). Diploid includes *A. konjac*, *A. albus*, *A. paeoniifolius*, etc. and triploid includes *A. muelleri*, *A. bulbifer*, etc.

Many research on *Amorphophallus* focus on its phylogeny, taxonomy, genetic diversity, flour and KGM (Grob *et al.* 2004; Diao *et al.* 2014; Jian *et al.* 2015; Pan *et al.* 2015; Huang *et al.* 2016; Gholave *et al.* 2017; Kite and Hetterscheid 2017; Gao *et al.* 2018; Wang *et al.* 2018; Liu *et al.* 2019; Zhu 2018). But there are only a few reports on the DNA content of *Amorphophallus*. Chauhan and Brandham has reported the DNA content of *A. paeoniifolius*,

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A. bulbifer, etc. (Chauhan and Brandham 1985). The DNA content of two species, *A. konjac* and *A. albus*, with the widest planting area and highest economic value and product quality in China has not yet been measured. This seriously hinders future work, such as hybridization, genome sequencing, etc., on the one hand, understanding the basic biological attribute of *Amorphophallus* DNA content, can provide a good reference for *Amorphophallus* genome sequencing (Pati *et al.* 2019), on the other hand, knowing the variation of DNA content can also reveal the interspecific and intraspecific genetic evolution relationships (Mabuchi *et al.* 2005), genetic diversity (Yan *et al.* 2016) and the geographical variation pattern (Sheng *et al.* 2016; Bennett 1976).

Flow cytometry is commonly used in determining the DNA content which has been successfully used in many plant species (Baack *et al.* 2005; Mabuchi *et al.* 2005; Pecinka *et al.* 2006; Smarda and Bures 2006; Caperta *et al.* 2018; Pati *et al.* 2019). In this study, we collected *A. konjac*, *A. albus* and *A. bulbifer* from China, *A. albus*, *A. paeoniifolius*, *A. muelleri* from Indonesia, and used flow cytometry to measure the contents of them. This is the first report determining the DNA content of *A. albus*, *A. konjac* and *A. muelleri*. In this study, we aimed to (i) determine the DNA content of *A. albus*, *A. konjac* and *A. muelleri*; (ii) understand the variation of DNA content intraspecies and interspecies of *A. albus*, *A. konjac* and *A. muelleri*; (iii) understand the geographical variation of DNA content.

Materials and methods

The following plant species: *A. konjac*, *A. albus* and *A. bulbifer* were used from in China; *A. albus*, *A. paeoniifolius* and *A. muelleri* were used from Indonesia. The plants were grown in a shelter greenhouse in Wuhan University, China. Young healthy tender buds of young plantlets (2–4 weeks old) were used for sample preparation. Buds, which were picked soon or stored in ice (< 2 days) were used for analyses. The internal standard used to quantify DNA were *Oryza sativa* L. spp. Var. Nipponbare with 0.9 pg of 2C nuclear DNA and *Zea mays* L. with 5.4 pg of 2C nuclear DNA (Bennett and Leitch 1995). Young, intact bud tissue of the analysed plants and an appropriate amount of young stem tissue of the internal reference standard were co-chopped by a sharp razor blade in a plastic Petri dish containing 2.0 mL of buffer solution (7.10 mg of KCl, 2.30 mg of $MgSO_4$, 2.30 mg of HEPES, 48.4 μL of 10% Triton X-100, and 0.2 g PVP and 50 μg RNase (1 mg/mL)) (Jaroslav *et al.* 2007; Sheng *et al.* 2016), mixed solution with plant cell nucleus was filtered through 400 mesh nylon membrane to dislodge plant residue. The filtered liquid was centrifuged at 3000 rpm at 4°C for 1.5 min to get single cells. Immediately after staining by propidium iodide (PI) for 15 min (Sheng *et al.* 2016), the relative fluorescence intensity of at least 10,000 particles was recorded on a CyFlow Space flow cytometer (Partec GmbH, Münster, Germany) equipped with a diode UV chip of 488 nm as an excitation light source. The DNA content of *Amorphophallus* can be calculated by the relative fluorescence intensity of the internal reference standard and the analysed plants, calculation method reference (Sheng *et al.* 2016). SPSS 22.0 software were used for all statistical calculations.

Results

By using the FlowJo software, we added co-chopped material results of six samples together, each histogram has two G1 peaks, the first represents internal standard (rice) and the second represent samples (figure 1).

The population averages of DNA content are listed in table 1. Diploid and triploid are concluded. DNA content is 12.95 ± 0.73 pg/2C for *A. konjac*, 10.51 ± 0.05 pg/2C for *A. albus* from China, 17.61 pg/2C for *A. bulbifer*, 14.16 ± 0.48 pg/2C for *A. albus* from Indonesia, 8.49 ± 0.2 pg/2C for *A. paeoniifolius*, 17.84 ± 1.46 pg/2C for *A. muelleri*. The highest DNA content was *A. muelleri*, the lowest was *A. paeoniifolius*, varying over two-fold, and the coefficient of variation was 27.6%.

Because of the differences in geographical environment, we use one-way ANOVA to calculate the differences between the three kinds of *Amorphophallus* in China and Indonesia, respectively (table 2). The DNA content of three kinds of *Amorphophallus* in China was significantly different ($P < 0.01$), and the coefficient of variation was 26.4%. The DNA content of three kinds of *Amorphophallus* in Indonesia was also significantly different ($P < 0.01$). The coefficient of variation was 34.9%.

A. muelleri from Indonesia is collected from seven random locations, i.e., representing seven populations. A Duncan's multiple range test indicated as which populations were significantly different at the alpha = 0.05 level (table 3).

We measured the DNA content of Chinese *A. albus* and Indonesian *A. albus* respectively, which were 10.51 ± 0.05 pg/2C in China and 14.16 ± 0.48 pg/2C in Indonesian. According to the results of Student's *t* test, the DNA content of Indonesia *A. albus* was significantly higher than that of Chinese *A. albus* ($P < 0.01$) (table 4).

Discussion

For all organisms, the DNA content is their basic biological attribute (Bennett and Leitch 2005). Understanding the DNA content of species can help to understand many biological processes, such as variation and evolution (Baack *et al.* 2005; Creber *et al.* 2006; Sheng *et al.* 2016; Inceer *et al.* 2018), speciation (Pellicer *et al.* 2018), species invasion (Guo *et al.* 2008), etc. At the same time, it can also help in practical work, such as cross breeding (Sisko 2003), genome

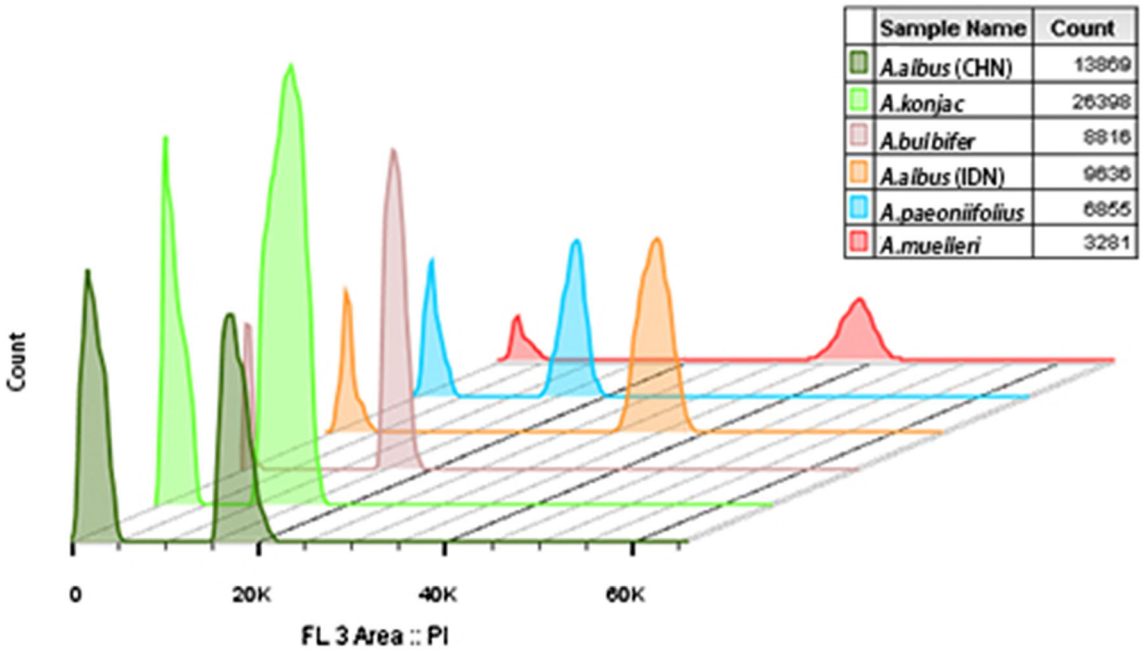

Figure 1. Estimation of nuclear DNA content in *Amorphophallus* using rice (*O. sativa*) cv Nipponbare as standard.

Table 1. DNA content of six kinds of *Amorphophallus* in China and Indonesia.

Sources	Species	DNA content (pg/2C)	Ploidy
China	*A. konjac*	12.95 ± 0.73	Diploid
China	*A. albus*	10.51 ± 0.05	Diploid
China	*A. bulbifer*	17.61	Triploid
Indonesia	*A. albus* (flowering)	14.16 ± 0.48	Diploid
Indonesia	*A. paeoniifolius*	8.49 ± 0.2	Diploid
Indonesia	*A. muelleri*	17.84 ± 1.46	Triploid

sequencing (Broderick *et al.* 2011; Pati *et al.* 2019), environmental protection (Ni and Guo 2005) and so on.

According to the DNA C-values database (https://cvalues.science.kew.org/), as of 18 February 2020, in angiosperms, the DNA content of 10,770 species has been measured, data are available for just *ca*. Three per cent of the *ca.* 352,000 species recognized (Lughadha *et al.* 2008). The average DNA content of 10,770 angiosperm species is 10.26 ± 17.88 pg/2C. The mean DNA content of the five *Amorphophallus* species measured in this study (13.85 ± 3.93 pg/2C) is about this average value, which indicates that the DNA content of *Amorphophallus* may be in the middle value in the angiosperm plants. The C-value of 149 Araceae species was recorded in the database, with an average value of 10.78 ± 8.28 pg/2C, which also indicated that the DNA content of *Amorphophallus* in the five species measured in this study might be in the middle value of Araceae. Around 15 kinds of *Amorphophallus* DNA content has been measured here (Chauhan and Brandham 1985; Zonneveld *et al*. 2005; Zhang *et al.* 2013), the smallest is *A. rivieri* 0.39 pg/2C, the largest is *A. johnsonii* 31.70 pg/2C. In this experiment, except *A. paeoniifolius* and *A. bulbifer* the other three kinds are the first measurement. The DNA content of *A. paeoniifolius* measured in this experiment is 8.49 ± 0.2 pg/2C, which is consistent with 8.42 pg/2C measured by Chauhan and Brandham (1985) and *A. bulbifer*

Table 2. Interspecific variation of DNA content of *Amorphophallus* in China and Indonesia.

				Significance of difference	
Species	Sources	Mean (pg/2C)	CV (%)	Alpha = 0.05	Alpha = 0.01
A. albus	China	10.51	26.4	a	A
A. konjac		12.95		b	B
A. bulbifer		17.61		c	C
A. paeoniifolius	Indonesia	8.49	34.9	a	A
A. albus		14.16		b	B
A. muelleri		17.84		c	C

CV, coefficient of variation. The lowercase letter abc indicates a significant difference of DNA content between species at the alpha = 0.05 level, and the upper case ABC indicates a significant difference of DNA content between species at alpha = 0.01 level.

Table 3. DNA content homogeneous subsets for the seven random places of *Amorphophallus* in Indonesia by Duncan analysis.

			Subsets (alpha = 0.05)		
Analytical methods	Random places	*n*	1	2	Duncans grouping
Duncan	4	4	16.976509900		a
	7	2	17.300310000	17.300310000	ab
	3	3	17.390850000	17.390850000	ab
	6	5		17.925838800	b
	2	2		17.936950000	b
	1	10		18.050084500	b
	5	10		18.146157200	b
	Significant		0.343	0.074	

Table 4. Intraspecific variation of DNA content of *A. albus* between China and Indonesia.

			Significance of difference	
Species	Sources	Mean (pg/2C)	Alpha = 0.05	Alpha = 0.01
A. albus	China	10.51	a	A
A. albus	Indonesia	14.16	b	B

(17.61 pg/2C) is also similar to what he measured (18.6 pg/2C), which shows that the results measured in this experiment are reliable.

C-value has been measured in other important cash crops. Rice (*O. sativa* L.) is 0.9 pg/2C (Bennett and Leitch 1995), corn (*Zea mays*) is 5.5 pg/2C (Greilhuber and Obermayer 1997), and soybean (*Glycine max*) is 2.26 pg/2C (Bennett and Leitch 1995), peanut (*Arachis hypogaea*) is 5.74 pg/2C (Temsch and Greilhuber 2000), Sorghum (*Sorghum bicolor*) is 3.40 pg/2C (Laurie and Bennett 1985). Compared with these species, the DNA content of *Amorphophallus* seems to be highest of all, which may be explained as *Amorphophallus* is a perennial plant, and the above species are annual plants. According to Ben's research (Bennett 1972), the DNA content of perennial plants is significantly larger than that of annual plants. Another hypothesis put forward by Huseyin Inceer (Inceer *et al.* 2018) is that taxa presenting rhodomes tend to present higher genome sizes. The possible reason is that the nutrient reserves availability in their storage organs, allowing genome expansion (Vesely *et al.* 2013). But there are also counterexamples, such as wheat (*Triticum aestivum*) which is 34.60 pg/2C (Bennett and Leitch 1995).

No matter whether its China or Indonesia, the variation of DNA content among *Amorphophallus* species very significantlly ($P < 0.01$), and the coefficient of variation was 26.4% and 34.9%, respectively. The variation of DNA content among species may be related to altitude, latitude, rainfall and other factors, Rayburn (Rayburn and Auger 1990) observed in maize that the DNA content of maize was positively correlated with the altitude. Bennett (1976) investigated the relationship between the DNA content of crop plants and the latitude. It was found that the species with high DNA content tend to distribute in the high latitude area. With the decrease of the latitude, the DNA content gradually decreases, and the crop DNA content in the tropical area was relatively lowest, which was naturally formed. Human activities modified and magnified it. Knight's (Knight and David 2002) survey of 401 species in the California flora found a positive correlation between DNA content and annual rainfall. In this study, because the sampling did not form a cline, we cannot accurately evaluate the variation of *Amorphophallus* genome size with environmental factors for the time being, and more research can be carried out in the future. As we expected, the DNA content of triploid *Amorphophallus* was significantly higher than that of diploid *Amorphophallus*. Triploid is vegetative reproduction, while diploid is sexual reproduction. The phenomenon that the vegetative reproduction species have larger genome is also found in other species, such as *tripleurospermum* taxa (Inceer *et al.* 2018), *Lolium* (Rees and Jones 1967). The DNA content of triploid *A. bulbifer* and *A. muelleri* are basically the same, which may indicate that they have a closer relationship. This result is the same as Liu's (Liu *et al.* 2019) study. They used DNA barcode to study the phylogenetic relationship of *Amorphophallus*, and found that *A. bulbifer* and *A. muelleri* are closely related.

A. muelleri comes from seven random natural populations, and its DNA content changes from 16.98 pg/2C to 18.15 pg/2C. We can divide *A. muelleri* into two subgroups by *t*-test, which shows that there are obvious variations in *A. muelleri* species. The factors that affect the variation of intraspecific DNA content may also be related to environmental factors, but the specific correlation needs more research. However, combining the coefficient of variation among the three kinds of *Amorphophallus* in Indonesia (34.9%) is greater than that in China (26.4%), it may indicate that there is a greater diversity in *Amorphophallus* population in Indonesia, and there may be more available *Amorphophallus* germplasm resources waiting for us to excavate.

Interestingly, the DNA content of *A. albus* from Indonesia (14.16 ± 0.48 pg/2C) is significantly higher ($P < 0.01$) than that of *A. albus* from China (10.51 ± 0.05 pg/2C), which may be caused by many factors. We speculate that there are two possible reasons: (i) Climate, Indonesia's

climate is a typical tropical rainforest climate, with an annual average temperature of 25–27°C, no four seasons, annual precipitation of 1600–2200 mm. China is located in the subtropical region, with complex climate change, four distinct seasons, and annual rainfall lower than Indonesia. *A. albus* 's most suitable growth temperature is 25°C, like to grow in a shaded place (Diao *et al.* 2006), and Indonesia's growth environment is more suitable for *A. albus* 's growth than China's. The research on *Microseris bigelovii* by Price *et al* (1981) found that plant populations with smaller genomes tend to be distributed in places where environmental conditions are relatively poor. To survive in such places, plants must quickly complete their life cycle, so that they have smaller genomes. Knight's (Knight and David 2002) study of California flora found that the species with larger genome tend to be distributed in the place with moderate maximum temperature (27°C) in July, and the DNA content decreases with the change of the maximum temperature to two ends. Bottini's (Bottini 2000) study on the distribution of natural population of *Berberis* found that the population distributed in the medium altitude and with high water use efficiency had higher DNA content. (ii) Annual growth time, as mentioned above, Indonesia has no four seasons, and the temperature is relatively uniform in a year, while China has four seasons. In China, due to the low temperature in the winter, *A. albus* can only grow from March to October. Therefore, *A. albus* has a longer annual growth time in Indonesia. Bennit (Bennett 1972) found that minimum generation time (MGT) is positively correlated with DNA content. His explanation is that the more DNA content, the slower the cell division, resulting in the longer MGT of the whole individual. Although he studied the relationship between MGT and DNA content, rather than the relationship between growth time and DNA content, a similar relationship may also exist between the two of them, but more accurate evidence needs further study.

In conclusion, this is the first study to measure the DNA content of *A. konjac*, *A. albus* and *A. muelleri*. The results showed that there were significant differences in DNA content among the species, which suggested that DNA content might be an effective method for *Amorphophallus* classification and identification of hybrids. This study found that even the DNA content of the same species in China and Indonesia is significantly different, indicating that DNA content may change adaptively with environmental conditions. The DNA content of *Amorphophallus* from Indonesia, whether intraspecific or interspecific has significant variation and the variation is larger than that of China, suggesting that *Amorphophallus* from Indonesia may have greater diversity and more potential *Amorphophallus* genetic resources. The understanding of DNA content is helpful for the genome sequencing of *Amorphophallus* species in the future. This study measured the DNA content of five species of *Amorphophallus*, and simply provided a trend of DNA content variation. Further research is needed to explore the relationship between geographical distribution and evolutionary origins.

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