HortScience 33(7):1247–1250. 1998.

Variation of the Nuclear DNA Content of Species of Subtribe Citrinae (Rutaceae)

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Additional index words. C value, flow cytometry

Abstract. **Laser flow cytometry was used to analyze nuclear DNA contents (2C values) of five genera (*Severinia* Ten., *Atalantia* Corrèa, *Fortunella* Swing., *Poncirus* Raf., and *Citrus* L.) taxonomically grouped in subtribe Citrinae (citrus fruit trees) of the Rutaceae. The genotypes analyzed had 2C values ranging from 0.67 pg for diploid *Severinia buxifolia* (Poir.) Ten. to 1.27 pg for tetraploid Hongkong *Fortunella hindsii* Swing. There was no significant difference in the 2C values within the sexually compatible diploid species of 11 "true citrus fruit trees" [*Citrus aurantium* L., *C. grandis* (L.) Osbeck, *C. limon* (L.) Burm. f., *C. limonia* Osbeck, *C. paradisi* Macf., *C. reshni* Hort. ex Tanaka, *C. sinensis* (L.) Osbeck, *C. volkameriana* Ten. & Pasq., *Poncirus trifoliata* (L.) Raf., and the intergeneric hybrid *C. sinensis* x *P. trifoliata*]. The species *Atalantia ceylanica* (Arn.) Oliv. (a "near-citrus fruit tree"), sexually incompatible with *Citrus* spp., had a 2C value significantly different from those of the true citrus fruit tree species. The 2C value of *Severinia buxifolia* (a "primitive citrus fruit tree"), another species sexually incompatible with the *Citrus* spp., also differed from those of some of the true citrus fruit tree species. The data largely corresponds with taxonomical differences between a) the genera *Citrus* and *Poncirus* and b) the genera *Severinia* and *Atalantia*, all assigned to subtribe Citrinae.**

Citrus L. species and related genera in the Rutaceae grow in tropical and subtropical regions of the world. They are thought to have originated in China, southeastern Asia, northeastern Australia, and New Caledonia (Vardi and Galun, 1989; Webber et al., 1967). The commonly cultivated citrus fruit trees belong to *Citrus* of subtribe Citrinae (Swingle and Reece, 1967). This subtribe consists of 65 species belonging to 13 genera, including *Severinia* Ten., *Pleiospermium* (Engl.) Swing., *Burkillanthus* Swing., *Limnocitrus* Swing., *Hesperethusa* Roem., *Citropsis* (Engl.) Swing. et M. Kell., *Atalantia* Corrèa, *Fortunella* Swing., *Eremocitrus* Swing., *Poncirus* Raf., *Clymenia* Swing., *Microcitrus* Swing., and *Citrus* (Swingle and Reece, 1967). Species in the Citrinae have been divided according to their morphological differences and sexual compatibilities into three different subtribal groups as "true citrus fruit trees," "near-citrus fruit trees," and "primitive citrus fruit trees" (Swingle and Reece, 1967). Some of the species belonging to these genera are a useful source of genetic traits for rootstock improvement. However, many species in the Rutaceae are difficult to hybridize sexually due to their sterility, incompatibility, and polyembryony (Ohgawara et al., 1989; Vardi and Galun, 1989).

Citrus trees and related species are usually diploid (2n = 2x = 18). Nevertheless, a few polyploids have been identified in nature, such as a tetraploid Hongkong wild kumquat (*Fortunella hindsii* Swing.), a triploid Tahiti lime [*Citrus aurantifolia* (Christm.) Swing.], and a tetraploid limeberry (*Triphasia* Lour. sp.) (Esen and Soost, 1972; Iwamasa and Nito, 1988). Also, polyploid cultivated lines have been produced experimentally (Esen and Soost, 1972).

A few earlier publications discuss the DNA contents (2C values) of *Citrus* and related species. Ingle et al. (1975) published the nuclear DNA content of *Citrus sinensis* as 1.8 pg, using comparative Feulgen photometry. Guerra (1984) analyzed *C. sinensis* using cytophotometry to measure its 4C value at 2.47 pg (2C = 1.23 pg). Flow cytometry is another tool for analyzing DNA content and ploidy levels. Arumuganathan and Earle (1991a) analyzed *C. sinensis* using flow cytometry and estimated the DNA content as 0.76 pg and 0.82 pg. They showed that the 2C value of *C. sinensis* was low in comparison with a hundred other higher plant species analyzed. A detailed study on the 2C values of different *Citrus* species and several varieties was carried out by Ollitrault et al. (1994), who obtained results very similar to those of Arumuganathan and Earle (1991a).

The objectives of the present study were: 1) to estimate the nuclear DNA contents in subtribe Citrinae by flow cytometry, sampling genera from three different subtribal groups, and 2) to compare the DNA contents of sexually compatible species with their close incompatible relatives. The species of "true citrus fruit trees" studied belonged to *Citrus*, *Fortunella* and *Poncirus*, of "near-citrus fruit trees" to *Atalantia*, and of "primitive citrus fruit trees" to *Severinia*.

Materials and Methods

Fifteen genotypes of the 13 species (*Severinia buxifolia*, *Atalantia ceylanica*, *Fortunella hindsii*, *Poncirus trifoliata*, *C. sinensis* x *P. trifoliata*, *Citrus aurantium*, *C. grandis*, *C. limon*, *C. limonia*, *C. paradisi*, *C. reshni*, *C. sinensis*, *C. volkameriana*) were grown either in the greenhouse as seedlings or as grafted plants (Table 1). The grafted plant material was received as scions from the Çukurova region in Turkey. Seeds were obtained from the Department of Plant Protection, the Univ. of Çukurova, Adana, Turkey, and from the USDA-ARS National Germplasm Repository for Citrus and Dates, Riverside, Calif. (Table 1). Seeds were surface-sterilized in 1% (v/v) sodium hypochloride with 0.1% (v/v) Tween 20 (polysorb. 20) (ICI, Essen, Germany) for 10 min, rinsed with sterile distilled H_2O, and then germinated in vitro on basic MS medium (Murashige and Skoog, 1962) free of sucrose. The plantlets were transferred into the greenhouse and grown for 4–6 weeks until they had small (5 cm in diameter) leaves. The mature leaves of grafted plants of *Citrus limon* 'Kütdiken' and *C. limon* 'Zagara Bianca' were used in the analyses.

Laser flow cytometry was applied to measure nuclear DNA contents (Arumuganathan and Earle, 1991b; Rokka et al., 1995). Leaves, 50 mg per sample, were chopped with a scalpel in Solution A (Arumuganathan and Earle, 1991b) containing propidium iodide (Calbiochem, La Jolla, Calif.) for nuclei staining, then filtered through a 48-µm nylon mesh. The filtrate was centrifuged at 16,000 g_n for 20 s in a microcentrifuge. The pellet was resuspended in Solution B, consisting of Solution A and RNase (Boehringer Mannheim, Mannheim, Germany). Chicken red blood cells (CRBC), diluted 1:200 in Alsever's solution, were added as an internal standard [2C value of 2.33 pg according to Galbraith et al. (1983)] to plant nuclei samples. The samples were incubated in the dark at 37 °C for 15 min. The samples were kept on ice until they were analyzed during the following 1–3 h using a flow cytometer with a 488 nm wavelength argon-ion laser (FACSort, Becton Dickinson,

Received for publication 30 Jan. 1998. Accepted for publication 19 June 1998. We wish to acknowledge the Research Foundation at the Univ. of Çukurova for part of the financial support. The Finnish Ministry of Agriculture and Forestry and the CIMO (Centre for International Mobility) are acknowledged for the financial support to Veli-Matti Rokka. Prof. Jari Valkonen (Swedish Univ. of Agricultural Sciences), Dr. Sirkka Immonen (Agricultural Research Centre of Finland), and Dr. Deana Namuth (Colorado State Univ.) are acknowledged for critical review of the manuscript. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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Table 1. Plant material of Citrinae analyzed with flow cytometry.

Species	Genotype	Origin	Common name	Source
Subtribal Group A. Primitive citrus fruit trees				
Severinia buxifolia	acc. PI 539794	USDA-ARS[z]	Chinese box-orange	seedling
Subtribal Group B. Near-citrus fruit trees				
Atalantia ceylanica	acc. PI 539144	USDA-ARS	Ceylon atalantia	seedling
Subtribal Group C. True citrus fruit trees				
Fortunella hindsii	'Hongkong'	Çukurova, TR[y]	Hongkong wild kumquat	seedling
Poncirus trifoliata	'Flying Dragon' acc. PI 539768	USDA-ARS	Trifoliate orange	seedling
Citrus aurantium		Çukurova, TR	Sour orange	seedling
Citrus grandis	'Shadox'	Çukurova, TR	Pummelo	seedling
Citrus limon	'Kütdiken'	Çukurova, TR	Lemon	grafted
Citrus limon	'Zagara Bianca'	Çukurova, TR	Lemon	grafted
Citrus limonia	'Brome Rangpur 2424'	USDA-ARS	Rangpur lime	seedling
Citrus paradisi	'Frost Marsh' (1005R)	Çukurova, TR	Grapefruit	seedling
Citrus reshni	'Cleopatra' acc. PI 539492	USDA-ARS	Cleopatra mandarin	seedling
Citrus sinensis	'Pineapple'	Çukurova, TR	Sweet orange	seedling
Citrus sinensis	'Sargoins Grosse Ronde'	Çukurova, TR	Sweet orange	seedling
Citrus volkameriana	3050	USDA-ARS	Volkamer lemon	seedling
C. sinensis x *P. trifoliata*	'Carrizo'	Çukurova, TR	Citrange	seedling

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San Jose, California). The "Low" flow rate was used in all measurements. Estimates of relative DNA amounts were based on the fluorescence intensities on a linear scale (FL2-H). The DNA content of each sample was calculated by comparing the position of the plant nuclear 2C peak with that of CRBC on the histogram scale (Arumuganathan and Earle, 1991b). According to DNA histogram plots, the 2C value of the plant nuclear DNA amount = (channel number of G_0/G_1 plant nuclear peak/channel number of CRBC nuclear peak) $\times$ 2.33 pg. Threshold was adjusted in each run to eliminate debris (broken nuclei and organelles) from the histogram plot. A total of 1000 to 4000 events per sample were analyzed. The mean of three samples was taken as an estimate of the 2C value for each genotype.

Data from flow cytometric readings were analyzed using analysis of variance (PROC ANOVA) (SAS, 1985). Tukey's Studentized range (HSD) test was used for mean separation.

Results and Discussion

Histograms from four flow cytometric measurements (Fig. 1) illustrate the 2C plant sample peaks and the internal CRBC DNA standard peaks.

The 2C values of *Citrus* spp. tested ranged from 0.76 pg to 0.88 pg (mean 0.81 pg/2C) (Table 2). There were no significant differences in DNA content among the diploid genotypes of *Citrus* ($P \leq 0.05$), but the interspecific variation in the 2C values among the analyzed species of *Citrus* was 14%. Nearly as much (11%) intraspecific variation was observed between the two *C. sinensis* cultivars tested (Table 2). All these *Citrus* species are closely related genetically and can hybridize sexually. Therefore their taxonomic classification is complicated (Vardi and Spiegel-Roy, 1978). The nuclear DNA contents of all *Citrus* spp. included in this study were close to those reported previously by Arumuganathan and Earle (1991a) and Ollitrault et al. (1994). Ollitrault et al. (1994) reported that the 2C values of various *Citrus* varieties ranged between 0.74 pg (*C. reticulata* Blanco) and 0.81 pg (*C. medica* L.). The interspecific variation in the DNA content in *Citrus* sp. was estimated to be 10% in their study, which was similar to our findings.

Atalantia ceylanica had significantly ($P \leq 0.05$) higher 2C DNA content (1.05 pg) than did *Citrus* spp. (mean 0.81 pg) (Table 2). *Atalantia ceylanica* is a diploid species related closely taxonomically to diploid *Citrus* spp., but classified as near-citrus fruit trees, a different subtribal group (Swingle and Reece, 1967). Although closely related, *Atalantia* and *Citrus* are incompatible sexually (Iwamasa et al., 1988). One reason for their sexual incompatibility could be morphological variation in their chromosome structures. Lapitan (1992) suggested that the nuclear DNA content depends on the amount of repetitive DNA, which is normally species-specific. Repetitive DNA creates species-specific differences in chromosome structure that can lead to irregularities in pairing of homoeologous chromosomes (Sybenga, 1992). However, tetraploid (2n = 4x = 36) somatic hybrids between *Atalantia* and *Citrus* have been obtained using protoplast fusion (Grosser et al., 1996; Louzada et al., 1993). Two different types of leaf morphology were observed among the *C. sinensis* + *A. ceylanica* somatic hybrids (Louzada et al., 1993), whereas, in general, morphology of somatic hybrids between *Citrus* species is uniform (Louzada et al., 1993). The different morphological types in *C. sinensis* + *A. ceylanica* somatic hybrids may be associated with different DNA contents of these two genera. Recently though, more vigorous somatic hybrids between *C. sinensis* and *A. ceylanica* have been obtained (Grosser et al., 1996).

Severinia and *Citrus* also are incompatible genera sexually (Cameron and Frost, 1968). *Severinia* is classified as primitive citrus fruit trees in the Citrinae (Swingle and Reece, 1967). *Severinia buxifolia* had a lower 2C DNA content (0.67 pg) than the studied species of *Citrus*, *Atalantia*, and *Poncirus*. As with *Atalantia* and *Citrus*, differences in chromosome structure may also be the reason for sexual incompatibility between *Severinia* and *Citrus*. Somatic hybridization between *S. buxifolia* and *Citrus* sp. is possible (Grosser et al., 1992; Grosser et al., 1996) and, as with *Citrus* + *Atalantia* somatic hybrids, *C. sinensis* + *S. buxifolia* somatic hybrids were low in vigor and had an unexpectedly low chromosome number (2n = 27) (Grosser et al., 1992; Grosser et al., 1996). The low chromosome number may have been the result of spontaneous chromosome elimination that is common in intergeneric somatic hybrids (Waara and Glimelius, 1995). *Citrus sinensis* also has been hybridized somatically with *Severinia disticha* (Blanco) Swing. (Grosser et al., 1988). These somatic hybrids were not viable, which may have been associated with negative genetic interaction between parental genomes (Grosser et al., 1996). This phenomenon may be due to a broader taxonomic distance between *Citrus* and *Severinia* than between *Citrus* and *Atalantia* (Swingle and Reece, 1967). In contrast, according to the DNA contents, *Citrus* sp. were related more closely to *Severinia buxifolia* than *Atalantia ceylanica* because the 2C values of only four of the 11 genotypes of true citrus fruit trees were different from *S. buxifolia* (Table 2).

Sexual and somatic hybridizations of *Citrus* are successful with *Poncirus* and *Fortunella* (Cameron and Frost, 1968; Deng et al., 1992; Grosser et al., 1996; Ohgawara et al., 1985). Morphologically uniform allotetraploid somatic hybrids also have been produced between *Citrus* and *Poncirus* (Grosser et al., 1996; Ohgawara et al., 1985) and *Citrus* and *Fortunella* (Deng et al., 1992). *Fortunella*, *Poncirus* and *Citrus* are classified as true citrus fruit trees (Swingle and Reece, 1967) (Table 1). In the present study, the DNA content of *P. trifoliata* was 0.77 pg and that of the sexual hybrid *C. sinensis* x *P. trifoliata* was 0.76 pg, which were similar to all species of *Citrus* (mean 2C value 0.81 pg) (Table 2). The similarity in 2C values of *Citrus* sp. and *Poncirus* was consistent with a close taxonomic relationship. However, isozyme polymorphism of *Fortunella* and *Poncirus* indicated that they are highly divergent from most cultivated spe-

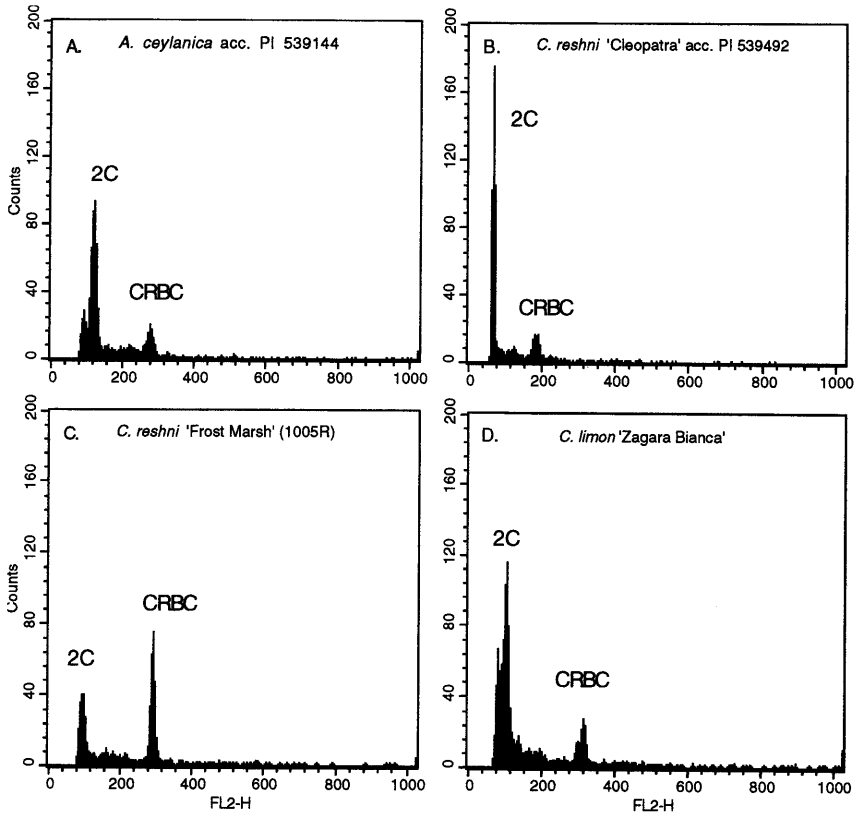

Fig 1. Flow cytometric DNA content (2C value) analysis of species of Citrinae. Chicken red blood cells (CRBC) were used as an internal DNA standard. 2C (G_1 phase) histogram peaks are shown for (**A**) *Atalantia ceylanica*, (**B**) *Citrus reshni* 'Cleopatra', (**C**) *C. reshni* 'Frost Marsh' and (**D**) *C. limon* 'Zagara Bianca'. The coefficients of variation (CV) for plant sample peaks ranged from 3.3% to 10.0%, but were generally 4.0% to 5.0%. The CRBC standards had CVs ranging from 1.4% to 5.6%.

Table 2. DNA contents of 15 genotypes belonging to Citrinae.

Species and cultivar	Subtribal group	Ploidy level (2n)	Mean 2C value[z]	SE[y]
Fortunella hindsii	True citrus fruit trees	4x	1.27 a[x]	0.00
Atalantia ceylanica	Near-citrus fruit trees	2x	1.05 b	0.07
Citrus aurantium	True citrus fruit trees	2x	0.88 c	0.03
Citrus sinensis 'Pineapple'	True citrus fruit trees	2x	0.85 c	0.01
Citrus reshni	True citrus fruit trees	2x	0.82 c	0.04
Citrus limonia 'Brome Rangpur'	True citrus fruit trees	2x	0.82 c	0.02
Citrus paradisi	True citrus fruit trees	2x	0.80 cd	0.05
Citrus limon 'Zagara Bianca'	True citrus fruit trees	2x	0.80 cd	0.01
Citrus volkameriana	True citrus fruit trees	2x	0.79 cd	0.03
Citrus limon 'Kütdiken'	True citrus fruit trees	2x	0.77 cd	0.01
Poncirus trifoliata	True citrus fruit trees	2x	0.77 cd	0.01
Citrus grandis	True citrus fruit trees	2x	0.77 cd	0.02
Citrus sinensis x *Poncirus trifoliata*	True citrus fruit trees	2x	0.76 cd	0.00
Citrus sinensis 'Sargoins Grosse Ronde'	True citrus fruit trees	2x	0.76 cd	0.01
Severinia buxifolia	Primitive citrus fruit trees	2x	0.67 d	0.02

[z]Mean of three flow cytometric measurements.
[y]Standard error of the mean.
[x]Mean separation by Tukey's standardized range HSD test, $P \leq 0.05$. Minimum significant difference = 0.1449, critical value of Studentized range = 5.211.

cies of *Citrus* (Raman and Dhillon, 1994). Still, relatively complete pairing of chromosomes in meiosis in intergeneric hybrids of *Citrus* with *Poncirus* and *Fortunella* has been observed, suggesting a common ancestor (Iwamasa and Nito, 1988).

The only polyploid species included in this study was the tetraploid (2n = 4x = 36) Hongkong wild kumquat (*Fortunella hindsii*) (Longley, 1925). It had a nuclear DNA content of 1.27 pg, which was lower than expected for a tetraploid species of Citrinae. Polyploidy actually is infrequent in the Rutaceae because of apomixis (nucellar polyembryony) that minimizes natural hybridization and production of unreduced gametes (Grosser et al., 1996).

Flow cytometric analysis revealed differences in the DNA contents between sexually incompatible genera *Citrus* and *Atalantia*, and *Citrus* and *Severinia*. Also, all of these have been classified previously in different taxonomic subtribal groups in Citrinae. Differences between the nuclear DNA contents also may explain some irregularities expressed frequently in the somatic hybrids between these species as compared with species of *Citrus* and *Poncirus* that have similar DNA contents. The observations of DNA content variations could be important to recognize in production of novel interspecific somatic hybrids.

Literature Cited

Arumuganathan, K. and E.D. Earle. 1991a. Nuclear DNA content of some important plant species. Plant Mol. Biol. Rpt. 9:208–218.

Arumuganathan, K. and E.D. Earle. 1991b. Estimation of nuclear DNA content of plants by flow cytometry. Plant Mol. Biol. Rpt. 9:229–241.

Cameron, J.W. and H.B. Frost. 1968. Genetics, breeding, and nucellar embryony, p. 325–381. In: W. Reuther, L.D. Batchelor, and H.J. Webber (eds.). The citrus industry, vol. 2. Anatomy, physiology, genetics, and reproduction. Univ. of California, Riverside.

Deng, X.X., J.W. Grosser, and F.G. Gmitter, Jr. 1992. Intergeneric somatic hybrid plants from protoplast fusion of *Fortunella crassifolia* cultivar 'Meiwa' with *Citrus sinensis* cultivar 'Valencia'. Scientia Hort. 49:55–62.

Esen, A. and R.K. Soost. 1972. Tetraploid progenies from 2x x 4x crosses of *Citrus* and their origin. J. Amer. Soc. Hort. Sci. 97:410–414.

Galbraith, D.W., K.R. Harkins, J.M. Maddox, N.M. Ayres, D.P. Sharma, and E. Firoozabady. 1983. Rapid flow cytometric analysis of the cell cycle in intact plant tissues. Science 220:1049–1051.

Grosser, J.W., F.G. Gmitter, Jr., and J.L. Chandler. 1988. Intergeneric somatic hybrid plants from sexually incompatible woody species: *Citrus sinensis* and *Severinia disticha*. Theor. Appl. Genet. 75:397–401.

Grosser, J.W., F.G. Gmitter, Jr., F. Sesto, X.X. Deng, and J.L. Chandler. 1992. Six new somatic hybrids and their potential for cultivar improvement. J. Amer. Soc. Hort. Sci. 117:169–173.

Grosser, J.W., F.A.A. Mourao-Fo, F.G. Gmitter, Jr., E.S. Louzada, J. Jiang, K. Baergen, A. Quiros, C. Cabasson, J.L. Schell, and J.L. Chandler. 1996. Allotetraploid hybrids between *Citrus* and seven related genera produced by somatic hybridization. Theor. Appl. Genet. 92:577–582.

Guerra, M. dos S. 1984. Cytogenetics of Rutaceae. II. Nuclear DNA content. Caryologia 37:219–226.

Ingle, J., J.N. Timmis, and J. Sinclair. 1975. The relationship between satellite deoxyribonucleic acid, ribosomal ribonucleic acid gene redundancy, and genome size in plants. Plant Physiol. 55:496–501.

Iwamasa, M. and N. Nito. 1988. Cytogenetics and the evolution of modern cultivated citrus, p. 265–274. In: R. Goren and K. Mendel (eds.). Proc. 6th Intl. Citrus Congr., Tel Aviv, Israel, 6-11 Mar. 1988. Balaban Publ. Israel, Margraf Publ. Germany.

Iwamasa, M., N. Nito, and J-T. Ling 1988. Intra- and intergeneric hybridization in the orange subfamily, Aurantioideae, p. 123–130. In: R. Goren and K. Mendel (eds.). Proc. 6th Intl. Citrus Congr., Tel Aviv, Israel, 6–11 Mar. 1988. Balaban Publ. Israel, Margraf Publ. Germany.

Lapitan, N.L.V. 1992. Organization and evolution of higher plant nuclear genome. Genome 35:171–182.

Longley, A.E. 1925. Polycary, polyspory and polyploidy in citrus and citrus relatives. Wash. Acad. Sci. 15:347–351.

Louzada, E.S., J.W. Grosser, and F.G. Gmitter, Jr. 1993. Intergeneric somatic hybridization of sexu-

ally incompatible parents: *Citrus sinensis* and *Atalantia ceylanica*. Plant Cell Rpt. 12:687–690.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473–497.

Ohgawara, T., S. Kobayashi, S. Ishii, K. Oshinaga, and I. Oiyama. 1989. Somatic hybridization in *Citrus*: Navel orange (*C. sinensis* Osb.) and grapefruit (*C. paradisi* Macf.). Theor. Appl. Genet. 78:609–612.

Ohgawara, T., S. Kobayashi, E. Ohgawara, H. Uchimiya, and S. Ishii. 1985. Somatic hybrid plants obtained by protoplast fusion between *Citrus sinensis* and *Poncirus trifoliata*. Theor. Appl. Genet. 71:1–4.

Ollitrault, P., D. Dambier, F. Luro, and C. Duperray. 1994. Nuclear genome size variations in *Citrus*. Fruits 49:390–393.

Raman, H. and B.S. Dhillon. 1994. Isozyme polymorphism and phylogenetic relationship among species of *Citrus*, *Fortunella* and *Poncirus*. Crop Improv. 21:37–43.

Rokka, V-M., J.P.T. Valkonen, and E. Pehu. 1995. Production and characterization of haploids derived from somatic hybrids between *Solanum brevidens* and *S. tuberosum* through anther culture. Plant Sci. 112:85–95.

SAS Institute. 1985. User's guide. version 5 ed. SAS Inst., Cary, N.C.

Swingle, W.T. and P.C. Reece. 1967. The botany of *Citrus* and its wild relatives, p. 190–430. In: W. Reuther, H.J. Webber, and L.D. Batchelor (eds.). The citrus industry, vol. 1. History, world distribution, botany, and varieties. Univ. of California, Riverside.

Sybenga, J. 1992. Cytogenetics in plant breeding, p. 469. In: R. Frankel, M. Grossman, and P. Maliga (eds.). Monographs on theoretical and applied genetics. Springer-Verlag, Berlin/Heidelberg, Germany.

Vardi, A. and E. Galun. 1989. Isolation and culture of *Citrus* protoplasts, p. 147–159. In: Y.P.S. Bajaj (ed.). Biotechnology in agriculture and forestry, vol. 8. Plant protoplasts and genetic engineering. Springer-Verlag, Berlin/Heidelberg, Germany.

Vardi, A. and P. Spiegel-Roy. 1978. Citrus breeding, taxonomy and the species problem. Proc. Intl. Soc. Citriculture 51–57.

Waara, S. and K. Glimelius. 1995. The potential of somatic hybridization in crop breeding. Euphytica 85:217–233.

Webber, H.J., W. Reuther, and H.W. Lawton. 1967. History and development of the citrus industry, p. 1–39. In: W. Reuther, H.J. Webber, and L.D. Batchelor (eds.). The citrus industry, vol. 1. History, world distribution, botany, and varieties. Univ. of California, Riverside.