Postprint of a Study on Nuclear DNA Content (2C-value) and Ploidy Levels in Chinese Enkianthus Species

Authors: Liang Hua, Jiang Lu, Zhu Dahai, Jianwei Zhou, Fan Dengmei, Zhang Zhiyong

Date: 2021-12-19T00:00:00+00:00

Abstract

Nuclear DNA content (2C-value) and ploidy level are important fundamental botanical characteristics that serve as powerful evidence for research in population evolution, species classification, and ecology. To determine the nuclear DNA content and ploidy level of various species in the Chinese Enkianthus Lour. genus and investigate interspecific and intraspecific differences in nuclear DNA content within this genus, this study utilized 60 samples from 23 populations across six Chinese Enkianthus species as experimental materials. Flow cytometry was employed to detect nuclear DNA content in these plants, with the rice variety 'Nipponbare' (Oryza sativa L. spp. japonica 'Nipponbare') serving as an internal reference. Furthermore, the diploid plant Enkianthus serrulatus was used as a ploidy reference to infer chromosome ploidy in other species, and the accuracy of ploidy was verified using the chromosome squash counting method. The results showed: (1) Nuclear DNA content in this genus ranged from 1.77-5.62 pg. (2) The four species in Section Enkianthus—Enkianthus quinqueflorus, E. serrulatus, E. serotinus, and E. perulatus—were all diploid (2n=2x=22); the two species in the Racemose section, E. chinensis and E. deflexus, exhibited both tetraploid and hexaploid forms. (3) Diploid plants in this genus showed significant interspecific and intraspecific differences in nuclear DNA content ($P<0.05$), whereas tetraploid and hexaploid plants showed no significant interspecific or intraspecific differences ($P>0.05$). This study establishes a foundation for research on phylogeny, biogeography, introduction and domestication, and genetic breeding in the Enkianthus genus.

Full Text

Nuclear DNA Content (2C-value) and Ploidy Level of *Enkianthus* Species (Ericaceae) from China

Hua LIANG[1,4], Lu JIANG[1,4], Dahai ZHU[2], Jianwei ZHOU[3]*, Dengmei FAN[4], Zhiyong ZHANG[4]

[1]College of Forestry, Jiangxi Agricultural University, Nanchang 330045, China
[2]Longxi-Hongkou National Natural Reserve, Chengdu 611830, China
[3]Science and Technology Park, Jiangxi Agricultural University, Nanchang 330045, China
[4]Laboratory of Subtropical Biodiversity, Jiangxi Agricultural University, Nanchang 330045, China

Abstract

Plant nuclear DNA content (2C-value) and ploidy level are fundamental botanical characteristics that provide powerful evidence for research on population evolution, biosystematics, and ecology. To determine the nuclear DNA content and ploidy level of *Enkianthus* Lour. species from China and explore interspecific and intraspecific differences in nuclear DNA content within this genus, we collected 60 samples from 23 populations across six Chinese *Enkianthus* species as experimental materials. Using rice variety 'Nipponbare' (*Oryza sativa* L. spp. japonica 'Nipponbare') as an internal reference, we measured nuclear DNA content via flow cytometry. Ploidy levels were inferred using the diploid species *Enkianthus serrulatus* as a reference and verified through chromosome squash counting. The results showed: (1) Nuclear DNA content in the genus ranged from 1.77–5.62 pg. (2) Four species in section *Enkianthus*—*E. quinqueflorus*, *E. serrulatus*, *E. serotinus*, and *E. perulatus*—were diploid (2n=2x=22), while two species in section *Racemus*—*E. chinensis* and *E. deflexus*—exhibited both tetraploid and hexaploid cytotypes. (3) Diploid species showed significant differences in nuclear DNA content both between and within species ($P<0.05$), whereas tetraploid and hexaploid plants showed no significant differences ($P>0.05$). This study establishes a foundation for future research on phylogenetics, biogeography, domestication, and genetic breeding in *Enkianthus*.

Keywords: *Enkianthus*, polyploid, genome size, flow cytometry, chromosome squash

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DNA carries the genetic information of organisms and is central to their survival and reproduction. DNA content correlates with cell size, metabolic rate, and leaf stomatal density (Beaulieu et al., 2007, 2008). The DNA content of each organism is often constant. To more intuitively describe DNA content, Swift (1950) proposed the term "C-value" to specifically refer to the DNA content in the nucleus of a gametophyte cell (chromosome number n). The Plant DNA C-

values Database (https://cvalues.science.kew.org/) has compiled C-value data for 12,273 species of angiosperms, gymnosperms, ferns, mosses, and algae. Since cells in the interphase of mitosis contain two unreplicated genomes, the nucleus contains 2C DNA content (2C-value) (Doležel & Bartoš, 2005). The 2C-value is a major characteristic parameter for describing biodiversity and holds significant importance in molecular biology, biosystematics, ecology, and population biology (Bennett et al., 2000; Ni & Guo, 2005; Kron et al., 2007).

Methods for measuring nuclear DNA content include chemical analysis (Schmidt & Thannhauser, 1945), reassociation kinetics (Britten & Kohne, 1968), Feulgen microspectrophotometry (Feulgen & Rossenbeck, 1924; Deeley, 1955), DNA image densitometry (Vilhar et al., 2001), and flow cytometry (Galbraith et al., 1983). Among these, flow cytometry has become the mainstream method for nuclear DNA content determination due to its simple operation, high efficiency, and accurate results (Bennett & Leitch, 2011). Additionally, flow cytometry offers broad application prospects for estimating ploidy in plants with small, numerous chromosomes and those for which chromosome squash materials are difficult to obtain (Bailey et al., 2008).

Enkianthus Lour. is a small East Asian endemic genus in the family Ericaceae comprising approximately 12 species. China is rich in *Enkianthus* species, with seven species recorded (Fang & Stevens, 2005): *E. pauciflorus*, *E. quinqueflorus*, *E. serrulatus*, *E. serotinus*, *E. perulatus*, *E. chinensis*, and *E. deflexus*. The genus is admired for its delicate bell-shaped flowers, particularly *E. quinqueflorus*, which is a prized ornamental in Guangzhou flower markets (Xu, 1982). *Enkianthus* exhibits substantial ploidy variation; Sax (1960) reported diploid (2n=22) and octoploid (2n=88) cytotypes in Japanese species. However, due to their small chromosome size and difficulty in cytological observation, ploidy research in this genus has seen limited progress. The genus also possesses medicinal value such as anti-inflammatory properties (Wang et al., 2020). Despite its high research value, studies on *Enkianthus* have primarily focused on taxonomy and phylogeny (Xu, 1982; Anderberg, 1994; Tsutsumi & Hirayama, 2012), cultivation and propagation (Yang et al., 2009; Pan et al., 2010; Li et al., 2020), and chemical composition (Ogawa et al., 1970; Sakakibara et al., 1983; Wang et al., 2014). Fundamental research on nuclear DNA content and ploidy levels in *Enkianthus* remains severely lacking, hindering advances in phylogenetics, plant resource utilization, and genetic breeding.

To address key questions—how many ploidy levels exist in Chinese *Enkianthus* species, whether multiple ploidies occur within a single species, whether nuclear DNA content differs among species with the same ploidy, and whether nuclear DNA content varies within a species across different distribution areas—this study employed flow cytometry to examine nuclear DNA content and ploidy levels in six Chinese *Enkianthus* species. Traditional chromosome squash observations were used to verify ploidy levels. We subsequently calculated genome sizes, explored relationships between nuclear DNA content variation (interspecific and intraspecific) and geographic environment, and investigated potential

phylogenetic relationships among different ploidy levels within the genus. This research fills a critical gap in knowledge of nuclear DNA content and ploidy in Chinese *Enkianthus* species, providing a foundation for future studies on resource utilization, polyploidization, phylogenetics, domestication, genetic breeding, and genomics.

Materials and Methods

1.1 Experimental Materials

We collected living plants and/or seeds of *Enkianthus* species from 27 counties across 10 provinces (autonomous regions) in China. Living plant materials comprised 60 individuals from 23 populations representing six species, while seeds were collected from eight populations across six species (Table 1). Voucher specimens are deposited at the Laboratory of Subtropical Biodiversity, Jiangxi Agricultural University (LSTB-JXAU). Seeds of the six *Enkianthus* species were germinated in a constant-temperature incubator at 20 °C, and young root tips were used for chromosome squash experiments. Living plants were transplanted to the ecological garden of Jiangxi Agricultural University, and young leaves were used for flow cytometry analysis. Based on previous research (Doležel et al., 2007), we selected rice variety 'Nipponbare' (*Oryza sativa* L. spp. japonica 'Nipponbare') (Sasaki & Burr, 2000) as the standard reference for 2C-values. Rice seeds were germinated and cultivated in an artificial climate chamber until leaf emergence. The diploid species *E. serrulatus* was used as the ploidy reference.

1.2.1 Chromosome Squash Counting

When seeds showed radicles approximately 0.5–1 cm long, 5–8 vigorous individuals were placed in 2.0 mL centrifuge tubes pre-chilled at 4 °C, and 0.6 mL of pre-chilled ultrapure water (4 °C) was added. Samples were pretreated in an ice-water mixture for 6 h. Pretreated materials were rinsed several times with ultrapure water, then transferred to freshly prepared Carnoy' s fixative (glacial acetic acid:anhydrous ethanol = 1:3) and fixed at 4 °C for 4 h. Fixed materials were washed several times, then placed in 1 $mol \cdot L^{-1}$ hydrochloric acid solution preheated to 60 °C for approximately 8 min of dissociation. After washing, root tips were placed on clean slides, and the meristematic region (the milky-white portion 2–5 mm from the root cap) was excised. One drop of improved phenol fuchsin stain was added, and after 10 min of staining, slides were prepared. Chromosomes were examined under an Aote Optical BK6000 microscope. Cells with clear, well-dispersed chromosome morphology were selected, and scales were added using OPTPro 3000 microscopic image analysis software. Finally, chromosome numbers were counted from more than 30 metaphase cells with good division images.

1.2.2 Flow Cytometry Detection

Following the method of Doležel et al. (2007), approximately 1 cm × 1 cm leaf tissue was placed in a 60 mm petri dish, and 1 mL of WPB isolation buffer (200 mM Tris; 4 mM $MgCl_2 \cdot 6H_2O$; 2 mM $Na_2EDTA \cdot 2H_2O$; 86 mM NaCl; 10 mM sodium metabisulfite; 1% PVP-10; 1% (v/v) Triton X-100, pH 7.5) was added. Using a sharp blade, samples were rapidly chopped vertically without scraping along the bottom of the dish to prevent nuclear rupture. Throughout the process, materials were kept submerged in isolation buffer to better liberate nuclei (Ye et al., 2015). Subsequently, 1 mL of DAPI stain (Sysmex) was added to the petri dish, and samples were incubated on ice in the dark for 10 min, with gentle mixing 2–3 times. The suspension was then filtered through a 400-mesh nylon screen to obtain stained nuclear suspension. A CyFlow® Cube 6 flow cytometer (Sysmex, Germany) with UV-LED excitation was used. Rice was employed as the internal standard for each sample, with at least 10,000 cells collected per detection. Each sample was analyzed in three replicates, and the mean value was calculated for all individuals within a population.

1.3 Data Analysis

Flow cytometry data were collected and analyzed using FlowJo 7.6 software. Results with a coefficient of variation (CV = standard deviation/mean × 100%) greater than 5% for the mean peak were discarded. The nuclear DNA content (2C-value) of samples was calculated as: sample 2C-value (DNA pg or Mbp) = (sample mean peak/standard mean peak) × standard 2C-value (Doležel et al., 2007). Genome size was calculated based on 1 pg DNA = 978 Mb (Doležel et al., 2003). Using diploid *E. serrulatus* as the ploidy reference, the ploidy level of other species was inferred using the formula: sample ploidy (integer) = (sample G1 mean peak/standard G1 mean peak) × standard ploidy (Doležel et al., 2007). SPSS 22.0 was used for statistical analysis of nuclear DNA content (2C-value) data, and OriginPro 9.0 was used for plotting.

Results

2.1 Nuclear DNA Content (2C-value) and Genome Size

The coefficient of variation (CV) for sample mean peaks remained within 5% (Figure 1 [Figure 1: see original paper]). Through further calculation, we obtained the 2C-values and genome sizes for six *Enkianthus* species. The hexaploid *E. deflexus* showed the highest nuclear DNA content (2C-value) with a mean of 5.62 pg, while *E. quinqueflorus* had the lowest at 1.77 pg. The hexaploid *E. deflexus* also had the largest genome size with a mean of 2,747.02 Mbp, whereas *E. quinqueflorus* had the smallest at 866.19 Mbp (Table 2).

2.2 Determination of Ploidy Levels

Chromosome squash results revealed that species in section *Enkianthus*—*E. serrulatus*, *E. serotinus*, *E. perulatus*, and *E. quinqueflorus*—all had 22 chromosomes and were diploid. Species in section *Racemus*—*E. deflexus* and *E. chinensis*—had 44 and 66 chromosomes, respectively, exhibiting both tetraploid and hexaploid cytotypes (Figure 2 [Figure 2: see original paper]). These results were consistent with ploidy levels inferred from flow cytometry (Table 2).

2.3 Interspecific and Intraspecific Differences in Nuclear DNA Content

One-way ANOVA of nuclear DNA content data revealed significant differences among the four diploid species ($P<0.05$). Specifically, *E. quinqueflorus* had significantly lower nuclear DNA content than other diploids, while *E. perulatus* had significantly higher content. However, no significant differences were detected between *E. chinensis* and *E. deflexus* at the same ploidy level ($P>0.05$) (Table 2). For species with larger sample sizes (*E. serrulatus*, *E. chinensis*, and *E. deflexus*), we performed one-way ANOVA on nuclear DNA content among different populations within each species. Significant differences were found among populations of diploid *E. serrulatus* (Figure 3 [Figure 3: see original paper]) ($P<0.05$), whereas no significant differences were observed among populations of polyploid species *E. chinensis* and *E. deflexus* at the same ploidy level (Figure 3) ($P>0.05$).

Discussion and Conclusion

Nuclear DNA content varies considerably among different plant taxa in nature (Chen et al., 2010), and this is also true for various groups within Ericaceae. According to the Plant DNA C-values Database, nuclear DNA content (2C-value) has been reported for 12 genera and 25 species in Ericaceae, ranging from 0.96 pg in *Erica multiflora* (Pellicer et al., 2010) to 59.80 pg in *Monotropa uniflora* (Bai et al., 2012). The nuclear DNA content of *Enkianthus* (1.77–5.62 pg) falls on the lower end within Ericaceae. Significant differences in nuclear DNA content have been documented among congeneric species with the same ploidy level, including *Vaccinium* (Costich et al., 1993), *Ziziphus* (Wu et al., 2013), and *Fragaria* (Chen et al., 2015). In this study, significant differences were detected among the four diploid species, with *E. quinqueflorus* and *E. serrulatus* showing significantly lower nuclear DNA content than *E. serotinus* and *E. perulatus* ($P<0.05$). Large differences in nuclear DNA content indicate substantial niche separation and diverse ecological adaptations among species (Li et al., 1999). Within the same family (or genus), species with lower nuclear DNA content among related taxa with similar life forms tend to have faster growth rates, shorter generation times, and can adapt to harsh environments, resulting in wider distribution ranges (Ni et al., 2005; Guo et al., 2008). Based on literature review and extensive field surveys, we confirmed that *E. quinqueflorus* and *E. serrulatus* indeed have broader distribution ranges than *E. serotinus* and

E. perulatus (Xu, 1982), suggesting stronger environmental adaptability in the former two species. For the narrowly distributed and less adaptable *E. serotinus* and *E. perulatus*, ex situ conservation strategies such as seed collection should be considered to protect these scarce plant resources.

To a certain extent, nuclear DNA content remains constant within a species to ensure genetic stability, as demonstrated in *Cajanus cajan* (Greilhuber & Obermayer, 1998), *Triticum aestivum* (Wetzel et al., 1999), and *Seleria albicans* (Lysák et al., 2000). In this study, polyploid species *E. chinensis* and *E. deflexus* showed no significant interspecific or intraspecific differences in nuclear DNA content at the same ploidy level ($P>0.05$), indicating a relatively stable genetic system that does not vary substantially with species or geographic factors. However, exceptions exist in nature. As early as 1966, Evans et al. (1966) found 16% variation in nuclear DNA content in flax (*Linum usitatissimum*) under different environmental conditions. Similar reports exist for maize (*Zea mays*) (Laurie & Bennett, 1985), perennial ryegrass (*Lolium perenne*) (Sugiyama et al., 2002), and bottle gourd (*Lagenaria siceraria*) (Achigan et al., 2008). These differences may be caused by climatic and geographic conditions, habitats, population density, and developmental stages (Guo et al., 2011). In this study, diploid *E. serrulatus* showed significant differences among populations, except between Chishui (Guizhou) and Xinning (Hunan). Comparing the climatic and geographic conditions of these four populations revealed that only the Chishui and Xinning populations grow in typical Danxia landform, which has poor water retention capacity and selects for strong drought resistance (Zhang, 2012). The Yongxiu population in Jiangxi borders Poyang Lake, where complex terrain and heterogeneous land-water surface properties influence mesoscale convective weather systems, significantly affecting the local climate for plant growth (Fu, 2013). The Lichuan population in Hubei lies in a transition zone between middle and north subtropical regions, with overlapping mountain ridges, crisscrossing valleys, large relative elevation differences, and pronounced mountain climate effects. These different geographic environmental conditions greatly influence plant growth and development and may explain the differences in nuclear DNA content among *E. serrulatus* populations.

Based on flow cytometry with diploid *E. serrulatus* as the ploidy reference, we inferred the ploidy levels of the other five species. Notably, tetraploid individuals of *E. chinensis* and *E. deflexus* exhibited non-integer ploidy issues, with tetraploid nuclear DNA content approximately 2.2 times that of diploid *E. serrulatus* and 2.0 times that of *E. perulatus*. Possible explanations include: (1) choice of ploidy reference, as significant differences in nuclear DNA content among diploid species lead to different inferred ratios when using different diploid references (Wang et al., 2009); and (2) tetraploid species may have originated from hybridization between species with larger nuclear DNA content (Yokoya et al., 2000). Therefore, when studying the origin and evolution of tetraploid *Enkianthus* species, attention should focus on diploid species with larger nuclear DNA content, particularly *E. perulatus* and *E. serotinus*. Additionally, we found that hexaploid nuclear DNA content in *Enkianthus* was less than 1.5 times that

of tetraploids and less than three times that of diploid *E. serotinus* and *E. perulatus*. Similar findings have been reported in *Agrostis*, where hexaploid nuclear DNA content was less than three times that of diploids (Bonos et al., 2002), and in *Rosa*, where tetraploid content was less than twice that of diploids (Jian et al., 2014). These differences may reflect intrinsic genomic variation among species or loss of repetitive DNA fragments during polyploid formation (Bonos et al., 2002).

Notably, section *Enkianthus* includes four diploid species distributed in China plus *E. perulatus* from Japan (Sax, 1960), while section *Racemus* includes *E. chinensis* and *E. deflexus* with both tetraploid and hexaploid cytotypes, and the Japanese species *E. campanulatus* as octoploid. No diploid species have been found in section *Racemus*, indicating substantial differences in ploidy levels between these two sections. The division of these sections is supported not only by macro-morphology (Xu, 1982), pollen micromorphology (Cheng & Lai, 1988), molecular markers (Tsutsumi & Hirayama, 2012), and chemical composition (Cheng et al., 1986), but is now further corroborated by nuclear DNA content and species ploidy levels. However, research on nuclear DNA content and ploidy in *Enkianthus* remains incomplete. For instance, *E. pauciflorus* from China and Japanese species *E. sikokianus*, *E. cernuus*, and *E. nudipes* have not been adequately studied due to difficulties in material acquisition. Additionally, five populations (E7, E8, E9, E12, E18) of three species had small sample sizes. Considering that nuclear DNA content from sparsely sampled populations may not represent the species-level value, we used multiple populations to assess species-level nuclear DNA content, providing a more robust representation (Bai et al., 2012; Pellicer et al., 2012).

Enkianthus chromosomes measure approximately 1–2 m, classifying them as small chromosomes (Lima-De-Faria, 1980) that are difficult for karyotype analysis. The consistency between flow cytometry ploidy inferences and chromosome squash results confirms the reliability of flow cytometry for large-scale ploidy determination in this genus, substantially improving detection efficiency. This study reports for the first time the nuclear DNA content (2C-value), genome size, and ploidy levels for five *Enkianthus* species (*E. quinqueflorus*, *E. serrulatus*, *E. serotinus*, *E. chinensis*, and *E. deflexus*), and nuclear DNA content (2C-value) and genome size for *E. perulatus*. The genus contains 2x, 4x, 6x, and 8x cytotypes, with multiple ploidies even within single species, indicating that polyploidization is widespread. Important questions remain unanswered: How did different polyploids form? What are the phylogenetic relationships among cytotypes? What role does ploidy variation play in ecological differentiation and speciation? This study provides an essential foundation for addressing these questions.

References

ACHIGAN-DAKO EG, FUCHS J, AHANCHEDE A, et al., 2008. Flow cytometric analysis in *Lagenaria siceraria* (Cucurbitaceae) indicates correlation of

genome size with usage types and growing elevation [J]. *Plant Syst Evol*, 276(1-2): 9-19.

ANDERBERG AA, 1994. Cladistic analysis of *Enkianthus* with notes on the early diversification of the Ericaceae [J]. *Nord J Bot*, 14(4): 385-401.

BAILEY JP, KAY QON, MCALLISTER H, et al., 2008. Chromosome numbers in *Sorbus* L. (Rosaceae) in the British Isles [J]. *Watsonia*, 27(1): 69-72.

BAI CK, ALVERSON WS, FOLLANSBEE A, et al., 2012. New reports of nuclear DNA content for 407 vascular plant taxa from the United States [J]. *Ann Bot*, 110(8): 1623-1629.

BEAULIEU JM, LEITCH IJ, KNIGHT CA, 2007. Genome size evolution in relation to leaf strategy and metabolic rates revisited [J]. *Ann Bot*, 99(3): 495-505.

BEAULIEU JM, LEITCH IJ, PATEL S, et al., 2008. Genome size is a strong predictor of cell size and stomatal density in angiosperms [J]. *New Phytol*, 179(4): 975-986.

BENNETT MD, BHANDOL P, LEITCH IJ, 2000. Nuclear DNA amounts in angiosperms and their modern uses: 807 new estimates [J]. *Ann Bot*, 86: 859-909.

BENNETT MD, LEITCH IJ, 2011. Nuclear DNA amounts in angiosperms: targets, trends and tomorrow [J]. *Ann Bot*, 107(3): 467-590.

BONOS SA, PLUMLEY KA, MEYER WA, 2002. Ploidy determination in *Agrostis* using flow cytometry and morphological traits [J]. *Crop Sci*, 42(1): 192-196.

BRITTEN RJ, KOHNE DE, 1968. Repeated sequences in DNA [J]. *Science*, 161(3841): 529-540.

CHEN BY, LI JF, HUO HZ, et al., 2015. Estimation of genome size in six wild strawberry species [J]. *J Fruit Sci*, 32(1): 51-56.

CHEN GQ, GUO SL, YIN LP, 2010. Applying DNA C-values to evaluate invasiveness of angiosperms: validity and limitation [J]. *Biol Invasions*, 12(5): 1335-1348.

CHENG XQ, SHAO H, WANG GX, et al., 1986. The chemotaxonomy of *Enkianthus* [J]. *Acta Bot Boreal-Occident Sin*, 6(3): 209-212.

CHENG ZB, LAI XR, 1988. Studies on the pollen morphology and evolution of the genus *Enkianthus* [J]. *J Sichuan Univ (Nat Sci Ed)*, 25(2): 221-229.

COSTICH DE, ORTIZ R, MEAGHER TR, et al., 1993. Determination of ploidy level and nuclear DNA content in blueberry by flow cytometry [J]. *Theor Appl Genet*, 86(8): 1001-1006.

DEELEY EM, 1955. An integrating microdensitometer for biological cells [J]. *J Sci Instrum*, 32(7): 263-267.

DOLEŽEL J, BARTOŠ J, 2005. Plant DNA flow cytometry and estimation of nuclear genome size [J]. *Ann Bot*, 95: 99–110.

DOLEŽEL J, BARTOŠ J, VOGLMAYR H, et al., 2003. Nuclear DNA content and genome size of trout and human [J]. *Cytom Part A*, 51(2): 127–128.

DOLEŽEL J, GREILHUBER J, SUDA J, 2007. Estimation of nuclear DNA content in plants using flow cytometry [J]. *Nat Protoc*, 2(9): 2233–2244.

EVANS GM, DURRANT A, REES H, 1966. Associated nuclear changes in the induction of flax genotrophs [J]. *Nature*, 212(5063): 697–699.

FANG RZ, STEVENS PF, 2005. *Enkianthus* [M]// WU ZY, RAVEN PH. *Flora of China*. Beijing: Science Press; St. Louis: Missouri Botanical Garden Press: 243–245.

FEULGEN R, ROSSENBECK H, 1924. Mikroskopisch-chemischer nachweis einer nukleinsäure von typus der thymonucleinsäure und die darauf beruhende selektive färbung von zellkernen in mikroskopischen präparaten [J]. *Hoppe-Seylers Z Physiol Chem*, 135(5–6): 203–248.

FU MN, 2013. Research on the impacts of Poyang lake on typical weather process and the characteristics of near surface boundary layer, Jiangsu China [D]. Nanjing: Nanjing University of Information Science and Technology: 8–10.

GALBRAITH DW, HARKINS KR, MADDOX JM, et al., 1983. Rapid flow cytometric analysis of the cell cycle in intact plant tissues [J]. *Science*, 220(4601): 1049–1051.

GREILHUBER J, OBERMAYER R, 1998. Genome size variation in *Cajanus cajan* (Fabaceae): A reconsideration [J]. *Plant Syst Evol*, 212: 135–141.

GUO SL, CHEN GQ, MAO LH, 2008. Relationship between DNA C-value and invasiveness in 539 angiosperm species in China [J]. *Acta Ecol Sin*, 28(8): 3698-3705.

GUO SL, ZHOU P, YIN LP, et al., 2011. Interspecific and intraspecific variations of plant DNA C-values and its biological significance [J]. *J Shanghai Norm Univ (Nat Sci)*, 40(1): 102-110.

JIAN H, ZHANG T, WANG Q, et al., 2014. Nuclear DNA content and 1Cx-value variations in genus *Rosa* L [J]. *Caryologia*, 67(4): 273–280.

KRON P, SUDA J, HUSBAND BC, 2007. Applications of flow cytometry to evolutionary and population biology [J]. *Annu Rev Ecol Evol Syst*, 38(1): 847–876.

LAURIE DA, BENNETT MD, 1985. Nuclear DNA C-content in the genera *Zea* and *Sorghum*. Intergeneric, interspecific and intraspecific variation [J]. *Heredity*, 55: 307–313.

LI C, LU T, LIU Y, et al., 2020. Effects of different treatments on seed germination of *Enkianthus chinensis* Franch [J]. *N Hortic*, 469(22): 72-78.

LI GQ, AN SQ, CHEN XL, et al., 1999. A primary research on relationship between C value paradox and its ecological adaptation of the life [J]. *Explor Nat*, 18(2): 61-66.

LIMA-DE-FARIA A, 1980. Classification of genes, rearrangements and chromosomes according to the chromosome field [J]. *Hereditas*, 93(1): 1-46.

LYSÁK MA, ALEXANDRA R, DIXON JM, et al., 2000. Limited genome size variation in *Sesleria albicans* [J]. *Ann Bot*, 86(2): 399-403.

NI LP, GUO SL, 2005. Review on relationship between invasiveness of plants and their DNA C-value [J]. *Acta Ecol Sin*, 25(9): 2372-2381.

OGAWA M, HISADA S, INAGAKI I, 1970. Studies on the constituents of *Enkianthus nudipes*. I. New flavan glucosides of the leaves and its absolute configuration [J]. *Yakugaku Zasshi*, 90(9): 1081-1085.

PAN W, ZHU BZ, XU B, et al., 2010. Nursery and twig-coiling techniques for raising seedlings of wild ornamental plants *Enkianthus quinqueflorus* Lour. [J]. *Guangdong For Sci Technol*, 26(6): 86-88.

PELLICER J, CLERMONT S, HOUSTON L, et al., 2012. Cytotype diversity in the *Sorbus* complex (Rosaceae) in Britain: sorting out the puzzle [J]. *Ann Bot*, 110(6): 1185-1193.

PELLICER J, ESTIARTE M, GARCIA S, et al., 2010. Genome size unaffected by moderate changes in climate and phosphorus availability in Mediterranean plants [J]. *Afr J Biotechnol*, 9(37): 6070-6077.

SAKAKIBARA J, KAIYA T, FUKUDA H, et al., 1983. 6β-Hydroxyursolic acid and other triterpenoids of *Enkianthus cernuus* [J]. *Phytochemistry*, 22(11): 2553-2555.

SASAKI T, BURR B, 2000. International rice genome sequencing project: The effort completely sequence the rice genome [J]. *Curr Opin Plant Biol*, 3(2): 138-141.

SAX HJ, 1960. Polyploidy in *Enkianthus* (Ericaceae) [J]. *J Arnold Arbor*, 41(2): 191-196.

SCHMIDT G, THANNHAUSER SJ, 1945. A method for the determination of desoxyribonucleic acid, ribonucleic acid, and phosphoproteins in animal tissues [J]. *J Biol Chem*, 161(161): 83-89.

SUGIYAMA S, YAMAGUCHI K, YAMADA T, 2002. Intraspecific phenotypic variation associated with nuclear DNA content in *Lolium perenne* L [J]. *Euphytica*, 128(2): 145-151.

SWIFT H, 1950. The constancy of desoxyribose nucleic acid in plant nuclei [J]. *P Natl Acad Sci USA*, 36(11): 643-654.

TSUTSUMI C, HIRAYAMA Y, 2012. The phylogeny of Japanese *Enkianthus* species (Ericaceae) [J]. *Bull Natl Mus Nat Sci, Ser. B*, 38(1): 11–17.

VILHAR B, GREILHUBER J, KOCE JD, et al., 2001. Plant genome size measurement with DNA image cytometry [J]. *Ann Bot*, 87: 719–728.

WANG HQ, MA SG, LIN MB et al., 2020. Hydroxylated ethacrylic and tiglic acid derivatives from the stems and branches of *Enkianthus chinensis* and their potential anti-inflammatory activities [J]. *J Nat Prod*, 83(10): 2867–2876.

WANG X, JIANG R, LIU Z, et al., 2014. Phytochemicals and biological activities of poisonous genera of Ericaceae in China [J]. *Nat Prod Commun*, 9(3): 427–442.

WANG Y, BIGELOW CA, JIANG Y, 2009. Ploidy level and DNA content of perennial ryegrass germplasm as determined by flow cytometry [J]. *Hortscience*, 44(7): 2049–2052.

WETZEL JB, AREF S, BALIGAR VC, et al., 1999. A lack of nuclear DNA C-content variability among wheat near isolines differing in aluminium response [J]. *Ann Bot*, 83(6): 725–728.

WU LP, TANG Y, LI YY, et al., 2013. Estimation of genome size of *Ziziphus jujuba* and *Z. acdiojujuba* [J]. *J Beijing For Univ*, 35(3): 77–83.

XU TZ, 1982. Classification, distribution and phylogeny of the genus *Enkianthus* [J]. *Acta Bot Yunnan*, 4(4): 355-362.

YANG JF, LIU XY, ZHANG SZ, et al., 2009. Tissue culture of *Enkianthus quinqueflorus* Lour. [J]. *J Trop Subtrop Bot*, 17(4): 383-387.

YE LJ, ZHANG ZR, SUN ZX, et al., 2015. The determination of nuclear DNA content (2C-value) on some representative genus and species of Magnoliaceae [J]. *Plant Divers Res*, 37(5): 605-610.

YOKOYA K, ROBERTS AV, MOTTLEY J, et al., 2000. Nuclear DNA amount in Roses [J]. *Ann Bot*, 85(4): 557–561.

ZHANG GZ, 2012. Study on plants of danxia landform in Hunan and Jiangxi, Hunan, China [D]. Changsha: Central South University of Forestry and Technology: 32-34.

Note: Figure translations are in progress. See original paper for figures.