

Research Report

FISH Karyotype Analysis of Four Wild Cucurbitaceae Species Using 5S and 45S rDNA Probes and the Emergence of New Polyploids in *Trichosanthes kirilowii* Maxim.

Nomar Espinosa Waminal^{1,2} and Hyun Hee Kim^{1*}

¹Plant Biotechnology Institute, Department of Life Science, Sahmyook University, Seoul 139-742, Korea

²Department of Plant Science, Plant Genomics and Breeding Institute, and Research Institute of Agriculture and Life Sciences, College of Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea

Abstract: Wild relative species of domesticated crops are useful genetic resources for improving agronomic traits. Cytogenetic investigations based on chromosome composition provide insight into basic genetic and genomic characteristics of a species that can be exploited in a breeding program. Here, we used FISH analysis to characterize the ploidy level, chromosome constitution, and genomic distribution of 5S and 45S ribosomal DNA (rDNA) in four wild Cucurbitaceae species, namely, *Citrullus lanatus* (Thunb.) Mansf. var. *citroides* L. H. Bailey ($2n = 22$), *Melothria japonica* Maxim. ($2n = 22$), *Sicyos angulatus* L. ($2n = 24$), and *Trichosanthes kirilowii* Maxim. ($2n = 66, 88, 110$ cytotypes), collected in different areas of Korea. All species were diploids, except for *T. kirilowii*, which included hexa-, octa-, and decaploid cytotypes ($2n = 6x = 66, 8x = 88, \text{ and } 10x = 110$). All species have small metaphase chromosomes in the range of 2–5 μm . The 45S rDNA signals were localized distally compared to the 5S rDNA. *C. lanatus* var. *citroides* and *M. japonica* showed one and two loci of 45S and 5S rDNA, respectively, with co-localization of rDNA signals in one *M. japonica* chromosome. *S. angulatus* showed two co-localized signals of 5S and 45S rDNA loci. The hexaploid *T. kirilowii* cytotype showed five signals each for 45S and 5S rDNA, with three being co-localized. This is the first report of hexaploid and decaploid cytotypes in *T. kirilowii*. These results will be useful in future Cucurbitaceae breeding programs.

Additional key words: *Citrullus lanatus* var. *citroides*, FISH karyotype, *Melothria japonica*, rDNA probe, *Sicyos angulatus*, *Trichosanthes kirilowii*

Introduction

The Cucurbitaceae family consists of economically important species such as *Cucumis melo* (melon), *Citrullus lanatus* (watermelon), *Cucurbita* spp. (pumpkin), and *Cucumis sativus* (cucumber) (Jeffrey, 2005), and breeding programs involving these plants have generated multi-billion dollar food, cosmetic, and medicine industries (Waminal et al., 2011; Weng and Sun, 2012). Genome assembly studies, particularly of cucumber, melon, and watermelon, have revealed phylogenetic relationships among members of this family and provided opportunities and resources to expedite crop improvement (Garcia-Mas et al., 2012; Guo et al., 2013; Huang et al.,

2009).

Introgression of important agronomic traits, such as biotic and abiotic stress resistance, from wild related species is an important tool for crop improvement (Anamthawat-Jónsson, 2001; Zamir, 2001). This approach has been demonstrated in wheat, rice, rapeseed, and other crops (Anamthawat-Jónsson, 2001; Brar and Khush, 1997; Humphreys et al., 1997; Jiang et al., 1993; Lashermes et al., 2000; Snowdon et al., 1997). Some cucurbits have also benefitted from this breeding approach. For example, recent introgression breeding from a wild relative of cucumber, *Cucumis hystris*, has increased the genetic diversity of cucumber (Delannay et al., 2010; Zhuang et al., 2006). Additionally,

*Corresponding author: kimhh@syu.ac.kr

✉ Received 15 June 2015; Revised 6 August 2015; Accepted 24 September 2015. This study was supported by a grant from the National Research Foundation of Korea (NRF: 20110023156).

introgression between cultivars and wild relatives of bitter gourds (*Momordica charantia*) (Liao et al., 2012) and *Citrullus* (Sain et al., 2002) have also been reported.

Other wild cucurbits possess economically and medicinally important traits. For example, *Melothria japonica* is a good source of proteolytic enzymes (Antão and Malcata, 2005, Uchikoba et al., 2001), and *Trichosanthes kirilowii*, which has long been used in China as a medicinal plant, is the source of the anti-HIV drug trichosanthin and the less cytotoxic protein TAP 29 (Lee-Huang et al., 1991; Mayer et al., 1992; McGrath et al., 1989; Qian et al., 2012). *Sicyos angulatus*, a wild invasive species introduced to Korea in the mid-20th century (Tzonev, 2005), is used as a rootstock for grafting cucumbers and watermelons to enhance resistance to nematodes and improve yield (Lee, 1994; Shengping et al., 2006). Like *S. angulatus*, *Citrullus lanatus* var. *citroides* also provides nematode- and fungus-resistant rootstock for watermelon (Thies and Levi, 2007). Efficient breeding programs that produce crops with established desirable traits will maximize the exploitation of these agriculturally and economically significant traits.

Cytogenetic information plays an important role in breeding programs for some of the world's most important crops, such as wheat, maize (Albert et al., 2010; Benavente et al., 2008; Icsó et al., 2014) and watermelon (Sain et al., 2002). Considering the potential of the four wild cucurbits (i.e., *Citrullus lanatus* (Thunb.) Mansf. var. *citroides* L. H. Bailey, *Melothria japonica* Maxim., *Sicyos angulatus* L., and *Trichosanthes kirilowii* Maxim.) as genetic resources, their limited cytogenetic information prompted us to investigate their chromosome characteristics to provide basic information of their genome structure. Most cucurbit species have small chromosomes that are difficult to distinguish (Waminal and Kim, 2012); however, fluorescence *in situ* hybridization (FISH) has revealed the identity of chromosome pairs and provided information that can be used in breeding programs (Huang et al., 2009; Waminal et al., 2011; Waminal and Kim, 2012).

Here, we determined the ploidy level, chromosome constitution, and rDNA distribution patterns of four wild cucurbit species using FISH analysis. Additionally, we report the hexaploid and decaploid cytotypes of *T. kirilowii* for the first time. The role of polyploidy in crop improvement is discussed.

Materials and Methods

Plants growing in uncultivated lands were collected from different areas in South Korea. Mature fruits of *Melothria*

japonica Maxim. and *Trichosanthes kirilowii* Maxim. were collected from South Jeolla Province, while those of *Sicyos angulatus* L. were from Sky Park, Seoul. The seeds of *Citrullus lanatus* (Thunb.) Mansf. var. *citroides* L. H. Bailey were kindly provided by Professor Gung Pyo Lee, Plant Genomics & Breeding Laboratory, School of Bioresource and Bioscience in Chung-Ang University, Ansong, Korea. Seeds were germinated and root tips with actively dividing cells were harvested.

Chromosome spread preparations and FISH procedures were performed according to Waminal and Kim (2012). Briefly, root tips treated with 2 mM 8-hydroxyquinoline and fixed with Carnoy's solution were enzymatically digested with pectolytic enzyme solution (2% Cellulase R-10 [C224, Phytotechnology Laboratories] and 1% Pectolyase Y-23 [P8004.0001, Duchefa] in 100 mM citrate buffer) for 1 h. Root tips were then squashed on slides pre-cleaned with 70% ethanol. Air-dried slides were fixed in 2% formaldehyde (Vrana et al., 2012) for 5 min and dehydrated with a series of ethanol treatments (70, 90, and 100%). A 5S rDNA probe was obtained by PCR amplification of *Brassica oleracea* 5S rDNA using primers from Waminal et al. (2014), while a 45S rDNA probe was obtained from a clone from Gerlach and Bedbrook (1979). DNA probes were labeled with either Alexa Fluor 488-5-dUTP (C11397, Life Technologies) or Texas Red-5-dUTP (NEL417001EA, Perkin Elmer). Images were captured with an Olympus BX53 epifluorescence microscope equipped with a Leica DFC365 FS CCD camera, and processed using Cytovision version 7.2 (Leica Microsystems, Germany). Further image enhancements were performed using Adobe Photoshop CS6.

Results and Discussion

FISH karyotype analysis is an excellent method for providing information about a genome's chromosomal organization. It has contributed to genomic studies and breeding programs by providing chromosomal information about a species of interest (Macas et al., 2007; Mayer et al., 2011; Szinay et al., 2010). In addition, chromosome composition can be used to verify and explain phylogenetic relationships of closely related taxa (Guerra, 2008; Mandáková et al., 2015). For instance, although the usual basic chromosome number of the family Cucurbitaceae is either 11 or 12 (Robinson and Decker-Walters, 1997), cucumber (*Cucumis sativus*) shows an atypical number of $x = 7$, where five of the seven chromosomes have resulted from a fusion of ten ancestral chromosomes after diverging from melons (Huang et al., 2009; Koo et al., 2010). Chromosome informa-

tion was also critical in identifying the taxonomic relationship of *C. hystrix*, a wild *Cucumis* species with morphological and biochemical features similar to those of cucumber, but with a basic chromosome number of 12, as observed for melon (Zhuang et al., 2006). Additionally, chromosome number information describes the association of certain phenotypic characteristics to the ploidy level (Qian et al., 2012; Singh, 1979; Soltis et al.; 2012, Xiong and Pires, 2011).

In this study, we analyzed four wild cucurbit species, i.e., *Citrullus lanatus* (Thunb.) Mansf. var. *citroides* L. H. Bailey, *Melothria japonica* Maxim., *Sicyos angulatus* L., and *Trichosanthes kirilowii* Maxim., using FISH karyotype analysis with 5S and 45S rDNA probes. All four species showed a typical basic chromosome number of 11 or 12. *C. lanatus*

var. *citroides* and *M. japonica*, which belong to the tribe Benincaseae, revealed diploid complements of $2n = 22$, and *S. angulatus* from the tribe Sicyeae showed $2n = 24$ (Fig. 1A-C). However, *T. kirilowii*, which is from the tribe Trichosantheae, appeared as polyploids of hexa-, octa-, and decaploid complements of $2n = 66$, 88, and 110, respectively, with a basic number of 11 (Figs. 1D, 2 and 3). Cucurbitaceae chromosomes are relatively small (Waminal and Kim, 2012; Waminal et al., 2011). The chromosome length of *C. lanatus* var. *citroides* ranged from 3.1 to 4.7 μm , while those of *M. japonica*, *S. angulatus*, and *T. kirilowii* ranged from 2.4 to 3.4 μm , 1.9 to 4.6 μm , and 2.3 to 4.5 μm , respectively (Table 1). Chromosome numbers were assigned based on decreasing order of total chromosome lengths. Our previous results of chromosome composition in Cucurbitaceae species

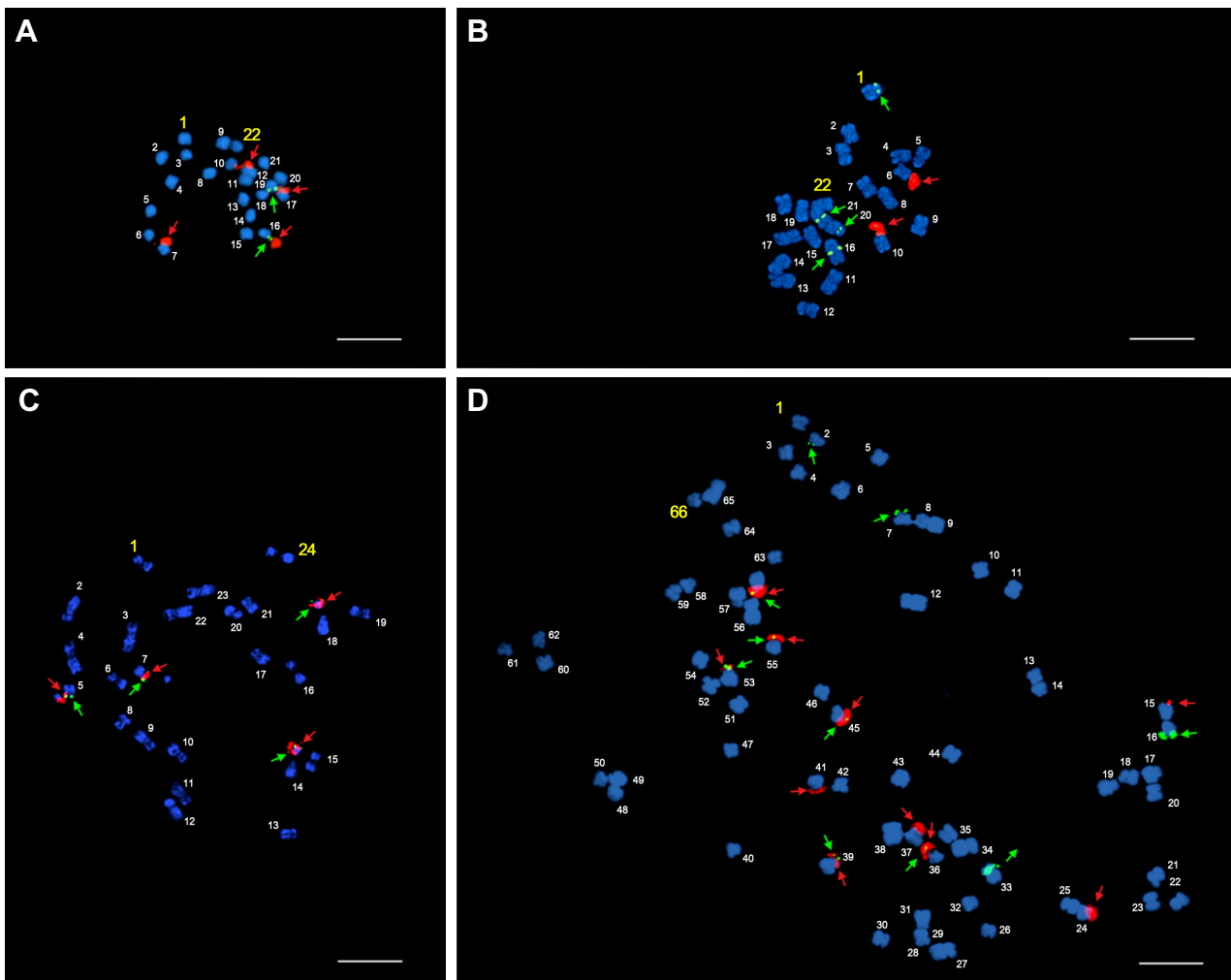


Fig. 1. Root mitotic chromosome spreads of the four wild cucurbits. Chromosome spreads of *Melothria japonica* (A), *Citrullus lanatus* var. *citroides* (B), *Sicyos angulatus* (C), and hexaploid *Trichosanthes kirilowii* (D), showing 5S and 45S rDNA loci as green and red signals, respectively. Arrows indicate the signals. Bars = 5 μm .

showed variation from the usual basic chromosome number of 11 and 12; for instance, *Luffa cylindrica* has 13 ($2n = 26$) and *Cucurbita moschata* has 10 ($2n = 40$), while others, such as *Lagenaria siceraria* and *Momordica charantia*, have the usual basic chromosome number of 11 ($2n = 22$) and *Benincasa hispida* has 12 ($2n = 24$). This suggests a dynamic genomic history laden with genome downsizing or amplification at the chromosomal level within Cucurbitaceae, and this is particularly exemplified by the genomic relationship between cucumber and melon (Huang et al., 2009, Koo et al., 2010). Genomic downsizing or amplification is not a rare phenomenon among angiosperms. In fact, it is commonly associated with genomic hybridizations that result in polyploids and their corresponding subsequent diploidization events, which can be demonstrated by rDNA loci reduction (Bruggmann et al., 2006; Clarkson et al., 2005; Kovarik et al., 2012; Mun et al., 2009).

The locations and distributions of ribosomal DNA can be useful for deducing species history and phylogenetic relationship (Clarkson et al., 2005). In several cucurbit species studied previously, 5S and 45S rDNA co-localization were commonly observed (Waminal et al., 2011; Waminal and Kim, 2012). Similarly, we observed co-localization of rDNA signals in three out of four wild species (Fig. 3, Table 1). *M. japonica* revealed one and two 5S and 45S rDNA loci, respectively, with one co-localized signal (1:2:1); while the corresponding numbers for *S. angulatus*, *T. kirilowii*, and *C. lanatus* var. *citroides* were 2:2:2, 5:5:3, and 2:1:0, respectively (Table 1). These co-localized signals could indicate syntenic regions from a shared origin (Bertioli et al., 2009; Kellogg 2013), and thus may carry some phylogenetic implications in the study of Cucurbitaceae species. Additionally, comparative genomics at the sequence level, similar to those done in grasses (Mayer et al., 2011), should provide insight into their phylogenetic relationships. Hexaploid *T. kirilowii* showed a disproportional rDNA loci number in relation to its ploidy. This can be explained by a loss of 45S rDNA loci after polyploidization, considering

that 45S rDNA loci have been shown to be rapidly gained or lost even after a single generation (Wendel, 2000; Waminal et al., 2012).

A hexaploid species has been reported in *Trichosanthes bracteata* (Karmakar et al., 2013) and diploid and octaploid cytotypes have been reported in *T. kirilowii* species (Qian et al., 2012). However, the hexaploid and decaploid cytotypes in *T. kirilowii* found here were not previously reported. Qian et al. (2012) reported diverse morphological polymorphism in diploids and octaploids of *T. kirilowii* collected from different regions of China. While leaf morphologies varied between the diploids and octaploids, there was even more variation among the octaploids. The fruit color, however, showed distinct difference between the two ploidy groups (Qian et al., 2012). Phenotypic variations need to be investigated in hexaploid and decaploid *T. kirilowii* cytotypes in a continued study. Most angiosperms are considered to have undergone ancient polyploidization events (McGrath and Lynch, 2012). While many cucurbits examined in previous studies exhibit disomic chromosome pairing, some have traces of ancient polyploidy in their chromosome number, such as the genus *Cucurbita*, which has $2n = 40$, showing a multiple basic chromosome number of 10 (Weeden and Robinson, 1986), and some exhibit an ongoing species diversification through polyploidization, as observed in *Gynostemma pentaphyllum* (Jiang, 2009).

Geographical distribution plays an important role in facilitating hybridization between related species, or cytotypes, and creating natural polyploids (Balao et al., 2011; Wendel, 2000). Several mechanisms could have resulted in the present polyploid genomes. Based on chromosome number, hexaploid *T. kirilowii* may be a result of hybridization between a diploid and a tetraploid. Furthermore, the decaploid could have resulted from a later hybridization between an octaploid and diploid, or tetraploid and hexaploid. Additionally, the disjunct distribution of this genus (Kocyan et al., 2007) may have facilitated several hybridization events, and those found in Korea may have established hexaploidy

Table 1. Summary of chromosome length ranges and rDNA distribution in four wild Cucurbitaceae species.

Species	2n	x	Ploidy	Chr. length range (μm)	5S rDNA loci	45S rDNA loci	Co-localized signals
<i>Citrullus lanatus</i> var. <i>citroides</i> L. H. Bailey	22	11	diploid	3.1-4.7	2	1	0
<i>Melothria japonica</i> Maxim.	22	11	diploid	2.4-3.4	1	2	1
<i>Sicyos angulatus</i> L.	24	12	diploid	1.9-4.6	2	2	2
<i>Trichosanthes kirilowii</i> Maxim.	66, 88, 110	11	hexa-, octa-, decaploid	2.3-3.5 ^z	5	5	3

^zMeasured only from hexaploid cytotype

and decaploidy in addition to the other established ploidies. The resulting genome reorganization after polyploidization (Bennetzen, 2005; Bennetzen et al., 2005; Chen and Yu,

2013) is a mechanism for plants to stabilize genomes and often leads to adaptive mechanisms to a vast array of environmental conditions. Hence, most polyploids have more efficient survival machinery as compared to their diploid counterparts (Otto, 2007; Ramsey, 2011; Wang et al., 2012) and this may explain their adaptation to a wide range of environmental conditions (Comai, 2005).

T. kirilowii has been used as a medicinal herb in oriental countries and is known to have important medicinal properties (Lee-Huang et al., 1991; Mayer et al., 1992; Qian et al., 2012). Breeding and cultivation of elite polyploid lines may be useful for the mass production of these medicinals. Presently, much work remains to be done to identify and develop the best cytogenetic lines with optimal medicinal and agronomic traits. The cytogenetic results presented here complement molecular and biochemical studies on *T. kirilowii* (Minh et al., 2015; Ni et al. 2015; Seo et al., 2015). Similarly, basic chromosome information of *C. lanatus* var. *citroides*, *M. japonica*, and *S. angulatus* may be useful in efforts to improve Cucurbitaceae crops. Understanding the genomic history of these species may further facilitate the efficient exploitation of their desirable agricultural and medicinal traits. As desirable traits are partially influenced by ploidy (Balao et al., 2011; Comai, 2005), the medicinal properties of these hexaploid and decaploid cytotypes should be further studied (Minh et al., 2015; Ni et al., 2015; Seo et al., 2015).

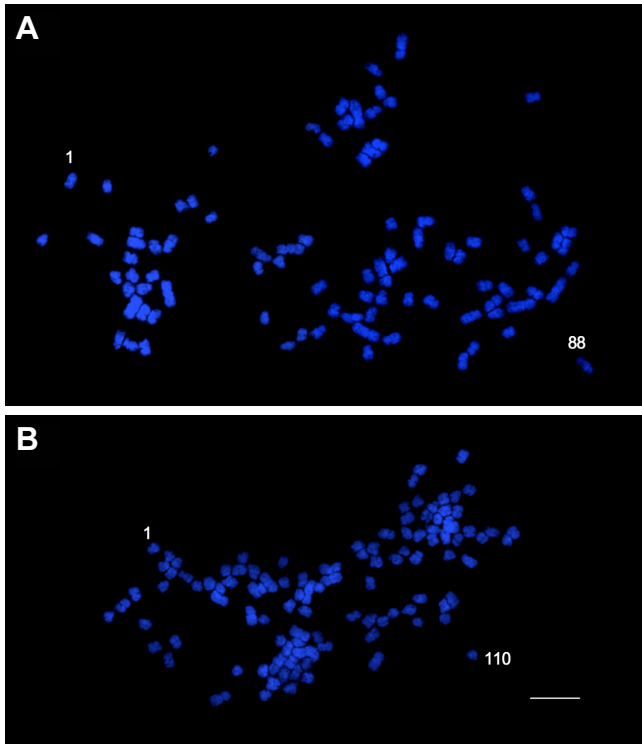


Fig. 2. Polyploid cytotypes of *Trichosanthes kirilowii*. The octaploid (A) and decaploid (B) are shown. Bars = 5 μ m.

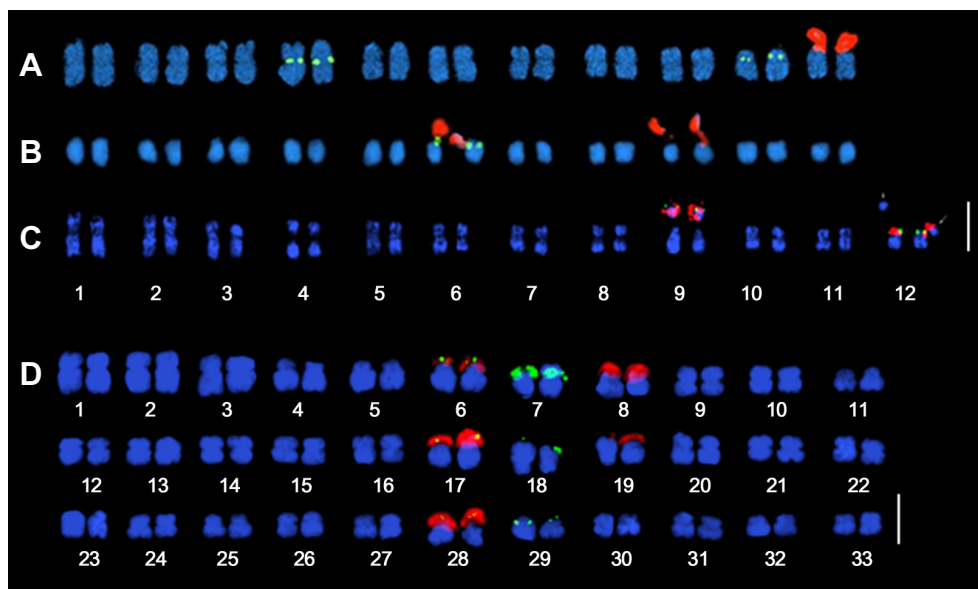


Fig. 3. Karyogram of the four wild cucurbit species. Karyograms of *Citrullus lanatus* var. *citroides* (A), *Melothria japonica* (B), *Sicyos angulatus* (C), and hexaploid *Trichosanthes kirilowii* (D). 5S and 45S rDNA loci are shown in green and red signals, respectively, and bars = 5 μ m.

Literature Cited

- Albert, P. S., Z. Gao, T. V. Danilova, and J. A. Birchler. 2010. Diversity of chromosomal karyotypes in maize and its relatives. *Cytogenet. Genome Res.* 129:6-16.
- Ananthawat-Jónsson, K. 2001. Molecular cytogenetics of introgressive hybridization in plants. *Methods Cell Sci.* 23:141-150.
- Antão, C. M. and F. X. Malcata. 2005. Plant serine proteases: biochemical, physiological and molecular features. *Plant Physiol. Biochem.* 43:637-650.
- Balao, F., J. Herrera, and S. Talavera. 2011. Phenotypic consequences of polyploidy and genome size at the microevolutionary scale: a multivariate morphological approach. *New Phytol.* 192: 256-265.
- Benavente, E., M. Cifuentes, J. C. Dusautoir, and J. David. 2008. The use of cytogenetic tools for studies in the crop-to-wild gene transfer scenario. *Cytogenet. Genome Res.* 120:384-395.
- Bennetzen, J. L. 2005. Transposable elements, gene creation and genome rearrangement in flowering plants. *Curr. Opin. Genet. Dev.* 15:621-627.
- Bennetzen, J. L., J. Ma, and K. M. Devos. 2005. Mechanisms of recent genome size variation in flowering plants. *Ann. Bot.* 95:127-132.
- Bertioli, D.J., M.C. Moretzsohn, L.H. Madsen, N. Sandal, S.C. Leal-bertioli, P.M. Guimaraes, B.K. Hougaard, J. Fredslund, L. Schauser, A.M. Nielsen, S. Sato, S. Tabata, S.B. Cannon, and J. Stougaard. 2009. An analysis of synteny of *Arachis* with *Lotus* and *Medicago* sheds new light on the structure, stability and evolution of legume genomes. *BMC Genomics* 10:45.
- Brar, D. S. and G. S. Khush. 1997. Alien introgression in rice. *Plant Mol. Biol.* 35:35-47.
- Bruggmann, R., A. K. Bharti, H. Gundlach, J. Lai, S. Young, A. C. Pontaroli, F. Wei, G. Haberer, G. Fuks, C. Du, C. Raymond, M. C. Estep, R. Liu, J. L. Bennetzen, A. P. Chan, P. D. Rabinowicz, J. Quackenbush, W. B. Barbazuk, R. A. Wing, B. Birren, C. Nusbaum, S. Rounsley, K. F. Mayer, and J. Messing. 2006. Uneven chromosome contraction and expansion in the maize genome. *Genome Res.* 16:1241-1251.
- Chen, Z. J. and H. H. Yu. 2013. Genetic and epigenetic mechanisms for polyploidy and hybridity, p. 335-354. In: Z. J. Chen and J.A. Birchler. *Polyploid and hybrid genomics*. John Wiley & Sons, Inc., Ames, Iowa, USA.
- Clarkson, J. J., K. Y. Lim, A. Kovarik, M. W. Chase, S. Knapp, and A. R. Leitch. 2005. Long-term genome diploidization in allopolyploid *Nicotiana* section *Repandae* (Solanaceae). *New Phytol.* 168:241-252.
- Comai, L. 2005. The advantages and disadvantages of being polyploid. *Nat. Rev. Genet.* 6:836-846.
- Delannay, I. Y., J. E. Staub, and J. F. Chen. 2010. Backcross Introgression of the *Cucumis hystrix* genome increases genetic diversity in US Processing cucumber. *J. Am. Soc. Hortic. Sci.* 135:351-361.
- Garcia-Mas, J., A. Benjak, W. Sanseverino, M. Bourgeois, G. Mir, V. M. Gonzalez, E. Henaff, F. Camara, L. Cozzuto, E. Lowy, T. Alioto, S. Capella-Gutierrez, J. Blanca, J. Canizares, P. Ziarso, D. Gonzalez-Ibeas, L. Rodriguez-Moreno, M. Droege, L. Du, M. Alvarez-Tejado, B. Lorente-Galdos, M. Mele, L. Yang, Y. Weng, A. Navarro, T. Marques-Bonet, M. A. Aranda, F. Nuez, B. Pico, T. Gabaldon, G. Roma, R. Guigo, J. M. Casacuberta, P. Arus, and P. Puigdomenech. 2012. The genome of melon (*Cucumis melo* L.). *Proc. Natl. Acad. Sci. USA* 109:11872-11877.
- Gerlach, W. and J. Bedbrook. 1979. Cloning and characterization of ribosomal RNA genes from wheat and barley. *Nucleic Acids Res.* 7:1869-1885.
- Guerra, M. 2008. Chromosome numbers in plant cytotoxicity: concepts and implications. *Cytogenet. Genome Res.* 120:339-350.
- Guo, S., J. Zhang, H. Sun, J. Salse, W. J. Lucas, H. Zhang, Y. Zheng, L. Mao, Y. Ren, Z. Wang, J. Min, X. Guo, F. Murat, B. K. Ham, Z. Zhang, S. Gao, M. Huang, Y. Xu, S. Zhong, A. Bombarely, L. A. Mueller, H. Zhao, H. He, Y. Zhang, Z. Zhang, S. Huang, T. Tan, E. Pang, K. Lin, Q. Hu, H. Kuang, P. Ni, B. Wang, J. Liu, Q. Kou, W. Hou, X. Zou, J. Jiang, G. Gong, K. Klee, H. Schoof, Y. Huang, X. Hu, S. Dong, D. Liang, J. Wang, K. Wu, Y. Xia, X. Zhao, Z. Zheng, M. Xing, X. Liang, B. Huang, T. Lv, J. Wang, Y. Yin, H. Yi, R. Li, M. Wu, A. Levi, X. Zhang, J. J. Giovannoni, J. Wang, Y. Li, Z. Fei, and Y. Xu. 2013. The draft genome of watermelon (*Citrullus lanatus*) and resequencing of 20 diverse accessions. *Nat. Genet.* 45:51-58.
- Huang, S., R. Li, Z. Zhang, L. Li, X. Gu, W. Fan, W. J. Lucas, X. Wang, B. Xie, P. Ni, Y. Ren, H. Zhu, J. Li, K. Lin, W. Jin, Z. Fei, G. Li, J. Staub, A. Kilian, E. A. van der Vossen, Y. Wu, J. Guo, J. He, Z. Jia, G. Tian, Y. Lu, J. Ruan, W. Qian, M. Wang, Q. Huang, B. Li, Z. Xuan, J. Cao, Asan, Z. Wu, J. Zhang, Q. Cai, Y. Bai, B. Zhao, Y. Han, Y. Li, X. Li, S. Wang, Q. Shi, S. Liu, W. K. Cho, J. Y. Kim, Y. Xu, K. Heller-Uszynska, H. Miao, Z. Cheng, S. Zhang, J. Wu, Y. Yang, H. Kang, M. Li, H. Liang, X. Ren, Z. Shi, M. Wen, M. Jian, H. Yang, G. Zhang, Z. Yang, R. Chen, L. Ma, H. Liu, Y. Zhou, J. Zhao, X. Fang, L. Fang, D. Liu, H. Zheng, Y. Zhang, N. Qin, Z. Li, G. Yang, S. Yang, L. Bolund, K. Kristiansen, S. Li, X. Zhang, J. Wang, R. Sun, B. Zhang, S. Jiang, and Y. Du. 2009. The genome of the cucumber, *Cucumis sativus* L. *Nat. Genet.* 41:1275-1281.
- Humphreys, M., H.-M. Thomas, J. Harper, G. Morgan, A. James, A. Ghamari-Zare, and H. Thomas. 1997. Dissecting drought-and cold-tolerance traits in the *Lolium-Festuca* complex by introgression mapping. *New Phytol.* 137:55-60.
- Icso, D., M. Molnar-Lang, and G. Linc. 2014. Constructing an alternative wheat karyotype using barley genomic DNA. *J. Appl. Genet.* 56:45-48.
- Jeffrey, C. S. 2005. A new system of Cucurbitaceae. *Bot. Zhurn.* 90:332-335.

- Jiang, J., B. Friebe, and B. Gill. 1993. Recent advances in alien gene transfer in wheat. *Euphytica* 73:199-212.
- Karmakar, K., R. K. Sinha, and S. Sinha. 2013. Karyological and electrophoretic distinction between sexes of *Trichosanthes bracteata*. *Am. J. Plant Sci.* 4:494.
- Kellogg, E.A. 2013. Grasses, Synteny, Evolution, and Molecular Systematics. In: S.M. HUGHES (ed.). *Brenner's Encyclopedia of Genetics* (Second Edition). San Diego: Academic Press.
- Kocyan, A., L. B. Zhang, H. Schaefer, and S. S. Renner. 2007. A multi-locus chloroplast phylogeny for the Cucurbitaceae and its implications for character evolution and classification. *Mol. Phylogenet. Evol.* 44:553-577.
- Koo, D. H., Y. W. Nam, D. Choi, J. W. Bang, H. de Jong, and Y. Hur. 2010. Molecular cytogenetic mapping of *Cucumis sativus* and *C. melo* using highly repetitive DNA sequences. *Chromosome Res.* 18:325-336.
- Kovarik, A., S. Renny-Byfield, M.-A. Grandbastien, and A. Leitch. 2012. Evolutionary implications of genome and karyotype restructuring in *Nicotiana tabacum* L., p. 209-224. In: P. S. Soltis and D. E. Soltis. *Polyploidy and genome evolution.*, Springer, Berlin Heidelberg, Germany.
- Lashermes, P., S. Andrzejewski, B. Bertrand, M. C. Combes, S. Dussert, G. Graziosi, P. Trouslot, and F. Anthony. 2000. Molecular analysis of introgressive breeding in coffee (*Coffea arabica* L.). *Theor. Appl. Genet.* 100:139-146.
- Lee-Huang, S., P. L. Huang, H. F. Kung, B. Q. Li, P. L. Huang, P. Huang, H. I. Huang, and H. C. Chen. 1991. TAP 29: an anti-human immunodeficiency virus protein from *Trichosanthes kirilowii* that is nontoxic to intact cells. *Proc. Natl. Acad. Sci. USA* 88:6570-6574.
- Lee, J.-M. 1994. Cultivation of grafted vegetables I. Current status, grafting methods, and benefits. *Hortscience.* 29:235-239.
- Liao, P.-C., C.-C. Tsai, C.-H. Chou, and Y.-C. Chiang. 2012. Introgression between cultivars and wild populations of *Momordica charantia* L. (Cucurbitaceae) in Taiwan. *Int. J. Mol. Sci.* 13: 6469-6491.
- Macas, J., P. Neumann and A. Navratilova. 2007. Repetitive DNA in the pea (*Pisum sativum* L.) genome: comprehensive characterization using 454 sequencing and comparison to soybean and *Medicago truncatula*. *BMC Genomics* 8:427.
- Mandáková, T., M. E. Schranz, T. F. Sharbel, H. de Jong, and M. A. Lysak. 2015. Karyotype evolution in apomictic *Boechera* and the origin of the aberrant chromosomes. *Plant J.* 82:785-793.
- Mayer, K. F. X., M. Martis, P. E. Hedley, H. Šimková, H. Liu, J. A. Morris, B. Steuernagel, S. Taudien, S. Roessner, H. Gundlach, M. Kubaláková, P. Suchánková, F. Murat, M. Felder, T. Nussbaumer, A. Graner, J. Salse, T. Endo, H. Sakai, T. Tanaka, T. Itoh, K. Sato, M. Platzer, T. Matsumoto, U. Scholz, J. Doležel, R. Waugh, and N. Stein. 2011. Unlocking the barley genome by chromosomal and comparative genomics. *Plant Cell* 23:1249-1263.
- Mayer, R. A., P. A. Sergios, K. Coonan, and L. O'Brien. 1992. Trichosanthin treatment of HIV-induced immune dysregulation. *Eur. J. Clin. Invest.* 22:113-122.
- McGrath, C. L. and M. Lynch. 2012. Evolutionary significance of whole-genome duplication, p.1-20. In: P. S. Soltis and D. E. Soltis (eds.). *Polyploidy and genome evolution.* Springer, Berlin Heidelberg, Germany.
- McGrath, M. S., K. M. Hwang, S. E. Caldwell, I. Gaston, K. C. Luk, P. Wu, V. L. Ng, S. Crowe, J. Daniels, J. Marsh, and et al. 1989. GLQ223: an inhibitor of human immunodeficiency virus replication in acutely and chronically infected cells of lymphocyte and mononuclear phagocyte lineage. *Proc. Natl. Acad. Sci. USA* 86:2844-2848.
- Minh, C. V., N. X. Nhiem, H. T. Yen, P. V. Kiem, B. H. Tai, H. Le Tuan Anh, T. T. T. Hien, S. Park, N. Kim, and S. H. Kim. 2015. Chemical constituents of *Trichosanthes kirilowii* and their cytotoxic activities. *Arch. Pharm. Res.* 1-6.
- Mun, J. H., S. J. Kwon, T. J. Yang, Y. J. Seol, M. Jin, J. A. Kim, M. H. Lim, J. S. Kim, S. Baek, B. S. Choi, H. J. Yu, D. S. Kim, N. Kim, K. B. Lim, S. I. Lee, J. H. Hahn, Y. P. Lim, I. Bancroft, and B. S. Park. 2009. Genome-wide comparative analysis of the *Brassica rapa* gene space reveals genome shrinkage and differential loss of duplicated genes after whole genome triplication. *Genome Biol.* 10:R111.
- Ni, L., X. Zhu, C. Gong, Y. Luo, L. Wang, W. Zhou, S. Zhu, and Y. Li. 2015. *Trichosanthes kirilowii* fruits inhibit non-small cell lung cancer cell growth through mitotic cell-cycle arrest. *Am. J. Chin. Med.* 43:349-364.
- Otto, S. P. 2007. The evolutionary consequences of polyploidy. *Cell* 131:452-462.
- Qian, C.-L., X.-H. Qi, J.-H. Yang, and M.-F. Zhang. 2012. Molecular phylogeny of Chinese snakegourd (*Trichosanthes kirilowii* Maxim.) based on cytological and AFLP analyses. *Caryologia* 65:216-222.
- Ramsey, J. 2011. Polyploidy and ecological adaptation in wild yarrow. *Proc. Natl. Acad. Sci.* 108:7096-7101.
- Robinson, R. W. and D. S. Decker-Walters 1997. *Cucurbits*. Wallingford, UK, CABI Publishing.
- Sain, R. S., P. Joshi and E. V. Divakara Sastry. 2002. Cytogenetic analysis of interspecific hybrids in genus *Citrullus* (Cucurbitaceae). *Euphytica* 128:205-210.
- Seo, C.-S. S., T.-W. Kim, Y.-J. Kim, S.-R. Park, H. Ha, H.-K. Shin, and J.-Y. Jung. 2015. *Trichosanthes kirilowii* ameliorates cisplatin-induced nephrotoxicity in both in vitro and in vivo. *Nat. Prod. res.* 29:554-557.
- Shengping, Z., G. Xingfang, and W. Ye. 2006. Effect of bur cucumber (*Sicyos angulatus* L.) as rootstock on growth physiology and stress resistance of cucumber plants. *Acta Hort.* Sin. 33:1231-1236.
- Singh, A. K. 1979. Cucurbitaceae and polyploidy. *Cytologia* 44: 897-905.
- Snowdon, R. J., W. Köhler, W. Friedt, and A. Köhler. 1997. Genomic *in situ* hybridization in *Brassica* amphidiploids and

- interspecific hybrids. *Theor. Appl. Genet.* 95:1320-1324.
- Soltis, D. E., R. J. A. Buggs, W. B. Barbazuk, S. Chamala, M. Chester, J. P. Gallagher, P. S. Schnable, and P. S. Soltis 2012. The early stages of polyploidy: Rapid and repeated evolution in *Tragopogon*, p.271-292. In: *Polyploidy and genome evolution*. P. S. Soltis and D. E. Soltis, Springer, Berlin, Heidelberg, Germany.
- Szinay, D., Y. Bai, R. Visser, and H. de Jong. 2010. FISH applications for genomics and plant breeding strategies in tomato and other solanaceous crops. *Cytogenet. Genome Res.* 129:199-210.
- Thies, J. A. and A. Levi. 2007. Characterization of watermelon (*Citrullus lanatus* var. *citroides*) germplasm for resistance to root-knot nematodes. *Hortscience.* 42:1530-1533.
- Tzonev, R. 2005. *Sicyos angulatus* (Cucurbitaceae): a new adventive species for the flora of Bulgaria. *Phytologia Balcanica* 11:67-68.
- Uchikoba, T., S. Hosoyamada, M. Onjyo, K. Arima, H. Yonezawa, and M. Kaneda. 2001. A serine endopeptidase from the fruits of *Melothria japonica* (Thunb.) Maxim. *Phytochemistry* 57:1-5.
- Vrana, J., H. Simkova, M. Kubalaková, J. Cihalikova and J. Dolezel. 2012. Flow cytometric chromosome sorting in plants: The next generation. *Methods* 57:331-337.
- Waminal, N., H. M. Park, K. B. Ryu, J. H. Kim, T. J. Yang, and H. H. Kim. 2012. Karyotype analysis of *Panax ginseng* C.A. Meyer, 1843 (Araliaceae) based on rDNA loci and DAPI band distribution. *Comp. Cytogenet.* 6:425-441.
- Waminal, N. E. and H. H. Kim. 2012. Dual-color FISH karyotype and rDNA distribution analyses on four Cucurbitaceae species. *Hortic. Environ. Biotechnol.* 53:49-56.
- Waminal, N. E., N. S. Kim, and H. H. Kim. 2011. Dual-color FISH karyotype analyses using rDNAs in three Cucurbitaceae species. *Genes Genom.* 33:521-528.
- Waminal, N. E., K. B. Ryu, B. R. Park, and H. H. Kim. 2014. Phylogeny of Cucurbitaceae species in Korea based on 5S rDNA non-transcribed spacer. *Genes Genom.* 36:57-64.
- Wang, Q., H. Liu, A. Gao, X. Yang, W. Liu, X. Li, and L. Li. 2012. Intergenomic rearrangements after polyploidization of *Kengyilia thoroaldiana* (Poaceae: Triticeae) affected by environmental factors. *PLoS one* 7.
- Weeden, N. F. and R. W. Robinson. 1986. Allozyme segregation ratios in the interspecific cross *Cucurbita maxima* x *C. ecuadorensis* suggest that hybrid breakdown is not caused by minor alterations in chromosome structure. *Genetics* 114:593-609.
- Wendel, J. F. 2000. Genome evolution in polyploids. *Plant Mol. Biol.* 42:225-249.
- Weng, Y. and Z. Sun 2012. Major cucurbit crops, p. 1-16. In: Y.-H. Wang, T. K. Behera, and C. Kole. *Genetics, genomics and breeding of cucurbits*. Science Publishers, New Hampshire, USA.
- Xiong, Z. Y. and J. C. Pires. 2011. Karyotype and identification of all homoeologous chromosomes of allopolyploid *Brassica napus* and its diploid progenitors. *Genet.* 187:37-49.
- Zamir, D. 2001. Improving plant breeding with exotic genetic libraries. *Nat. Rev. Genet.* 2:983-989.
- Zhuang, F. Y., J. F. Chen, J. E. Staub, and C. T. Qian. 2006. Taxonomic relationships of a rare *Cucumis* species (*C. hystrix* Chakr.) and its interspecific hybrid with cucumber. *Hortscience* 41:571-574.