

Chromosome number and secondary constriction variation in 51 accessions of a citrus germplasm bank

Marcelo Guerra¹, Andrea Pedrosa¹, Ana Emília Barros e Silva¹,
Maria Tereza Marquim Cornélio¹, Karla Santos¹ and
Walter dos Santos Soares Filho²

ABSTRACT

The mitotic chromosomes of 51 citrus accessions from the Centro Nacional de Pesquisa em Mandioca e Fruticultura Tropical, Cruz das Almas, BA, Brazil, were analyzed. The sample included representatives of 20 *Citrus* species, one of *Poncirus* and seven hybrids. All accessions showed $2n = 18$ without any evidence of numerical variation. The most clearly variable karyotype feature was the number and position of secondary constrictions (SECs). In 19 accessions the SECs were not identified, mainly due to the degree of chromatin condensation. In the remainder they varied in number from one to three per karyotype. They were found in the proximal region of one of the three largest chromosome pairs, in the terminal/subterminal region of a smaller chromosome or, more seldom, terminally in a larger chromosome. Only in a few cases were such constrictions observed simultaneously in both homologues of the same chromosome pair. The high variability of this karyotype feature may be due to the activation of this region in the previous interphase but may also indicate a high structural variability and heterozygosity of citrus germplasms.

INTRODUCTION

Karyotype analysis using conventional staining techniques in species with small chromosomes is almost always limited to the identification of the number and a few morphological features of the chromosomes (see, e.g., Frahm-Leliveld, 1965; Lin, 1987). Measurement of chromosome size and centromeric index in such cases is often only an approximation, because the error in such methods increases inversely with the chromosome size. On the other hand, the characterization of a large number of individuals or

varieties using techniques like C-banding or fluorochrome analysis, which can provide more details, is very time consuming and expensive. *Citrus* species have small chromosomes and a diversified banding pattern after C-banding or fluorochrome staining (Guerra, 1993). The cytogenetical characterization of citrus accessions could help in the identification of a particular genomic variant, or for the detection of true hybrids in breeding programs, as well as for studies of karyotype evolution of the group. In order to analyze a large number of individuals from a germplasm bank of citrus, we decided to first conduct a conventional analysis of the chromosome number, position and number of secondary constrictions (SECs) and satellites, which are easily observed by conventional methods. This first overview provides some information about

¹ Departamento de Botânica, Centro de Ciências Biológicas, Universidade Federal de Pernambuco, 50670-901 Recife, PE, Brasil. Send correspondence to M.G.

² Centro Nacional de Pesquisa em Mandioca e Fruticultura Tropical, EMBRAPA, Cruz das Almas, BA, Brasil.

the karyotype variability in the genus and will make it easier to select which materials should be analyzed by banding methods.

The chromosome number of *Citrus* species was correctly established by Frost (1925a) as $n = 9$ or $2n = 18$. However, polyploid seedlings and adult plants have often been reported (Frost, 1925b; Krug, 1943; Esen and Soost, 1971; Hutchinson and Barrett, 1981). Several authors have emphasized the importance of such polyploids in citrus breeding programs (Krug and Bacchi, 1943; Esen and Soost, 1971). Iwamasa and Nito (1988) observed less than 1% to over 20% polyploid seedlings, depending on the variety. The origin of such polyploids may be chromosome complement duplication of nucellar cells before somatic embryogenesis (Frost, 1925b) or during megasporogenesis (Esen *et al.*, 1979). Some other factors, such as interspecific hybridization, ploidy level or the mono/polyembryonic nature of the variety in question may also contribute to the frequency of polyploids (Cameron and Soost, 1969; Oiyama *et al.*, 1981; Wakana *et al.*, 1981).

Aneuploidy, on the other hand, occurs only at a very low frequency (Krug and Bacchi, 1943; Wakana *et al.*, 1981). Aneuploid adults, as opposed to seedlings, have been reported only by Sharma and Bal (1957). Nevertheless, several types of meiotic irregularities capable of producing aneuploid spores have been observed in some varieties (Raghuvanshi, 1962a,b; Naithani and Raghuvanshi, 1963; Sellito-Boaventura and Pio, 1989).

The aim of this work was to investigate the occurrence of variations in the secondary constrictions or satellites and chromosome number in some accessions of the germplasm bank of citrus from the Centro Nacional de Pesquisa em Mandioca e Fruticultura Tropical (CNPMPF), from EMBRAPA, Cruz das Almas, BA, Brazil.

MATERIAL AND METHODS

All material was obtained from the living collection of the germplasm bank of CNPMPF (Table I). Cytogenetic analysis was conducted in the Department of Botany, Universidade Federal de Pernambuco.

Root tip cells were analyzed from most cultivars, whereas young flower buds were used for meiotic analysis in others. For mitotic analysis the material was pretreated with 8-hydroxyquinoline (0.002 M) for 1 h at room temperature and then 20-23 h at 10°C. Cytological preparations were made following the method described by Guerra (1983). Mitotic and meiotic cells were hydrolyzed in 5N HCl for 20 min at

room temperature. The material was squashed in a drop of 45% acetic acid, frozen in liquid nitrogen to permit removal of the coverslip, air-dried, stained in a 2% Giemsa solution and mounted in Euparal. Photographs were taken with Agfa Copex Pan 25 ASA.

RESULTS AND DISCUSSION

The 51 accessions analyzed included 20 species of *Citrus*, the very closely related *Poncirus trifoliata* and seven interspecific hybrids. This is the most extensive cytogenetic study of citrus germplasm thus far carried out. All germplasms presented $2n = 18$, with very similar karyotypic morphology and size, although a single seedling of *C. sinensis* cv. Berna was triploid. In some accessions the pretreatment produced highly condensed metaphase chromosomes hindering the observation of primary and secondary constrictions. Stickiness was also very often observed, mainly between metaphase chromosomes. As observed by Guerra (1987), at the interphase stage all the material analyzed showed a very similar chromatin structure with a variable number of chromocenters in a faintly stainable chromatin reticulum (Figures 1a,b, 2a,b,e-h).

In some cells of certain varieties the chromosome number seemed to be higher than 18 due to the presence of long or very distended proximal SECs. Because such SECs stain very weakly, the two stained parts adjacent to the SEC may be misinterpreted as two small chromosomes. In fact, one of these parts is a large satellite, constituted of nearly the whole chromosome arm, and the other one is the remaining chromosome (centromere plus the other arm). This artifact is probably the origin of reports of unusual chromosome numbers in *Citrus*, such as $2n = 19$ in *C. acida aurantifolia* cv. Columbia and $2n = 21$ in *C. decumana* cv. Monster Kalsia (Sharma and Bal, 1957).

Proximal SECs, inducing apparent variation in chromosome number, have been detected in many other genera and may represent one of the most common sources of error in chromosome counts of angiosperms (Favarger, 1978). It is almost restricted to mitotic analysis, mainly when counts are done in prophase or prometaphase cells. Giemsa dye solution makes it possible to avoid this error because it produces a deep staining of chromatin without loss of contrast with the background. Such a contrast is not always possible with the more commonly used aceto-stains or Feulgen method (Guerra, 1983).

Secondary constrictions were observed in most material and seemed to be one of the most important

Table I - List of the citrus accessions analyzed with the species name (according to Tanaka, 1954), cultivar and/or common name, experimental station code (when available) and reference code for cytogenetic analysis.

Species	Common name/Cultivar	Code	Reference
<i>Citrus aurantifolia</i> (Christm.) Swing.	Acid lime/'West Indian' or 'Galego'	BRA 000019	RUT-658
	'Malay Lemon'	BRA 000744	RUT-737
<i>C. aurantium</i> L.	Sour orange/'Double Calice'		RUT-728
<i>C. deliciosa</i> Ten.	Mandarin/'Mediterranean	BRA 004219	RUT-519,
	Montenegrina'		RUT-719
<i>C. depressa</i> Hay.	Shekwasha	BRA 004545	RUT-716,
			RUT-717
<i>C. hystrix</i> DC.			RUT-1036
<i>C. jambhiri</i> Lush.	Rough lemon	BRA 000418	RUT-670
<i>C. karna</i> Raf.			RUT-707
<i>C. keraji</i> Hort. ex Tan.			RUT-709
<i>C. limon</i> (L.) Burm.	Lemon/'Cook Eureka'	BRA 000299	RUT-738
	'Frost Eureka'	BRA 000256	RUT-739
	'Eureka IPEACS'		RUT-715
	'Lisbon'	BRA 000264	RUT-704
<i>C. limonia</i> Osbeck x <i>C. reshni</i> Hort. ex Tan.	Rangpur lime x Cleopatra mandarin		RUT-669
<i>C. longispina</i> Wester			RUT-742
<i>C. medica</i> L.	Citron		RUT-1037
<i>C. nobilis</i> Loureiro	Mandarin/'King'	BRA 000469	RUT-727
<i>C. pennivesiculata</i> (Lush.) Tan.			RUT-712
<i>C. reshni</i> Hort. ex Tan.	Cleopatra		RUT-666
<i>C. reshni</i> Hort. ex Tan. x <i>C. limonia</i> Osbeck	Cleopatra mandarin x Rangpur lime		RUT-671
<i>C. reshni</i> Hort. ex Tan. x <i>P. trifoliata</i> (L.) Raf.	Cleopatra mandarin x Trifoliolate orange		RUT-889
	Barnes selection		
<i>C. reticulata</i> Blanco	Common mandarin/'Cravo'	BRA 000744	RUT-719
<i>C. sinensis</i> (L.) Osbeck	Sweet orange/'Bahia'		RUT-692
	'Bahia Vale del Cauca'	BRA 004073	RUT-1074
	'Berna'	BRA 005045	RUT-1080
	'Comuna'		RUT-1083
	'Early Oblong'	CRC 267	RUT-1078
	'Finiki'	BRA 005258	RUT-1071
	'Gardner'		RUT-1119
	'Hamlin'	BRA 005347	RUT-1068,
			RUT-1069
	'Kona'	BRA 005185	RUT-1077
	'Lue Gim Gong'	BRA 000116	RUT-720
	'Natal'	BRA 000124	RUT-1082
	'Olivelands'	BRA 005126	RUT-1079
	'Parson Brown'	BRA 000141	RUT-1073
	'Pêra'	BRA 005380	RUT-1075
	'Pêra Vacinada'		RUT-772
	'Pineapple'	BRA 000167	RUT-1070
	'Rubi'	BRA 000183	RUT-1085
	'Shamouti'	BRA 001368	RUT-1086
	'Trepadeira'		RUT-1072
	'Valencia'	BRA 000213	RUT-1084
<i>C. sinensis</i> (L.) Osbeck x <i>C. reticulata</i> Blanco	Tangor/'Murcott'	BRA 000370	RUT-668
<i>C. sunki</i> Hort. ex Tan.	Sunki	BRA 000051	RUT-713
<i>C. sunki</i> Hort. ex Tan. x <i>P. trifoliata</i> (L.) Raf.	Sunki x Trifoliolate orange English selection		RUT-1087
<i>C. sunki</i> Hort. ex Tan. x <i>P. trifoliata</i> (L.) Raf.	Sunki x Trifoliolate orange Swingle selection		RUT-885
<i>C. tangerina</i> Hort. ex Tan.	Mandarin/'Dancy'	BRA 000035	RUT-1089
<i>C. tangerina</i> Hort. ex Tan. x <i>C. paradisi</i> Macf.	Tangelo/'Orlando'	BRA 003981	RUT-730,
			RUT-1088
<i>C. volkameriana</i> Ten. et Pasq.	'Volkamer' Catânea 2 selection		RUT-729
<i>C. webberi</i> Wester			RUT-1038
<i>Poncirus trifoliata</i> (L.) Raf.	Trifoliolate orange Pomeroy selection		RUT-741

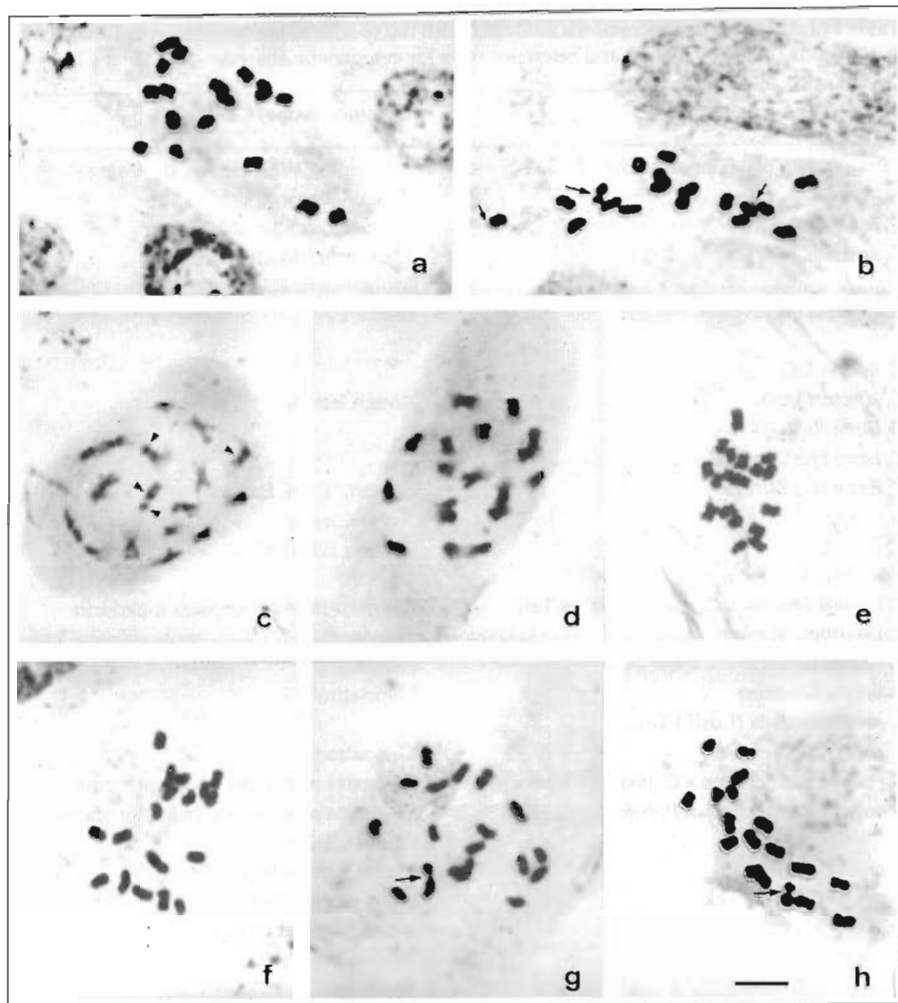


Figure 1 - Chromosomal variability in *Citrus* species. (a, b) Metaphases of *Citrus depressa* showing in a no secondary constrictions (SECs) and in b one proximal and two terminal SECs. (c, d, e) Prophase (c), prometaphase (d) and metaphase (e) chromosome complement of *C. deliciosa*. (f, g, h) Metaphase of *C. pennivesiculata* (f), *C. volkameriana* (g) and *Poncirus trifoliata* (h). Larger arrows, proximal SECs; smaller arrows, terminal SECs; arrowheads, heteropycnotic blocks. Bar in h represents 5 μm for all figures.

karyological features in this group. Although the number of observable SECs may vary from cell to cell, even in a stable karyotype, the highest number of SECs observed is generally stable (Sato *et al.*, 1980; Yonenaga-Yassuda *et al.*, 1983). In some cells, the SECs were not observed because the chromosomes were too condensed (Figure 1a,b). However, in a few cultivars, in spite of an ideal level of chromatin condensation, the SECs were not visible (Figure 1f). In our sample, considering only the maximum number and chromosome position of SECs observed, there was a small but significant variation between karyotypes.

On the other hand, the number of SECs may be overestimated due to the presence of weakly stained or distended regions associated with large blocks of terminal heterochromatin (Figure 1c-e and prophase in Figure 3a). Sharma and Bal (1957) analyzed 10 *Citrus* cultivars and described the occurrence of numerous secondary constrictions in all of them (six SECs in most of the cases), including in three cultivars of *C. aurantifolia*, whereas in this species we found only two SECs in each. These conflicting results may be due to the interpretation of prophase/prometaphase chromo-

somes. Since all citrus species present many terminal heterochromatic blocks (Liang, 1988; Wei *et al.*, 1988; Guerra, 1993), the prophase and prometaphase chromosomes may show, even with conventional staining, a pattern of weakly and deeply stained regions. Some of these regions can easily be misinterpreted as SECs and satellites. Since these authors presented only chromosome drawings we cannot compare our results with theirs. Furthermore, no other author has reported satellites in citrus chromosomes (see, e.g., Krug and Bacchi, 1943; Agarwal, 1987; Ito *et al.*, 1993).

The secondary constrictions observed here were located in proximal, subterminal or terminal position in chromosomes of different sizes. The highest number of SECs per karyotype was three, which may be less than the real number of rDNA sites. In general, the number of SECs is far smaller than the number of rDNA sites. In a sample of 33 cultivars of *Vigna unguiculata* karyotypically analyzed by Frahm-Leliveld (1965) the maximum number of SECs per karyotype observed was two, whereas 10 rDNA sites were conspicuously localized by *in situ* hybridization (Guerra *et al.*, 1996).

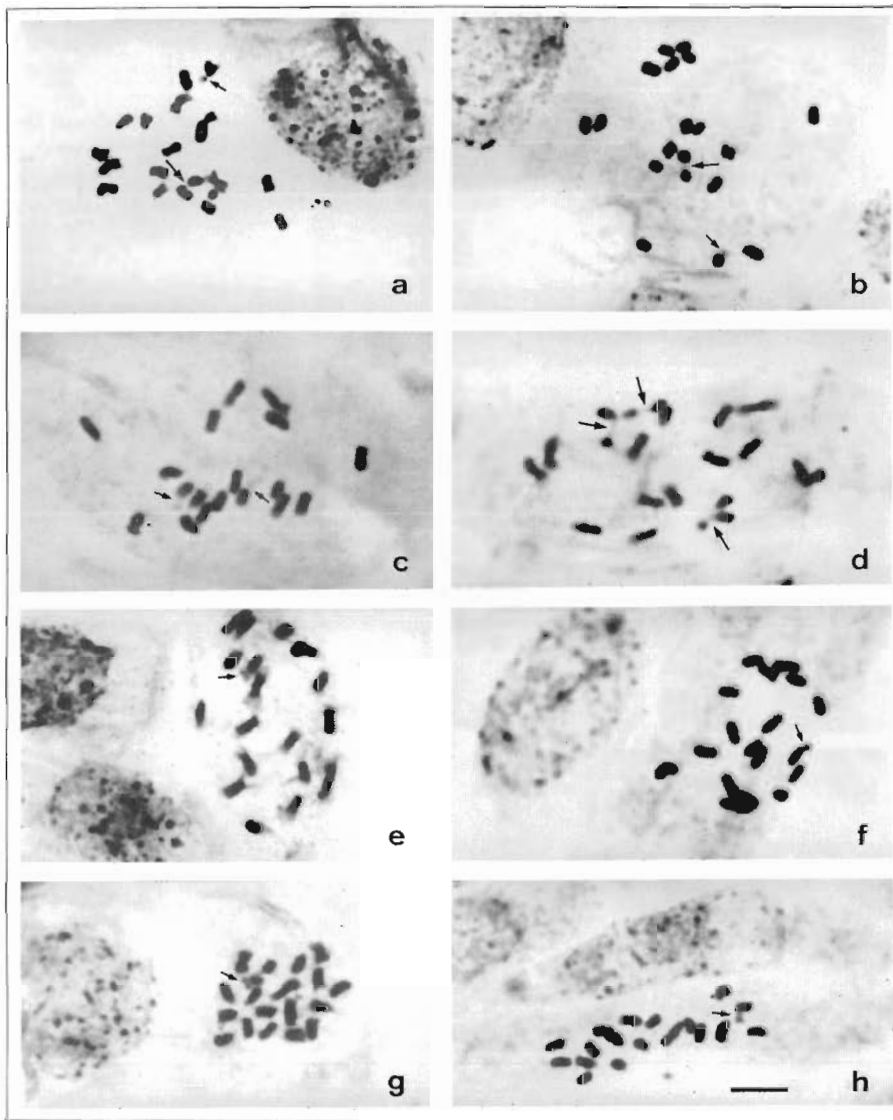


Figure 2 - Karyotype variability in number and position of secondary constrictions in some citrus accessions. (a, b, c, d) Chromosome complement of *Citrus keraji* (a), *C. reticulata* x *C. paradisi* (b), *C. sunki* (c), *C. longispina* (d). (e, f, g, h) Chromosome complement of *C. limon* cv. Cook Eureka (e), cv. Frost Eureka (f), cv. Eureka IPEACS (g), cv. Lisbon (h). Larger arrows, proximal SECs; smaller arrows, terminal SECs. Bar in h represents 5 μm for all figures.

In the karyotypes of six accessions only a single constriction was observed: in *Citrus volkameriana* (Figure 1g), *Poncirus trifoliata* (Figure 1h) and the hybrid *C. reshni* x *P. trifoliata*, a single proximal SEC in one of the largest chromosomes; in *C. reshni* and *C. sunki* x *P. trifoliata*, a single terminal satellite in a small-sized chromosome, and in *C. nobilis*, a subterminal SEC in a large chromosome.

Two SECs were observed in *C. aurantifolia*, *C. sinensis*, *C. limon*, *C. sunki*, *C. keraji* and the hybrid *C. reticulata* x *C. paradisi*. In the first three species more than one cultivar per species was analyzed. *C. keraji* (Figure 2a) and *C. reticulata* x *C. paradisi* (Figure 2b) presented a proximal constriction in a large-sized chromosome and a terminal SEC in a medium-sized one. *C. sunki* also had two SECs, but both were terminally located in a pair of medium size chromosomes, presumed to be homologues (Figure 2c).

Three SECs were observed in only two species. *C. longispina* presented a rare karyotype, with three of

the largest chromosomes bearing a proximal SEC. Two of them seemed to form a homozygous pair whereas there was no obvious homologue for the third, smaller one (Figure 2d). *C. depressa* had a proximal SEC in a single large chromosome and a terminal SEC in two smaller ones (Figure 1b).

C. sinensis was the species with the highest number of cultivars investigated. Twenty orange cultivars were analyzed and SECs were not identified in four of them: 'Trepadeira', 'Bahia', 'Pêra Vacinada' and 'Ruby'. In the remaining 16 cultivars, the highest number of SECs observed was two: a proximal SEC in a large chromosome and a subterminal SEC in a medium-sized one (Figure 3a, b, d-h). Rarely, the SECs were observed only in both presumed homologues of a large chromosome pair (Figure 3c) or in these two homologues and in the medium-sized chromosome.

From these data, we hypothesized that there are at least two rDNA sites in the chromosome complement of *C. sinensis*, one in the proximal region of a

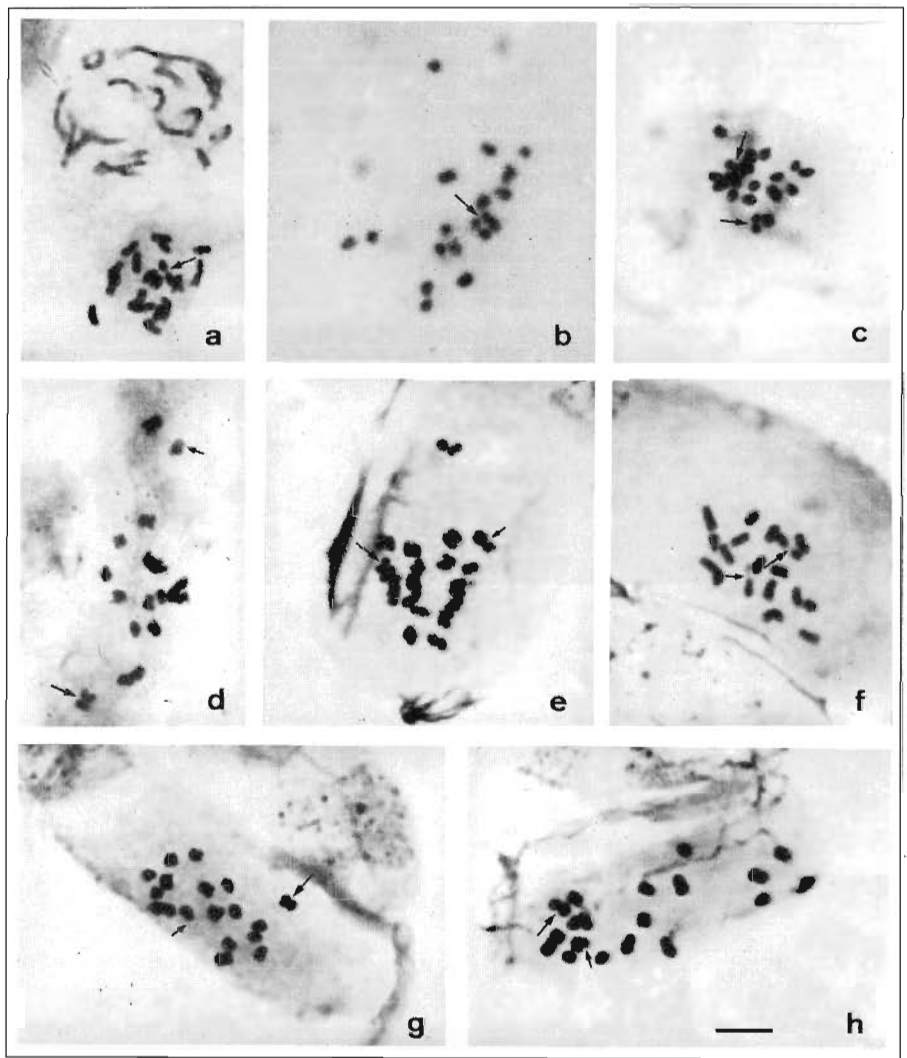


Figure 3 - Karyotype variability between *Citrus sinensis* cultivars. (a) 'Bahia Vale del Cauca'; (b) 'Comuna'; (c) 'Olivelands'; (d) 'Shamouti'; (e, f) 'Valencia', metaphases with different levels of chromosome condensation; (g) 'Parson Brown'; (h) 'Gardner'. Observe heteropycnotic blocks in prophase chromosomes (a). Larger arrows, proximal SECs; smaller arrows, terminal SECs. Bar in h represents 5 μ m for all figures.

large metacentric/submetacentric chromosome and another terminally located in a medium-sized submetacentric/acrocentric chromosome. However, there may be three rDNA sites, with the proximal one being present in both homologues of the pair. One terminal and two proximal rDNA sites have been detected by *in situ* hybridization in another *C. sinensis* cultivar (Matsuyama *et al.*, 1996). Their activation seemed to be controlled by a special kind of hierarchy, where the proximal SEC of the large chromosome is commonly activated and the terminal SEC of the smaller one is only activated as an additional resource. The notable feature in this case is that only one SEC of each chromosome pair is preferentially activated, pointing to a form of structural heterozygosity and giving additional support to the putative hybrid origin of *C. sinensis* and many other *Citrus* species (Swingle and Reece, 1967).

Further evidence of dominance between heterologous rDNA sites was observed in the hybrid *C. reshni* x *P. trifoliata*, where the proximal SEC of the large chromosome of *P. trifoliata* was visible but not the

terminal SEC of *C. reshni*. A similar hierarchy has been described in many other species and hybrids (Lacadena *et al.*, 1988).

In two other species, *C. aurantifolia* and *C. limon*, more than one cultivar was analyzed. In *C. aurantifolia* two cultivars were investigated. In one of them ('Malay Lemon') two proximal SECs were observed whereas in the other ('Galego') no SEC was found, probably due to over-condensed chromosomes. In each of four cultivars of *C. limon* (Figure 2e-h) only a subterminal SEC was observed, in either one or both chromosomes of the largest pair, together with either one or two small satellites respectively in each.

In the remaining varieties no SEC was observed, although all cells should contain at least one active NOR during interphase and therefore a SEC in metaphase. Probably in some of them the SEC was very short or terminally located and hence not detected (Moscone *et al.*, 1995).

The SECs are probably the most variable karyotype feature in citrus as well as in most eukaryotes. The

instability of SECs is commonly attributed to their activity in the previous interphase (Miller *et al.*, 1976; Sato *et al.*, 1980; Yonenaga-Yassuda *et al.*, 1983), although it may also indicate true structural polymorphism (Schubert and Wobus, 1985). The high frequency of SECs in heterozygosity, especially in the case of *C. keraji*, *C. sinensis* and *C. reticulata* x *C. paradisi*, where two different pairs were heterozygous, suggests that structural variation may be involved in this kind of heterozygosity. Comparing the karyotype of these 20 species, it is evident that at least some accessions have the major rDNA sites fixed in different positions in the chromosome complement. In this case, the number and position of SECs in *Citrus* cultivars might be a consistent karyotype feature.

No other structural type of karyotype variation was clearly identified in the cultivars analyzed here. Differences in chromosome size or centromeric index among metaphases of different cultivars (see Figure 1b,e or Figure 3c,h) were not consistently observed. Indeed, such variations are very difficult to establish for small chromosomes, except when they are very pronounced or a quantitative analysis has been carried out. Polyploids, which have often been reported (Frost, 1925b; Esen *et al.*, 1979; Wakana *et al.*, 1981), were found at a very low frequency (one in about 500 seedlings) and may be attributed to the genetic characteristics of the cultivars (see, Iwamasa and Nito, 1988). In *Citrus* species CMA banding analysis (Guerra, 1993) may be more effective to detect structural chromosome variation than conventional staining methods. However, particularly in the case of change in number and position of SECs, which has not been detected by banding methods, conventional staining seems to be more useful.

ACKNOWLEDGMENTS

The authors are very grateful to Dr. Simon Mayo (Kew Gardens, England) for correcting the English manuscript. This research was supported by CNPq and FACEPE.

RESUMO

Foram analisados 51 acessos de citros do banco ativo de germoplasma do Centro Nacional de Pesquisa de Mandioca e Fruticultura Tropical (CNPq/EMBRAPA), em Cruz das Almas, Bahia. A amostra incluiu representantes de 20 espécies de *Citrus*, uma de *Poncirus* e sete híbridos interespecíficos. Todos os acessos mostraram $2n = 18$ sem nenhum indício de alteração numérica. A característica cariotípica mais claramente variável foi o número e posição das constrições secundárias (SECs). Em 19 acessos não foi possível localizar as SECs devido principalmente ao grau de

condensação cromossômica. Nos demais acessos elas variaram em número de um a três e estavam localizadas na região proximal de um dos três maiores pares cromossômicos, na região terminal-subterminal de um dos pares de tamanho médio ou, mais raramente, em um dos cromossomos maiores. Em apenas uns poucos casos essas constrições foram observadas simultaneamente em ambos os homólogos de um par cromossômico. A alta variabilidade dessa característica cariotípica pode em parte ser devida à ativação diferencial dessa região na interfase anterior, mas pode também estar relacionada à grande heterozigosidade estrutural cromossômica conhecida em espécies de citros.

REFERENCES

- Agarwal, P.K. (1987). Karyotype of *Citrus tamurana* (Tan.). *Chrom. Inf. Serv.* 42: 3-5.
- Cameron, J.W. and Soost, R.K. (1969). Characters of new populations of *Citrus* polyploids, and the relation between tetraploidy in the pollen parent and hybrid tetraploid progeny. *Proc. First Int. Citrus Symp.* 1: 199-205.
- Esen, A. and Soost, R.K. (1971). Unexpected triploids in *Citrus*: Their origin, identification, and possible use. *J. Hered.* 62: 329-333.
- Esen, A., Soost, R.K. and Geraci, G. (1979). Genetic evidence for the origin of diploid megagametophytes in *Citrus*. *J. Hered.* 70: 5-8.
- Favarger, C. (1978). Philosophie des comptages de chromosomes. *Taxon* 27: 441-448.
- Frahm-Leliveld, J.A. (1965). Cytological data on some wild tropical *Vigna* species and cultivars from cowpea and asparagus bean. *Euphytica* 14: 251-270.
- Frost, H.B. (1925a). The chromosomes of *Citrus*. *J. Washington Acad. Sci.* 15: 1-3.
- Frost, H.B. (1925b). Tetraploidy in *Citrus*. *Proc. Natl. Acad. Sci. USA* 2: 535-537.
- Guerra, M. (1983). O uso do corante Giemsa na citogenética vegetal: comparação entre a coloração simples e o bandeamento. *Ciênc. Cult.* 35: 190-193.
- Guerra, M. (1987). Cytogenetics of Rutaceae IV. Structure and systematic significance of interphase nuclei. *Cytologia* 52: 213-222.
- Guerra, M. (1993). Cytogenetics of Rutaceae. V. High chromosomal variability in *Citrus* species revealed by CMA/DAPI staining. *Heredity* 71: 234-241.
- Guerra, M., Kenton, A. and Bennett, M.D. (1996). rDNA sites in mitotic and polytene chromosomes of *Vigna unguiculata* (L.) Walp. and *Phaseolus coccineus* L. revealed by *in situ* hybridization. *Ann. Bot.* 78: 157-161.
- Hutchinson, D.J. and Barrett, H.C. (1981). Tetraploid frequency in nucellar seedlings from single trees of Carrizo and Troyer *Citrus* hybrids. *Proc. Int. Soc. Citric.* 1: 27-29.
- Ito, Y., Omura, M. and Nesumi, H. (1993). Improvement of chromosome observation methods for *Citrus*. In: *Techniques on Gene Diagnosis and Breeding in Fruit Trees* (Hayashi, T., Omura, M. and Scott, N.S., eds.). FTRS, Tsukuba, pp. 31-38.

- Iwamasa, M. and Nito, N.** (1988). Cytogenetics and the evolution of modern cultivated *Citrus*. *Proceedings of the sixth International Citrus Congress* (Goren, R. and Mendel, K., eds.). Margraf Scientific Books, Weikersheim, pp. 265-275.
- Krug, C.A.** (1943). Chromosome numbers in the subfamily Aurantioideae with special reference to the genus *Citrus*. *Bot. Gaz.* 104: 602-611.
- Krug, C.A. and Bacchi, O.** (1943). Triploid varieties of *Citrus*. *J. Hered.* 34: 277-283.
- Lacadena, J.R., Cermeno, M.C., Orellana, J. and Santos, J.L.** (1988). Nucleolar competition in Triticeae. In: *Kew Chromosome Conference III* (Brandham, P., ed.). HMSO, London, pp. 151-165.
- Liang, G.L.** (1988). Studies on the Giemsa C-banding patterns of some *Citrus* and its related genera. *Acta Genet. Sin.* 15: 409-416.
- Lin, B.-Y.** (1987). Número cromossômico de exemplares da família Bromeliaceae. *Rev. Bras. Frutic.* 9: 49-55.
- Matsuyama, T., Akihama, T., Ito, Y., Omura, M. and Fukui, K.** (1996). Characterization of heterochromatic regions in 'Trovita' orange (*Citrus sinensis* Osbeck) chromosomes by the fluorescent staining and FISH methods. *Genome* 39: 941-945.
- Miller, D.A., Dev, V.G., Tantravahi, R. and Miller, O.J.** (1976). Suppression of human nucleolus organizer activity in mouse-human somatic hybrid cells. *Expl. Cell Res.* 101: 235-243.
- Moscone, E.A., Loidl, J., Ehrendorfer, F. and Hunziker, A.T.** (1995). Analysis of active nucleolus organizing regions in *Capsicum* (Solanaceae) by silver staining. *Am. J. Bot.* 82: 276-287.
- Naithani, S.P. and Raghuvanshi, S.S.** (1963). Cytogenetical studies in *Citrus*. Part I. *Genetica* 33: 301-312.
- Oiyama, I., Okudai, N. and Takahara, T.** (1981). Ploidy levels of seedlings obtained from 2x X 4x crosses in *Citrus*. *Proc. Int. Soc. Citric.* 1: 32-34.
- Raghuvanshi, S.S.** (1962a). Cytogenetical studies in genus *Citrus*. *Citrus assamensis*. *Caryologia* 15: 143-149.
- Raghuvanshi, S.S.** (1962b). Cytogenetical studies in genus *Citrus*. IV. Evolution in genus *Citrus*. *Cytologia* 27: 172-188.
- Sato, S., Ohta, S. and Kuroki, Y.** (1980). Heteromorphic appearance of acrocentric nucleolus organizer regions in *Nothoscordum fragrans*. *Cytologia* 45: 87-96.
- Schubert, I. and Wobus, U.** (1985). *In situ* hybridization confirms jumping nucleolar organizing regions in *Allium*. *Chromosoma* 92: 143-148.
- Sellito-Boaventura, Y.M. and Pio, R.M.** (1989). Análise citogenética em três cultivares de laranja doce (*Citrus sinensis* (L.) Osbeck). *Rev. Bras. Genet.* 12: 117-126.
- Sharma, A.K. and Bal, A.K.** (1957). Chromosome studies in *Citrus*. I. *Agron. Lusit.* 19: 101-126.
- Swingle, W.T. and Reece, P.C.** (1967). The botany of *Citrus* and its wild relatives. In: *The Citrus Industry, Vol. I., History, World Distribution, Botany and Varieties* (Reuter, W., Weber, H.J. and Batchelor, L.D., eds.). University of California Press, Berkeley, pp. 190-430.
- Tanaka, T.** (1954). Species problem in *Citrus* (Revisio aurantiacearum IX). *Japanese Soc. Promotion Sci.* Ueno, Tokio, pp. 152.
- Wakana, A., Iwamasa, M. and Uemoto, S.** (1981). Seed development in relation to ploidy of zygotic embryo and endosperm in polyembryonic *Citrus*. *Proc. Int. Soc. Citric.* 1: 35-39.
- Wei, W.N., Chen, Y.C., Li, R.T.L. and Duan, I.C.** (1988). Studies on the evolution of *Citrus* based on karyotype and Giemsa patterns. *Acta Hort. Sin.* 15: 222-228.
- Yonenaga-Yassuda, Y., Assis, M. de F.L., Kasahara, S., L'Abbate, M. and Souza, M.J.** (1983). Nucleolar organizer regions in *Akodon arviculoides* (Cricetidae, Rodentia): evidence for the activity of rDNA genes in both X chromosomes of females. *Cytogenet. Cell Genet.* 35: 143-147.

(Received May 21, 1996)