Pak. J. Bot., 50(4): 1517-1520, 2018.

GENOME SIZE VARIATION IS CORRELATED WITH ALTITUDE WITHIN CHINESE SPECIES OF *ALLIUM* L.

FENGLING GUO, JINPING WU, LEIFU CHEN, JIE ZHOU, YANXU YIN AND ZHENGMING QIU*

Industrial Crops Institute of Hubei Academy of Agricultural Sciences, Wuhan 430064, China
**Corresponding author's email: guofenglingok@163.com*

Abstract

Species in the genus *Allium* L. (Alliaceae) are important economic crops. They exhibit variations in genome size and basic chromosome number. In the present study, the nuclear genome size of plants sampled from *Allium* populations was determined using flow cytometry occuring at altitudes from 43 m to 1530 m above sea level (asl) in Hubei province, China. Our results revealed that the values of 2C genome of these *Allium* populations distributed from 15.10 to 64.65 pg, the significant interspecific variation in the nuclear DNA contents. There is a positive correlation among these *Allium* population between altitude and genome size. This study will deepen the understanding of the relationship between the nuclear genome size and the habitat altitude in high altitude-dependent plants.

Key words: Allium, C-Value, Flow Cytometry, Altitude

Introduction

The C-value is often adopted to describe Genome size, which is defined to evaluate the DNA content of a haploid chromosome set of a living organism (Bennett and Leitch, 2005). The C-value within a species is usually constant, although genome size shows a great diversity and some genuine intraspecific variations have been observed (Greilhuber, 2005). It has been shown that the C-value is generally not positively correlated with organism complexity and phylogenetic positions,which is termed the "C-value enigma" (Gregory, 2005). The nuclear DNA contents range from 0.13 to 304.4 pg, varing by >2000-fold, in higher plants (Greilhuber *et al.*, 2010; Pellicer *et al.*, 2010). The genome size of plants is often influenced by the chromosome number and the ploidy level (Pellicer *et al.*, 2010) . Besides, altitude, temperature and water availability are also associated with genome size (Bennett, 1972; Bennett, 1987; Auger, 1990; Garcíafernández *et al.*, 2012). Larger genome sizes sometimes reflect the ability to grow at low temperatures or at high latitudes or elevations for some cereals, grasses, and legumes (Bennett, 1987). Water availability plays a role in genome size variation between hydrophytes and chamaephytes, which are thought to contain a variety of changes in ploidy levels and basal chromosome numbers (Les & Philbrick, 1993). The relatively low nuclear DNA contents in plants from high altitudes and other extreme environments are basically consistent with the hypothesis about large genome restriction (Knight *et al.*, 2005). It was proposed that the non-coding region of the genome affects not only cell cycle duration but also life cycle type of plants (Grime & Mowforth, 1982). Vos *et al.,* used the molecule marker technique to show that intraspecific variation in C-value and the altitude of habitat was basically consistent with genetic differences between some altitudinally contrasting populations (Vos *et al.*, 1995).

Edible species in the genus *Allium* L. (Alliaceae), onions, garlic, leeks, etc., are of great economic value. And exhibit a great differences in bulb and rhizome shape and size. Various *Allium* species live in particular ecological niches at different altitudes. At present, there are no reports on the possible link between the habitat altitude and the genome size in edible species of *Allium*. This study aimed to measure the nuclear genome size of twenty-eight major edible *Allium* populations from Hubei province, China, and to examine possible potential links between genome size and habitat altitude in species of *Allium*.

Materials and Methods

Plant materials: The plant material was collected mainly from wild *Allium* species in Hubei province, central China. The taxonomic status, accession numbers and origins of twenty-eight populations studied in this research are given in Table 1. Flow cytometry analysis was performed according to the method described by Oriane *et.al,* (2015). Leaf materials taken from five individuals in each population were wrapped separately in a slightly damp tissue paper and stored at 4℃ for 4 days prior to flow cytometric analysis.

Flow cytometry: Leaf tissues from each sampled individual were chopped with a razor blade together with an standard species leaf tissue in 1 ml of nuclei isolation buffer (50 mM KCl, 1 mg/ml dithiothreitol 10 mM MgSO4, 5 mM Hepes and 0.2% Triton X-100 supplemented with 100 µg/ml of ribonuclease A). The resulting slurry was filtered with a 33 µm nylon mesh, and the nuclear suspension was stained with a final concentration of 50 µg/ml propidium iodide (PI, Sigma-Aldrich) and kept on ice for 5-20 min. Flow cytometry assay with a 488 nm laser for excitation (Becton Dickinson) was used to analyze the cell-cycle profile. Flow cytometry was performed at the instrument sharing center in the College of Life Sciences, Wuhan University. The flow cytometry results were quantitatively analyzed with a Flowjo 7.6 software. One run was done per preparation, with >10,000 nuclei measured. Leaf tissue of broad bean (*Vicia faba* L.) was used as the internal standard of genome size. The nuclear DNA content was calculated following a published method based on the results of fluorescence intensities (Doležel, 1991).

Statistical analyses

The significant differences of the genome size among the *Allium* in different altitude habitats were alanyzed using *t*-test method.

Table 1. Nuclear DNA contents of plants from the twenty-eight populations in the statistical analyses.

Allium species.	Collection location	Altitude (m)	DNA content (2C, pg)
A. chinense G. Don. Monogr	Stone buddhist temple town, Wuxue city	43	25.95
A. schoenprasum L.	Stone buddhist temple town, Wuxue city	43	35.39
A. satevum L.	Big temple town, Wuxue city	53	26.9
A.chinense G. Don. Monogr	Cai He town, Xishui county, Huanggang city	61	43.41
A. prrum L.	Wuan town, Xiangyang city	92	35.43
A. chinense G. Don. Monogr	Guankou town, Huanggang city	97	23.59
A. satevum L.	Big temple town, Wuxue city	113	22.65
A.chinense G. Don. Monogr	Dupi town, Huanggang city	120	32.09
A. tulosum L.	Guishan town, Macheng city	122	24.54
A. satevum L.	Sizhuang town, Xianning city	137	41.53
A. satevum L.	Yangfanglin town, Xianning city	137	22.65
A. schoenprasum L	Yangfanglin town, Xianning city	137	38.69
A. schoenprasum L	Zhangji town, Zhongxiang city	143	24.54
A. schoenprasum L	Kedian town, Zhongxiang city	197	23.59
A. schoenprasum L	Datong town, Huanggang city	229	22.65
A. schoenprasum L.	Limiao town, Xiangyang city	407	27.37
A. satevum L.	Huolongping town, Enshi city	854	28.31
A. ovalifolium Hand.-Mzt.	Huolongping town, Enshi city	854	25.01
A.chrysanthum	Pingbaying town, Enshi city	854	64.65
A. ascalonicum	Xiaocun town, Enshi city	860	37.75
A. chrysanthum	Zhongbao town, Enshi city	867	54.27
A. ascalonicum	Pingbaying town, Enshi city	1032	54.21
A. satevum L.	Yerengu town, Shiyan city	1065	27.37
A. satevum L.	Songbai town, Shiyan city	1127	31.62
A. schoenprasum L.	Huolongping town, Enshi city	1154	21.54
A. satevum L.	Qiuyang town, Enshi city	1260	25.09
A. satevum L.	Shangkan town, Shiyan citu	1429	55.21
A. satevum L.	Ziqiu town, Yichang city	1530	24.54

Results

Ecological parameters, including altltude, have been shown to affect genome size in plants (Auger, 1990). Therefore, the nuclear DNA amounts of *Allium* populations collected at different altitudes were analyzed by flow cytometry. Fluorescence histograms of three representative native garlics (*Allium sativum* L.) from different altitudes and the internal reference standards are shown in Fig. 1, where the x-axis indicates genome size. The mean nuclear DNA content for each individual in all of the analyzed populations is summarized in Table 1. These results showed that the holoploid DNA content values (2C) varied from 15.10 to 64.65 pg, which was above 4-fold variation, with a mean of 32.28 pg. In one species, native garlic, the histogram obtained from the flow cytometric analysis indicated that increased genome size was positively related to incremental changes in altitude (Fig. 1).

Populations of *Allium* species were gathered occuring at various altitudes between 43 m and 1530 m above sea level (asl). Comparative analysis of DNA contents combined with altitude showed that the genome size of native garlic collected at 1429 m asl is 55.21 pg (2C content), which was significantly larger than that of native garlic collected at 53 m asl, which is 26.9 pg, and the genome size of native garlic collected at 854 m asl, which was 28.31 pg (Fig. 2). Populations at 854 m elevation tended to cluster at the smaller end of the DNA content than those at 1429 m elevation, although they had a higher DNA content than those collected at 53 m elevation. The results showed highly significant differences among native garlic populations indicating that nuclear DNA content was positively correlated with altitude for this species. Detailed analysis of the distribution of genome size according to altitude revealed of three different clusters in these populations. In contrast, bitter shallots growing at 407 m elevation had a higher nuclear DNA content than plants collected at 1154 m elevation, but a lower DNA content than those growing at 43 m elevation, indicating that the DNA content was negatively correlated with altitude for bitter shallot (Fig. 3).

Discussion

The genus *Allium* shows a great difference in morphological charactersand ecological habitat, and the *Allium* species had a significantly high nuclear DNA amounts. The relationship betweenDNA content and environmental factors such as altitude has been discussed in a range of plant species with varying conclusions. Some contradictory results have suggested a different linear relationship between altitude and genome size with different local adaptation mechanisms (Knight *et al.*, 2005; Ohsawa and Ide, 2008)). Ploidy level and genome size across an altitudinal gradient have been studied in locally adapted populations of *Silene ciliata* (Garcíafernández *et al.*, 2012). The correlate between genome size and the altitude habitat has been reported in Mexican maize(Bennett, 1976). Comparing their habitat altitudes, 23 populations of *Zea mays* in Mexico were found to have both positive and

negative correlationswith altitude (Auger, 1990) . Rivin *et al.*, (1986) showed that DNA sequence repeats in the maize genome might fluctuate dramatically in copy number. At present, no detailed study was conducted to examine the relationship between altitude habitats and nuclear genome size in species of *Allium* L. from China. In this study, we explored the intraspecific DNA content variations in *Allium* as it relates to the altitude, and found that there were both positive and negative correlations. Intraspecific variations in the nuclear DNA amounts in the *Allium* populations examined here represented an increase of more than 4 times of lowe altitude compared to the high altitute *Allium.* These increases in the genome size may impact on the duration of cell cycle that is involved in local adaption. Cells with smaller genome sizes show a rapid rate of replication in the nuclear DNA and cell division, and an increase in C-value was related to an elevation in cell cycle duration (Kidd *et al.*, 1987).

Many studies suggested that plants with tlarge genome size might be excluded from extreme environments (Levin & Funderburg, 1979; Bennett, 1987). A hypothesis presented by Knight *et al.*, about the constraint of large genome postulates that the plant species with a large genome size are under-represented in an extreme environment since the large genome size is a problem for reproducing and differentiation of plant species (Knight *et al.*, 2005). Nonetheless, our data suggest that *Allium* plants growing at high altitudes have tended to evolve towards larger genomes, and high altitude ecosystems can be considered to be extreme. We guess that these species have adapted to the challenges of vegetative growth and reproductive growth encountered in high altitude habitats. The possibility that the extreme climates typical of high altitude environments favour genotypes of *Allium* in which the cell exhibits a lower proportion of rapid fission, as compared to populations from lower altitudes and this needs to be further examined. In conclusion, our study provides estimates of the nuclear DNA contents for plants from 28 populations of *Allium* collected from different altitudes in China. Our results are not completely consistent with the large genome constraint hypothesis. However, more plant species living at low and high altitudes need to be studied to examine the relationships between habitat altitude and nuclear genome size.

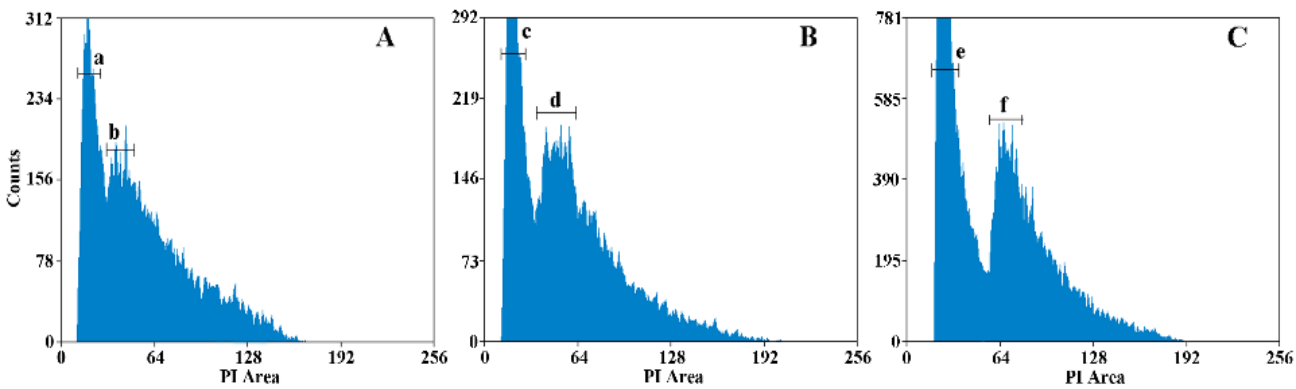

Fig. 1. Fluorescence histograms of propidium iodide-stained nuclei for genome size estimates by flow cytometry.

A. (a) *Vicia faba* (C-value = 26.9 pg) was used as the internal reference genome size standard; (b) native garlic from a population growing at 53 m asl. Its C-value was estimated to be 39.17 pg; **B.** (c) *Vicia faba*; (d) native garlic from a population at 854 m. C-value = 55.21 pg; **C.** (e) *Vicia faba*; (f) native garlic from a population at 1429 m asl. C-value = 64.65 pg.

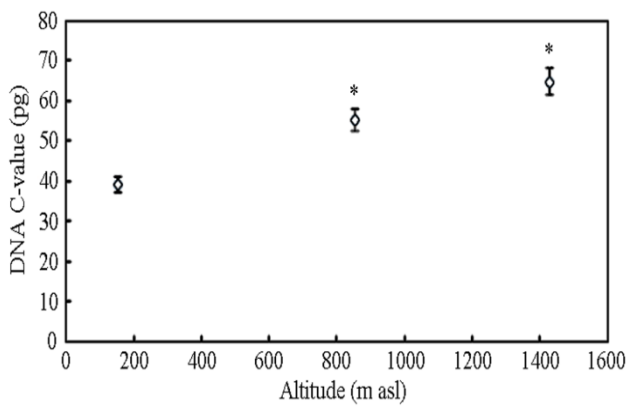

Fig. 2. The relationship between mean DNA C-values (pg) and altitude of origin for plants from three natural populations of native garlic. * indicates a significant difference between the high altitude and the low altitude populations at $p<0.05$ using the Student's *t*-test. asl: above sea level.

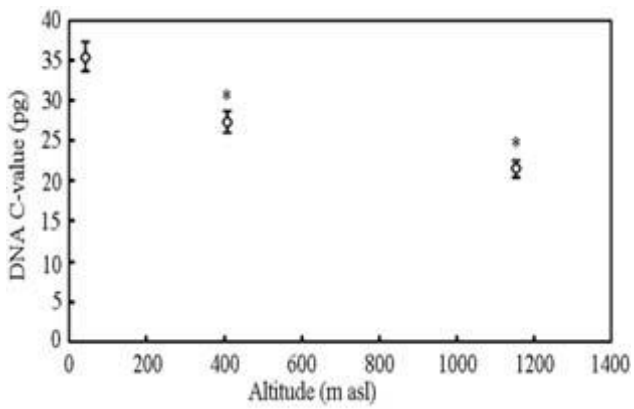

Fig. 3. The relationship between mean DNA C-value (pg) and altitude of origin for plants from three natural population of bitter shallot. * indicates a significant difference between the high altitude group and the low altitude population at $p<0.05$ using Student's *t*-test. asl: above sea level.

Acknowledgments

The native English speaking scientists of Elixigen Company (Huntington Beach, California) helped for editing our manuscript.

Funding

This study was supoorted by characteristics of vegetable industry system [grant number CARS-25-G-13], the major special project of technology innovation in Hubei province [grant number 2016ABA098] and special project of germplasm resources protection in Ministry of Agriculture [grant number S878]

Conflict of interest

The authors declared that there is no conflict of any interest.

References

Auger, J.A., 1990. Genome size variation in zea mays ssp. Mays adapted to different altitudes. *Theoretical & Applied Genetics*, 79(4): 470-474.

Bennett, M.D., 1972. Nuclear DNA content and minimum generation time in herbaceous plants. *Proce. Royal Soc. Lon.*, 181(63): 109-135.

Bennett, M.D., 1976. DNA amount, latitude, and crop plant distribution. *Environm. & Experim. Bot.*, 16(2-3): 93-98.

Bennett, M.D., 1987. Variation in genomic form in plants and its ecological implications. New Phytologist, 106(s1): 177-200.

Bennett, M.D. and I.J. Leitch, 2005. Nuclear DNA amounts in angiosperms: Progress, problems and prospects. *Ann Bot*, 95(1): 45-90.

Doležel, J., 1991. Flow cytometric analysis of nuclear DNA content in higher plants. *Phytochem. Anal.*, 2(4): 143-154.

Garcíafernández, A., J.M. Iriondo, J. Vallès, J. Orellana and A. Escudero, 2012. Ploidy level and genome size of locally adapted populations of silene ciliata across an altitudinal gradient. *Plant System. & Evol.*, 298(1): 139-146.

Gregory, T.R., 2005. The c-value enigma in plants and animals: A review of parallels and an appeal for partnership. *Ann. Bot.*, 95(1): 133-146.

Greilhuber, J., 2005. Intraspecific variation in genome size in angiosperms: Identifying its existence. *Ann. Bot.*, 95(1): 91-98.

Greilhuber, J., T. Borsch, K. Müller, A. Worberg, S. Porembski and W. Barthlott, 2010. Smallest angiosperm genomes found in lentibulariaceae, with chromosomes of bacterial size. *Plant Biol.*, 8(6): 770-777.

Grime, J.P. and M.A. Mowforth, 1982. Variation in genome size-an ecological interpretation. *Nature*, 299(5879): 151-153.

Kidd, A.D., D. Francis and M.D. Bennett, 1987. Replicon size, mean rate of DNA replication and the duration of the cell cycle and its component phases in eight monocotyledonous species of contrasting DNA c values. *Ann. Bot.*,59(6): 603-609.

Knight, C.A., N.A. Molinari and D.A. Petrov, 2005. The large genome constraint hypothesis: Evolution, ecology and phenotype. *Ann. Bot.*, 95(1): 177-190.

Les, D.H. and C.T. Philbrick, 1993. Studies of hybridization and chromosome number variation in aquatic angiosperms: Evolutionary implications. *Aquatic Bot.*, 44(2-3): 181-228.

Levin, D.A. and S.W. Funderburg. 1979. Genome size in angiosperms: Temperate versus tropical species. *Amer. Naturalist*, 114(6): 784-795.

Ohsawa, T. and Y. Ide. 2008. Global patterns of genetic variation in plant species along vertical and horizontal gradients on mountains. *Glo. Ecol. & Biogeog.*, 17(2): 152-163.

Oriane, H., J. Vallès, A. Romo, M. Á.. Canela and T. Garnatje. 2015. Genome size variation in gymnosperms under different growth conditions. Caryologia, 68(2): 92-96.

Pellicer, J., M.F. Fay and I.J. Leitch. 2010. The largest eukaryotic genome of them all? *Bot. J. Linn. Soc.*, 164(1): 10-15.

Pellicer, J., S. Garcia, M.A. Canela, T. Garnatje, A.A. Korobkov, J.D. Twibell and J. Vallès, 2010. Genome size dynamics in Artemisia l. (Asteraceae): Following the track of polyploidy. *Plant Biol.*, 12(5): 820-830.

Rivin, C.J., C.A. Cullis and V. Walbot, 1986. Evaluating quantitative variation in the genome of zea mays. *Genetics*, 113(4): 1009-1019.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, d.L.T. Van, M. Hornes, A. Frijters, J. Pot, J. Peleman and M. Kuiper, 1995. Aflp: A new technique for DNA fingerprinting. *Nucl. Acids Res.*, 23(21): 4407.

(Received for publication 7 July 2017)