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Apresentação

A hybridization program based on its own Active Germplasm Bank, in order to obtain new citrus varieties, better adapted to tropical conditions, has started at Embrapa – National Cassava & Fruits Research Center (CNPMF), in September 1988. As immediate objectives, the program aims at selection of genotypes, particularly rootstocks, tolerant to drought conditions and aluminum toxicity, resistant to diseases such as *Phythophthora* root rot and Citrus Tristeza Virus - CTV, and adapted to high density tree population, to increasing the longevity of the citrus orchards.

A multidisciplinary research group is involved in these studies and several research activities and results obtained are discussed in this paper.

Mario Augusto Pinto da Cunha
General Director
Embrapa Cassava&Fruits

Summary

Citrus Breeding Program at *Embrapa Cassava & Fruits*: development of hybrids

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Abstract – In order to obtain new citrus varieties, better adapted to tropical conditions, the Embrapa – National Cassava & Fruits Research Center (CNPMF), as known as ***Embrapa Cassava&Fruits***, has started in September 1988 a hybridization program based on its own Active Germplasm Bank, which shows a great genetic variability, represented for about 700 accesses, comprising several *Citrus* species and varieties, besides close relative genera, among which are included *Poncirus*, *Fortunella*, *Microcitrus*, *Eremocitrus* and *Severinia*, among others of smaller importance, under the point of view of genetic improvement.The program was set up due to the relatively low longevity of the citrus orchards, whose life span is around 15 to 18 years in the the main producing areas of São Paulo State, that represents about 75% of the Brazilian production, and from 12 to 15 years in the North and Northeast regions of the country. As immediate objectives, the program aims at selection of genotypes, particularly rootstocks, tolerant to drought conditions and aluminum toxicity, resistant to diseases such as *Phytophthora* root rot and Citrus Tristeza Virus - CTV, and adapted to high density tree population.

A multidisciplinary research group is involved in these studies and several research activities are underway, mainly: a) hybridization, including varieties of high agronomic value and/or adaptive potential to grow in abiotic and biotic stresses; b) identification of hybrid embryos and early identification of zygotic seedlings by analysis of foliar morphology, isoenzymes, polymorphic DNA segments and chromosome banding; c) studies concerned to in vitro cultivation of citrus embryos, as well as to the complement of the embryogenesis of zygotic embryos starting from early stages, avoiding or restricting the presence of nucellar embryos, particularly in polyembryonic varieties, used as female parents in artificial hybridizations; d) identification of promising parents regarding to obtaining new hybrid varieties, rootstocks and scions; e) definition of methodologies for early selection of hybrids tolerant to drought and aluminum toxicity, as well as resistant to *Phytophthora* root rot and CTV; f) establishment of methodologies for early flowering induction, aiming at shortening the pre-reproductive period of selected hybrids; g) development of somatic hybrids by protoplast fusion.

Index terms: hybridization, polyembryony, drought tolerance, tolerance to aluminum toxicity, *Phytophthora* root rot, Citrus Tristeza Virus, tissue culture, molecular markers, protoplast fusion, chromosome banding, early flowering induction.

Programa de Melhoramento Genético de Citros da *Embrapa Mandioca e Fruticultura:* obtenção de híbridos

Resumo - Visando a obtenção de novas variedades cítricas, melhor adaptadas aos trópicos, a Embrapa – Centro Nacional de Pesquisa de Mandioca e Fruticultura Tropical (CNPMF), também denominada ***Embrapa Mandioca e Fruticultura***, iniciou em setembro de 1988 um programa de hibridações, tendo como base seu Banco Ativo de Germoplasma de Citros. Dotado de grande variabilidade genética, o referido banco de germoplasma possui cerca de 700 acessos, compreendendo diversas espécies e variedades de *Citrus*, além de gêneros afins a este, dentre os quais incluem-se *Poncirus*, *Fortunella*, *Microcitrus*, *Eremocitrus* e *Severinia*, entre outros de menor importância, sob o ponto de vista de seu uso em melhoramento genético. Esta iniciativa teve como estímulo a relativamente baixa longevidade dos pomares brasileiros, cuja vida útil está em torno de 15 a 18 anos nas principais regiões produtoras do Estado de São Paulo, onde se concentram cerca de 75% da citricultura nacional, e de 12 a 15 anos no Norte e Nordeste do País. Como objetivos imediatos, o referido programa busca a seleção de genótipos, particularmente porta-enxertos, tolerantes à seca e ao alumínio, resistentes à gomose de *Phytophthora* e ao complexo do Vírus da Tristeza dos Citros – CTV (Citrus Tristeza Virus), além de adaptados a altas densidades populacionais. Contando com o apoio de uma equipe multidisciplinar e multiinstitucional, diversas ações de pesquisa encontram-se em curso, destacando-se: a) hibridações envolvendo variedades de comprovado valor agronômico e/ou de adaptação a condições de estresse causadas por fatores bióticos e abióticos; b) reconhecimento de embriões de natureza híbrida e identificação precoce de *seedlings* zigóticos, mediante análises de morfologia foliar, izoenzimas, segmentos polimórficos de DNA e bandeamento cromossômico; c) estudos dirigidos ao cultivo in vitro de embriões de citros, bem como ao complemento da embriogênese de embriões zigóticos a partir de estádios iniciais de desenvolvimento, evitando ou restringindo a presença de embriões de origem nucelar, particularmente em variedades altamente poliembriônicas, utilizadas como parentais femininos em hibridações controladas; d) identificação de parentais promissores no

tocante à obtenção de variedades híbridas com valor comercial, copas e porta-enxertos; e) definição de metodologias que permitam a seleção precoce de híbridos tolerantes à seca e ao alumínio, bem como resistentes à gomose de *Phytophthora* e ao CTV; f) estabelecimento de metodologias dirigidas à indução precoce de florescimento, no intuito de encurtar o período pré-reprodutivo de híbridos selecionados em trabalhos preliminares de avaliação; g) obtenção de híbridos somáticos mediante fusão de protoplastos.

Termos para indexação: hibridação, poliembrionia, tolerância à seca, tolerância ao alumínio, gomose de *Phytophthora*, vírus da tristeza dos citros, cultura de tecidos, fusão de protoplastos, marcadores moleculares, bandeamento cromossômico, indução de florescimento.

Introduction

The great genetic variability presented by *Citrus* (L.) and their close related genera can be useful in improvement programs based in hybridization, particularly those aiming at obtaining new rootstocks adapted to high density tree population and adverse environment such as abiotic and biotic stress. In that sense, the genera *Microcitrus* (Swing.) and *Eremocitrus* (Swing.), found in wild form almost exclusively in Australia, constitute important sources of genes for adaptation to stressful environment. *Eremocitrus* is strongly xerophytic, being able to grow in semi-arids regions, in soils with little or any nitrogen, besides resisting to relatively high salt concentration in soil solution, while *Microcitrus* is semi-xerophytic and could support long drought periods. These genera, as well as *Poncirus* (Raf.) and *Fortunella* (Swing.), also show great cold tolerance, presenting adaptation to habitats where no species of *Citrus* could survive. *Severinia* (Ten.), more primitive gender, supports boron levels sufficiently high to eliminate species of *Citrus*, being surprising the fact that under such conditions the roots of *Severinia* uptake and transport very low amounts of that element, allowing the establishment of healthy scions of *Citrus* commercial varieties, even if was used scions quite sensitive to boron, like as lemons [*C. limon* (L.) Burm. f.]. *Citropsis gilletiana* (Swing. & M. Kell.), native wild *Citrus* relative from the Republic of Congo, used as rootstock in that country, it is immune to the attack of a trunk borer weevil (coleoptera: curculionidiae) whose larvae dig the plant collar of *Citrus*, besides being resistant to the fungal disease *Phytophthora* root rot (Swingle, 1967). Additionally, *Poncirus*, *Fortunella*, *Microcitrus*, *Eremocitrus* and *Severinia*, as well as *Citropsis* [(Engl.) Swing. & M. Kell.], show great resistance to *Phytophthora* root rot. Species such as *Severinia buxifolia* [(Poir.) Ten.] and *Eremocitrus glauca* [(Lindl.) Swing.] have shown tolerance to soil salinity. *Citropsis gilletiana* presents resistance to the burrowing nematode. *Poncirus trifoliata* [(L.) Raf.] and *Severinia buxifolia* are considered resistant to the common complex of Citrus Tristeza Virus – CTV. *Eremocitrus glauca* can be used in improvement programs aiming at obtaining rootstocks adapted to sandy soils. *Microcitrus australis* [(Planch.) Swing.] and *M. australasica* [(F. Muell.) Swing.] are well adapted to areas related to heavy rain conditions and low fertility soils (Hearn *et al.*, 1974). *Severinia buxifolia*, as well as several seletions of *Poncirus trifoliata*, show resistance to the citrus nematode

[*Tylenchulus semipenetrans* (Cobb)] (Hutchison & O'Bannon, 1972). *Poncirus trifoliata* presents as source of tolerance to flooding soils (Yelenosky *et al.*, 1974). *Citropsis*, *Eremocitrus*, *Microcitrus* and *Clymenia* (Swing.) can be used in genetic improvement programs aiming at obtaining rootstocks that induce tree dwarfing, in order to provide commercial groves with high density tree population (Castle, 1979).

According to these above-mentioned possibilities, in order to obtain new citrus varieties, better adapted to tropical conditions, the Embrapa - National Research Center for Cassava and Tropical Fruit Crops (CNPMF), as known as ***Embrapa Cassava&Fruits***, has started in September 1988 a hybridization program based on its own Active Germplasm Bank, which shows a great genetic variability, represented for about 700 accesses, comprising several *Citrus* L. species and varieties, besides close relative genera, among which are included *Poncirus* Raf., *Fortunella* Swing., *Microcitrus* Swing., *Eremocitrus* Swing. and *Severinia* Ten., among others of smaller importance, under the point of view of genetic improvement. The program was set up due to the relatively low longevity of the citrus orchards, whose life span is around 15 to 18 years in the the main producing areas of São Paulo State, that represents about 75% of the Brazilian production, and from 12 to 15 years in the North and Northeast regions of the country.

As immediate objectives, the program aims at selection of genotypes, particularly rootstocks, tolerant to drought conditions and aluminum toxicity, resistant to diseases such as *Phytophthora* root rot and the complex of Citrus Tristeza Virus - CTV, and adapted to high density tree population.

Hybridizations and Selection of Promising Individuals

Thousand of hybrids were obtained from crossings involving *Citrus* species and interspecific hybrids, besides related genera and intergeneric hybrids, including lemons (several especies), sweet oranges [*C. sinensis* (L.) Osb.] and sour oranges (*C. aurantium* L.), tangerines/mandarins (several species), *Poncirus trifoliata* and their hybrids (APPENDIX, Table 1). Field evaluations, aiming at selection of new rootstocks, have involving seedlings and rootstocks grafted with scions of commercial importance, mainly 'Pera' sweet *orange* (*C. sinensis*), due to its importance for the Brazilian citriculture. Regarding to hybrids obtained to use as scions, these material have been preliminarly evaluated in combination with rootstocks that induce their growth and fruit production, such as 'Volkamer' lemon (*C. volkameriana* Ten. et Pasq.), in order to allow the identification of individuals with commercial importance in a shortest period of time.

Criteria for parent selection are based on agronomic value and/or adaptable potential to adverse conditions such as tolerance to drought and aluminum toxicity and resistance to diseases. Among related *Citrus* genera, *Poncirus*, *Microcitrus* and *Eremocitrus* are most important due to their relative potential to provide new dwarfing rootstocks (Castle, 1979) and resistance to *Phytopthora* root rot (Hearn et al., 1974), fungal disease that has caused serious losses in Brazilian orchards. *Microcitrus* and *Eremocitrus* may also allow development of rootstocks adapted to extended water stress periods (Swingle, 1967), as above-mentioned.

The parent choice also obey a dynamic process based on information provided by the Citrus Breeding Program of ***Embrapa Cassava&Fruits*** – CBP. This include data regarding to crossing combination capacity in relation to fruit set, after controlled pollination, polyembryony level (preference is given to female monoembryonic parent or material that shows polyembryony level between low to moderate, in way to avoid or to reduce the presence of individuals form nucellar origin) and frequency of promising hybrids that present the before mentioned desirable traits.

In this sense, 'Robinson' tangerine-tangelo ['Clementine' mandarin *C. clementina* Hort. ex Tan. x 'Orlando' tangelo ('Duncan' grapefruit *C. paradisi* Macf. x 'Dancy' tangerine *C. tangerina* Hort. ex Tan.)], 'Sunki' mandarin (*C. sunki* Hort. ex Tan.), 'Rangpur' lime (*C. limonia* Osb.) and 'Dweet' tangor ('Mediterranean' sweet orange *C. sinensis* x 'Dancy' tangerine) are recommended for crossing as female parents since they show high fruit set rate in controlled crossings (Soares Filho *et al.*, 1995a), and, respectively, none, low and moderate (latter two varieties) polyembryony (Soares Filho *et al.*, 1995b). ‘Clementine’ mandarin, due to its monoembryonic condition, and ‘Double Calice’ sour orange, that presents relatively high percentage of fruit set rate in controlled hybridizations (Medrado, 1998; Soares Filho *et al.*, 2002), also can be recommended as female parents.

Regarding to the frequency of zygotic individuals in controlled crossings, hybridizations were carried out by the CBP, involving monoembryonic female parents, e. g. ‘Clementine’ mandarin, only resulting in hybrid individuals, as expected. Hybrid frequency is in inverse proportion to the polyembryonic level when female parents are polyembryonic (Vásquez Araujo, 1991; Soares Filho *et al.*, 1994; Moreira, 1996; Medrado, 1998; Soares Filho *et al.*, 2000 e 2002). Among the female parents used by CBP, ‘Sunki’ mandarin deserves special attention due to its relatively high frequency of hybrid formation in controlled crossings, approximately 50% (Table 1).

Table 1. Polyembryony percentage (% P) of several *Citrus* varieties and percentage of zygotic seedlings (% ZS), obtained from controlled crossings between these varieties, used as female parents, and *Poncirus trifoliata* (L.) Raf. and hybrids of this species. Citrus Breeding Program of ***Embrapa Cassava & Fruits***, Cruz das Almas, Bahia, Brazil.

Varieties	**% P**	**% ZS**
Sunki mandarin (*Citrus sunki* Hort. ex Tan.)[1]	15.9	53.6
Sunki mandarin[2]	8.3	51.1
Rangpur lime (*C. limonia* Osb.)[3]	56.8	18.6
Rangpur lime[1]	35.2	18.6
Volkamer lemon (*C. volkameriana* Ten. *et* Pasq.)[1]	58.6	14.2
Volkamer lemon[2]	79.2	16.3
Sour orange (*C. aurantium* L.)[2]	76.4	17.4
Pêra sweet orange [*C. sinensis* (L.) Osb.][3]	90.7	6.0
Cleopatra mandarin (*C. reshni* Hort. ex Tan.)[3]	98.8	5.2

[1]Source: Moreira (1996), Soares Filho *et al.* (2000);
[2]Source: Medrado (1998), Soares Filho *et al.* (2002);
[3]Source: Vásquez Araujo (1991), Soares Filho *et al.* (1994).

In additon, greenhouse and field observations in hybrid seedlings within progenies obtained from crossings involving 'Sunki' mandarin and sour orange as female parent, indicate, due to their likeness and relative uniformity, that such varieties should present relatively high levels of homozygous, qualifying them as important parents in the development of new rootstocks, what is reinforced by their excellent horticultural characteristics. Disadvantages are the susceptibility to *Phytophthora* root rot, presented by 'Sunki' mandarin, and to the CTV, showed by sour orange. Both, however, are complementary in what concerns to overcome these deficiencies, with possible formation of resistant hybrids to these diseases that cause considerable damages to the citrus groves.

Regarding to the vigour of hybrid seedlings obtained from the CBP, considering early stages of development, results show that controlled crossings between the female parents 'Rangpur' lime (Soares Filho *et al.*, 1991) and 'Sunki' mandarin (Soares Filho *et al.*, 1999), using *P. trifoliata* and its hybrids as male parents, most of individuals identified as zygotic presented similar or superior vigour to others from nucellar origin. These results suggest an excellent relative potential to obtain promising hybrids from crossings between *Citrus* and *Poncirus*, which can develop new rootstocks varieties.

Field evaluations, comprising more than 400 hybrid seedlings obtained from controlled pollinations, allowed identification of approximately 60 individuals that show improvement in vigour, from regular to good, better phytosanitary behaviour and less sensitivity to water stress. In this group, among those in the fruit set stage, evaluations related to polyembryony level and average number of seeds per fruit allowed to identify 19 promising hybrids. Positive characteristics were considered the suitable level of polyembryony and amount of appropriate seeds in order to be used as rootstocks. They are: LVK ('Volkamer' lemon) x LCR ('Rangpur' lime) – 010, HTR (trifoliate hybrid) – 069, HTR – 112, HTR – 116, HTR – 166 (moderate to low polyembryony, ranging from 25% to 40%); TSK ('Sunki' mandarin) x [(TR (*Poncirus trifoliata*) x LCR)] – 001, LVK x LCR – 038, HTR – 144 (moderate polyembryony, ranging from 50% to 65%); TSK x (TR x LCR) – 010, TSK x CTTR ['Troyer' citrange (*C. sinensis* x *P. trifoliata*)] – 002, CTYM ('Yuma' citrange) x LCR – 005, HTR –

051 (high polyembryony, ranging from 75% to 80 %); TSK x CTTR – 017, CTYM x LCR – 003, LCR x TR – 001, HTR – 010, HTR – 053, HTR – 070 and HTR – 127 (very high polyembryony, above 90%).

Two years after transplanting in the field, under Table Coastlands conditions, 'Pêra' sweet orange scions grafted in LVK x LCR – 038, CTYM x LCR – 005, HTR – 051, HTR – 112, HTR – 116 and HTR – 127 hybrids, as well as in 'Rangpur' lime, 'Volkamer' lemon, 'Cleopatra' mandarin (*C. reshni* Hort. ex Tan.), 'Troyer' and 'Rusk' citranges (*C. sinensis* x *P. trifoliata*) and 'Swingle' citrumelo (*C. paradisi* x *P. trifoliata*), showed more vigorous plants (plant height and stem diameter 10 cm above and below grafting) when the rootstocks were these lemons and citranges. Among the rootstock hybrids obtained in CBP, LVK x LCR - 038, HTR - 051, HTR - 112 and HTR – 116 showed similar behaviour to 'Cleopatra' mandarin. Considering these preliminary results, 'Swingle' citrumelo and the trifoliate hybrids CTYM x LCR - 005 and HTR – 127, although have presented similar values for the 'Cleopatra' mandarin, regarding to vigour characters, were more related to less vigorous scion/ rootstock combinations. The inferior behaviour of 'Swingle' citrumelo was expected due to its graft incompatibility with 'Pêra' sweet orange. It has to be pointed out the interest for rootstock selections that determine plant dwarfing without lost in fruit production, to make possible higher tree population densities.

Identification of Hybrids: Embryos and Seedlings

Analysis of morphologic characters

Results obtained by CBP indicate that embryo size can be used as auxiliary tool in the identification of sexual embryos, because there is a tendency of the zygotic embryos be found among those of big size, being this situation so much more evident as minor is the polyembryony degree, according to Table 2. There is an inverse relationship between polyembryony percentage and big size zygotic embryos.

Table 2. Polyembryony percentage (% P) and distribution of zygotic embryos (number and percentage) within size classes in several citrus varieties. Citrus Breeding Program of ***Embrapa Cassava&Fruits***, Cruz das Almas, Bahia, Brazil.

Var.[1]	% P	Embryo size classes[2]							
		B		M		S		VS	
		Nº	%	Nº	%	Nº	%	Nº	%
TSK[3]	15.9	244	96.8	6	2.4	2	0.8	-	-
LCR[3]	35.2	37	94.9	2	5.1	-	-	-	-
LCR[4]	56.8	323	86.8	27	7.3	18	4.8	4	1.1
LVK[3]	58.6	43	82.7	7	13.5	2	3.8	-	-
LPE[4]	90.7	33	46.5	26	36.6	11	15.5	1	1.4
CLEO[4]	98.8	49	50.0	9	9.2	26	26.5	14	14.3

[1]Varieties: TSK – 'Sunki' mandarin (*Citrus sunki* Hort. ex Tan.), LCR – 'Rangpur' lime (*C. limonia* Osb.), LVK – 'Volkamer' lemon (*C. volkameriana* Ten. *et* Pasq.), LPE – 'Pêra' sweet orange [*C. sinensis* (L.) Osb.], CLEO – 'Cleopatra' mandarin (*C. reshni* Hort. ex Tan.);
[2]B – big (≥ 5.0 mm), M – medium (3.0 mm to 4.9 mm), S – small (1.0 mm to 2.9 mm), VS – very small (< 1.0 mm);
[3]Source: Moreira (1996), Soares Filho *et al.* (2000);
[4]Source: Vásquez Araujo (1991), Soares Filho *et al.* (1994).

Cotyledon color is another character that can be employee in the identification of embryos of hybrid nature, in reason of influences of the pollinizer on its manifestation (metaxeny effect). It is important to observe, in controlled crossings, the use of parents with coloration of contrasting cotyledons amongst themselves (Vásquez Araujo, 1991; Vásquez Araujo *et al.*, 1994). For example: female parent whose seeds have embryos with white cotyledons, in which can be included the sweet oranges, sour oranges and grapefruits, crossed with male parent, whose seeds have green cotyledons, situation common to a lot of mandarins, like 'Cleopatra' and 'Sunki', they will give formation to zygotic embryos with greenish cotyledons, while those of nucellar origin will present white cotyledons.

In relation to the position of the embryo in the seed, studies carried out by CBP showed that both zygotic and nucellar embryos present strong tendency to be located in the micropylar area, indicating, therefore, that this character does not facilitate the recognition of the hybrid embryos (Vásquez Araujo, 1991; Soares Filho *et al.*, 1994; Moreira, 1996).

For identification of hybrid seedlings from young stages of development, observations of the leaf lamina morphology have been used predominantly by CBP, giving emphasis to trifoliate morphology, dominant, present in *P. trifoliata* and its hybrids.

Isozymes analyses

Isozymes analyses were used to identify hybrid seedlings obtained by CBP. Due to their costs relatively low, the system glutamate-oxaloacetate-transaminase (GOT) it was quite used (Souza Jr. *et al.*, 1993). Considering the limitations of that system for identifying zygotic seedlings in many crossings of interest, other isozymes systems are being appraised, for example, leucine-amino-peptidase (LEP), fosfoglucose-isomerase (PGI) and peroxidase (PRX).

Analyses of polymorphic DNA

Analyses of polymorphic DNA segments are in process, standing out the RAPD (Random Amplified Polymorphic DNA) due its better feasibility to routine activities and less demanding in equipments. The advantages of this technique are the speed, the limitless number of existent markers, the possibility for automation, no interference of environmental factors, among others, what turns it excellent for works that involve a great number of analyses. Besides, the analyses of polymorphic DNA segments have the advantage, in relation to those based on isozymes markers, of allowing the identification of zygotic seedlings even within intraespefic hybridizations. The isozymes, however, will be considered, whenever possible, in reason of their high simplicity of application and low costs.

Analyses of chromosomal banding

Analyses of chromosomal banding accomplished in different mandarin species (C. *reshni*, *C. reticulata* Blanco and *C. nobilis* Lour.), using fluorchromes CMA/DAPI (Santos *et al.*, 1993), as well as in lemons [‘Siciliano’ *C. limon* (L.) Burm. f., ‘Volkamer’, ‘Rough’ *C. jambhiri* Lush. and ‘Ponderosa’ (probable natural hybrid between *C. medica* and *C. limon*)], ‘Rangpur’ lime, ‘Tahiti’ lime (*C. latifolia* Tan.), ‘Persian’ lime (C. *limettioides* Tan.), grapefruit and citron (*C. medica* L.), based on the characterization of banding CMA and in the identification of ribosomal DNA (rDNA) regions (Carvalho *et al.*, 2001), showed heteromorphism for one or more pairs of chromosomes. These results indicate the possibility to use chromosomes markers as auxiliaries in the identification of zygotic seedlings, in controlled crossings involving parents with different chromosomic band patterns.

Cultivation of Embryos

For female parents whose seeds present high polyembryony degree (higher than 70%), it is advisable that the cultivation of embryos, originated from fruits obtained by controlled pollinations, are performed under aseptic conditions, in vitro, in order to favor their germination, increasing the survival of most of the seedlings of hybrid nature. In the situations in which the female parents are monoembryonic or they present polyembryony degrees between moderate and low, the cultivation in vitro of embryos, due to their relatively high costs and high labor demand, can be avoided, without evident damages concerning the survival of the zygotic seedlings (Soares Filho *et al.*, 1995c; Medrado, 1998; Soares Filho *et al.*, 2002). This situation is due to the fact that zygotic embryos, originated from polyembryonic varieties, tend to be among those of big size, what facilitates their germination, being this particularity so much more evident as minor is the polyembryony degree, according to Table 2.

In varieties whose seeds present high polyembryony degree, is common to find immature embryos (< 3.0 mm), that don't germinate on MT medium (Murashige & Tucker, 1969), recommended for citrus, indicating the need for adjustments. So, studies performed by CBP (Morais, 1997) with 'Cleopatra' mandarin, which is characterized by its high polyembryony degree, resulted in the following modifications in the MT medium, in order to have good germination for both immature and mature (> 6.0 mm) embryos, as well as to the normal development of seedlings: maintenance of the original concentrations of micronutrients and vitamins, reduction to half the macronutrient concentration, reduction of sucrose concentration to 40 g/L, suplementation of the medium with 0.08 mg/L of BAP (benzylaminopurine), 0.01 mg/L of NAA (naphthalene acetic acid) and 20 mg/L of adenine. These modifications are suitable to the cultivation of dettached embryos with four to five months of formation, in relation to the ovule fertilization, since that in mature fruits the small embryos (< 3.0 mm) are commonly dehydrated and/or damaged. This protocol should be applied to other polyembryonic varieties, to verify the need of specific adjustments.

Based on the hypothesis that the zygotic embryo is formed previously to the nucellar(s) in polyembryonic seeds, the results of these studies will serve as base for the methodology definition capable to allow the complementation of in vitro embryogenesis of first embryos formed after fertilization, in level of each seed, in order to avoid or to restrict the manifestation of polyembryony, particularly in varieties highly polyembryonic.

Early Selection of Genotypes Tolerant to Aluminum and Drought

Studies were accomplished by CBP to identify genotypes tolerant to aluminum, utilizing uniform seedlings (nucellars) of several varieties, submitted to the cultivation in nutrient solution of Furlani & Hanna (1984), with adjustments (Pinto, 1999). The stress of aluminum was applied by aluminum chloride ($AlCl_3$) and the evaluations of the stress effects were performed 30 days after the beginning of the hydroponic cultivation. Observations were emphasized to the radicular system of the seedlings to identify the best parameter to explain the tolerance to aluminum during the juvenile stage of development. ‘Rangpur’ lime, ‘Florida’ rough and ‘Volkamer’ lemons were less tolerant than ‘Cleopatra’ mandarin and sour orange. There was a reduction of the growth of the most sensitive rootstoks starting from the concentration of 10 mg/L of Al^{+3}, being this reduction more evident in the radicular system than in the shoots (Pinto, 1999).

Complementary studies were performed with ‘Rangpur’ and ‘Galego’ [C. *aurantifolia* (Christm.) Swing.] limes, ‘Volkamer’ lemon, sour orange and ‘Cleopatra’ mandarin, as well as five hybrids obtained by CBP: HTR - 002, HTR - 010, HTR - 144, LCR x TR - 001 and LCR x LRF (‘Florida’ rough lemon) - 005. It was confirmed that the visual symptom that best characterizes the phytotoxic effect of the aluminum in seedlings of citrus varieties is the reduction of the growth of the radicular system, being verified, however, that the 'Volkamer' lemon was the most tolerant among the commercial varieties studied. Among hybrids, the following presented larger tolerance to the aluminum: HTR - 002, HTR - 144 and LCR x TR-001 (Lima *et al.*, 2001).

In relation to drought tolerance, preliminars studies are being planned to establish a methodology that allow the selection, in early stages, of genotypes tolerant to drought. The efficiency of the method will depend on how the water deficit will be applied so that the plant can respond in a short time by physiologic changes which can be easily evaluated. In that sense, the water deficit will be evaluated based on soil water content, in relation to full soil field capacity, considering the gravimetric method. The tests will be carry out in greenhouse, using contrasting genotypes for drought tolerance.

Selection of Genotypes Resistant to *Phytophthora* Root Rot

Studies were conducted to compare citrus genotypes with respect to resistance to *Phytophthora* root rot in laboratory and under field conditions. Evaluation was based on the mean length of stem lesions caused by *Phytophthora* in seven-year-old trees, considering five plants of each crossing: 'Rangpur' lime x 'Volkamer' lemon, 'Rangpur' lime x 'Florida' rough lemon, 'Volkamer' lemon x 'Palmeiras' sweet orange (*C. sinensis*), 'Florida' rough lemon x 'Hamlin' sweet orange (*C. sinensis*), 'Rangpur' lime x 'Palmeiras' sweet orange, 'Volkamer' lemon x 'Valencia' sweet orange (*C. sinensis*), 'Sunki' mandarin x ('Rangpur' lime x *P. trifoliata*), 'Rangpur' lime x *P. trifoliata*, 'Rangpur' lime x 'Swingle' citrumelo, 'Rangpur' lime x 'Yuma' citrange, 'Cleopatra' mandarin x ('Rangpur' lime x *P. trifoliata*). Propagule densities were determined using soil samples collected in four different sites under trees (at 20 cm depth) near to plants. The highest propagule counts was found in the rhizosphere of plants obtained from crossings which lemons/ lime and sweet oranges (susceptible to the pathogen) were used as parents. The extent of soil infestation was significantly lower in samples collected near the plants originated from hybridization using *P. trifoliata* and their hybrids as parents. Hybrids of 'Yuma' citrange and 'Swingle' citrumelo, as well as those involving *P. trifoliata*, except 'Rangpur' lime x *P. trifoliata* and 'Sunki' mandarin x ('Rangpur' lime x *P. trifoliata*), neither show stem lesions caused by *Phytophthora* nor death plants (Oliveira *et al.*, 2000).

Reaction of Hybrids to the Citrus Tristeza Virus

Field investigation was conducted in 195 hybrids and varieties used as their parents, in which were collected up to 10 branches in each plant sampled. The study involved four to seven-years-old hybrid seedlings developed by the CBP. Branches were evaluated according to Citrus Tristeza Virus – CTV symptoms using a 1-5 scale (1: without stem pitting; 5: all surface covered by severe stem pitting, independent of presence of superficial stem pitting) (Meissner Filho *et al.*, 2002).

Promising crossings regarding to CTV resistant hybrids were: 'Rangpur' lime x 'Swingle' citrumelo, 'Volkamer' lemon x 'Rangpur' lime, 'Sunki' mandarin x ('Rangpur' lime x *P. trifoliata*), 'King' mandarin (*C. nobilis*) x 'Swingle' citrumelo, 'Clementine de Nules' mandarin x C-35 citrange and 'Sunki' mandarin x 'Swingle' citrumelo.
Among the crossings which showed high frequency of non resistant hybrids are: 'Volkamer' lemon x 'Palmeiras' sweet orange, 'Volkamer' lemon x 'Valencia' sweet orange, 'Clementine de Nules' mandarin x 'Swingle' citrumelo and 'Clementine de Nules' mandarin x 'Hybrid' (*Poncirus trifoliata* hybrid) (Diamantino, 2001).

Induction of Flowering

In order to shorten the prereproductive period of selected hybrids, in juvenile phases of development, the flowering induction constitutes an objective of great importance. In this sense, it was studied the effect of methanol on the increase of carbohydrates concentration in the plant by inhibition of the photorespiration, supported by the hypothesis that one of the factors that affect positively the flowering in fruit crops is the concentration of carbohydrates. The experiment utilized nucellar seedlings of ‘Rangpur’ lime and ‘Cleopatra’ mandarin one year old. These varieties present different periods of flowering, respectively precocious and late. The results indicate that the methanol can favor the vegetative development of the studied varieties, with effects more expressive in ‘Cleopatra’ mandarin, indicating the existence of interactions between methanol and different genotypes. The seedlings vigor was measured by plant height and size of leaves (length and width) randomly selected (Souza *et al.*, 2000).

Somatic Hybridization by Protoplast Fusion

Initialy, the studies performed by CBP have been concentrated in the steps of obtaining and cultivation of embryogenic calli, germination of seeds and in vitro cultivation of nucellar seedlings. Later, the studies will include the stages of cultivation of cell suspensions, germination of embryos, isolation, purification and culture of protoplasts from materials used as parents in the somatic hybridizations, protoplast fusion, cultivation and selection of the fused products, regeneration and identification of somatic hybrids.

Obtaining and cultivation of embryogenic calli

Preliminary experiments, using several species and varieties of citrus, showed that the formation of embryogenic calli is more efficient when is used abortive ovules, when compared to calli obtained from nucellus.

The extraction of not fertilized ovules, according to variety, have been accomplished in fruits with four to five weeks after fertilization. In general, the induction of callus has occurred between the sixth and the eighth weeks of cultivation. In ‘Hamlin’ sweet orange, selection CNPMF 20, the formation of callus was more precocious, occurring between three to four weeks after the establishment in vitro of the explants. Percentages of callus formation higher than 40% were obtained in the mandarins ‘Sunki’ (42%), ‘Swatow’ (*C. reticulata*) (50%) and ‘Cleopatra’ (66%), ‘Hamlin’ sweet orange selections CNPMF 04 (52%) and CNPMF 20 (58%), ‘Troyer’ citrange (56%) and

'Rangpur' lime selection CNPMF 03 (60%). The smallest percentages of callus formation were obtained in the lemons 'Mazoe' rough (0%) and 'Volkamer' (0%), C. *amblycarpa* Ochse (4%) and 'Common' sour orange (6%). Those calli are being subcultivated on MT medium plus BAP (10 mg/L) and malt extract (500 mg/L).

In another experiment, it was evaluated the percentage and fresh weight of calli formed from abortive ovules of 'Hamlin' sweet orange selection CNPMF 04, on MT medium, suplemented with several combinations of BAP and 2.4-D levels. The treatment with 5 mg/L of BAP, in the absence of 2.4-D, caused the largest percentage (35%) of ovules that formed calli (Table 3) and the highest index of callus fresh weight (246.7 g) (Table 4). According to Spiegel-Roy & Vardi (1984), the nucellar cells of the polyembryonic citrus species are naturally embryogenic and, having an appropriate hormonal balance, they don't need growth regulators for callus induction. However, for recalcitrant genotypes, adjustments are necessary in the balance of the growth regulator substances, since the internal demands vary among species.
The addition of 2.4-D reduced significantly the formation of callus. This result shows that these tissues have an endogenous level of this auxin and that an increment in this level could be higher than the optimum limit for callus formation. This hypothesis was proved by the fact that the addition of inhibitors of the auxins and cytocinins synthesis stimulate highly the embryogenic process (Kochba & Spiegel-Roy, 1977).

Table 3. Percentage of calli formation from abortive ovules of 'Hamlin' sweet orange [*Citrus sinensis* (L.) Osb.] selection CNPMF 04, in different levels of BAP and 2.4-D. Citrus Breeding Program of ***Embrapa Cassava&Fruits***, Cruz das Almas, Bahia, Brazil.

BAP (mg/L)	2.4-D (mg/L)			$\bar{x}$
	0	5	10	
0	30.00	0.00	0.00	10.00
5	35.00	5.00	10.00	16.70
10	5.00	0.00	0.00	1.67
$\bar{x}$	23.33	1.66	3.33	

Table 4. Fresh weight (g) of calli originated from abortive ovules of 'Hamlin' sweet orange [*Citrus sinensis* (L.) Osb.] selection CNPMF 04, in different levels of BAP and 2.4-D. Citrus Breeding Program of ***Embrapa Cassava&Fruits***, Cruz das Almas, Bahia, Brazil.

BAP (mg/L)	2.4-D (mg/L)			$\bar{x}$
	0	5	10	
0	167.7	0.0	0.0	55.9
5	246.7	12.2	17.3	92.0
10	86.6	0.0	0.0	28.9
$\bar{x}$	167.0	4.1	5.8	

Seed germination

For obtaining nucellar seedlings, seeds of mature fruits were used in the following varieties: 'Mazoe' rough lemon and 'Rangpur'lime (selections 'Santa Bárbara' and 'Santa Cruz'), 'Troyer'citrange, 'Sunki', 'Cleopatra' and 'Swatow' mandarins, 'Common' and 'Narrow Leaf' sour oranges, *Fortunella* 'Jin Dan' and *Poncirus trifoliata* 'Flying Dragon' selection. Under aseptic conditions, the seeds, with teguments, were inoculated in flasks containing 30 mL of the RMAN medium (MT medium plus 25 g/L of sucrose) and then transferred to growth room with 27 ± 1º C, 16 hours of fotoperiod and 1500 lux. In all varieties the germination indexes were higher than 90%. The seedlings presented good initial development, having leaves well expanded and intense green coloration. The sproutings presented little leaf expansion and were subcultivated in RMAN medium.

In vitro cultivation of nucellar seedlings for protoplasts isolation

In order to obtain high quality leaf material for protoplast extraction, the growth of nucellar seedlings, micropropagated from lateral and apical buds, was evaluated in 'Rangpur' lime and 'Mazoe' rough lemon. The effects of IAA (indol acetic acid) and BAP, in the concentrations 0.0 and 5.0 mg/L and 0.0, 0.5 and 1.0 mg/L, respectively, on in vitro seedlings growth, were studied. Explants were cultivated in flasks containing 35 mL of RMAN medium, under the same growth conditions mentioned previously. 'Mazoe' rough lemon was superior to 'Rangpur' lime in some parameters such as: number of sprouting, height of the seedlings, fresh weigh of the aerial part, leaf length, and fresh weight of roots (Table 5). In relation to types of used explants, seedlings originated from stem apexes were superior to those originated from lateral buds, regarding to the parameters number of sprouting, number of roots, root length, and fresh weight of roots (Table 6).

Table 5. In vitro performance of micropropagated nucellar seedlings of 'Rangpur' lime (*Citrus limonia* Osb.) and 'Mazoe' rough lemon (*C. jambhiri* Lush.). Citrus Breeding Program of ***Embrapa Cassava&Fruits***, Cruz das Almas, Bahia, Brazil.

Parameters	'Rangpur' lime	'Mazoe' rough lemon
Number of sprouting	2.69	2.95
Height of aerial part (cm)	1.90	2.15
Fresh weight of aerial part (mg)	184.96	315.89
Leaf length (cm)	1.94	2.39
Number of roots	0.68	0.37
Root length (cm)	1.43	1.23
Fresh weight of roots (mg)	13.62	21.67

Table 6. Response of two types of explants for micropropagation of nucellar seedlings of 'Rangpur' lime (*Citrus limonia* Osb.) and 'Mazoe' rough lemon (*C. jambhiri* Lush.). Citrus Breeding Program of ***Embrapa Cassava&Fruits***, Cruz das Almas, Bahia, Brazil.

Parameters	Explants	
	Lateral bud	Stem apex
Number of sprouting	2.73	2.88
Height of aerial part (cm)	2.07	1.74
Fresh weight of aerial part (mg)	286.81	242.19
Leaf length (cm)	2.49	2.32
Number of roots	0.44	0.60
Root length (cm)	1.10	1.55
Fresh weight of roots (mg)	14.25	20.59

In absence of AIA and BAP the results were higher than those without growth regulators, especially in relation to the parameters height of the aerial part, leaf length, number and length of roots. In the culture medium without both growth regulators the seedlings presented a more intense green coloration. According to Spiegel-Roy & Vardi (1984), this happens because, in general, the citrus varieties easily adapt to the absence of growth regulators, becoming an exception among most of the plant species.

Complementary evaluations were performed in 'Dancy' tangerine, 'Sunki' mandarin, 'Common' sour orange, *C. amblycarpa*, *C. macrophylla* Wester, 'Carrizo' citrange, 'Swingle' citrumelo, 'Rangpur' lime selection 'Santa Cruz' and in the hybrids LCR x TR - 001 and LVK x LCR - 038, obtained by CBP. Test tubes of 25 mm x 150 mm and flasks containing 10 mL and 35 mL of RMAN medium, respectively, were used. 'Dancy' tangerine was superior to the other varieties concerning the parameters height, fresh and dry weight of the aerial part. The leaves of this tangerine showed a more intense green coloration. In relation to fresh and dry weight of roots, the largest values were found in the hybrid LVK x LCR - 038, which also presented the largest root length. The 'Common' sour orange didn't form roots. The highest number of leaves was observed in 'Sunki' mandarin. Regarding to the explants, seedlings originated from stem apexes confirmed their superiority in relation to those originated from lateral buds, considering the parameters seedling height, fresh and dry weight of the aerial part, fresh and dry weight of root, number of leaves and root length. Seedlings cultivated in flasks presented better results than that cultivated in test tubes, in relation to fresh and dry weight of the aerial part, fresh and dry weight of root and number of leaves. On the other hand, the seedlings cultivated in test tubes presented larger height and root length. According to Grattapaglia & Machado (1998), the flask type and the amount of medium used are variable that affect directly the superficial area of the interface medium-atmosphere, the volume of air on medium and the depth of the medium. Those factors also affect the composition of the gaseous phase of the flask and, consequently, the plant growth.

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Appendix

Table 1. Hybrids obtained in the Citrus Breeding Program of ***Embrapa Cassava&Fruits***, 1988 – 2002. Cruz das Almas, Bahia, Brazil.

Rootstock varieties	
Crossings	**N^0 of hybrids**
TCL ('Clementine' mandarin) x CTC25 (C-25 citrange)	249
TCL x CTCG ('Cunninghan' citrange)	200
TCL x CTARG ('Argentina' citrange)	187
TCL x CTC35 (C-35 citrange)	168
TCL x CTSF ('Sanford' citrange)	156
TCL x TRBK (*Poncirus trifoliata* Beneke)	149
TCL x CTSW ('Swingle' citrumelo)	98
TCL x HYD (trifoliate Hybrid)	70
TCL x CTTR ('Troyer' citrange)	38
TCL x [CLEO ('Cleopatra' mandarin) x TRBN (*P. trifoliata* Barnes)]	32
TCL x [LCR ('Rangpur' lime) x TR (*P. trifoliata*)]	23
TCL x TRDP (*P. trifoliata* Diploid)	22
TCL x TRPO (*P. trifoliata* Pomeroy)	16
TCL x CTRK ('Rusk' citrange)	11
TCL x LVK ('Volkamer' lemon)	10
TCL x LRM ('Mazoe' rough lemon)	8
TCL x CTQT (citrangequat)	6
TCL x CTCM ('Coleman' citrange)	2
TCL x TRRD (*P. trifoliata* Rubidoux)	1
TSK ('Sunki' mandarin) x CTARG	203
TSK x (LCR x TR)	77
TSK x CTSW	65
TSK x CTQT	53
TSK x CTC13 (C-13 citrange)	44
TSK x CTTR	42
TSK x LHA ('Hamlin' sweet orange)	41
TSK x TRDP	39
TSK x LRM	24
TSK x CTC25	20
TSK x TRBK	19
TSK x CTRK	17
TSK x CTCM	11
TSK x TRFD (*P. trifoliata* Flying Dragon)	10
TSK x TRBN	7
TSK x CTC35	7
TSK x CWEB (*Citrus webberi*)	7
TSK x LVK	5

Continues

Continuation

Rootstock varieties	
Crossings	**N^0 of hybrids**
TSK x LPA (‘Palmeiras’ sweet orange)	4
TSK x LPE (‘Pêra’ sweet orange)	2
TSK x LCR	1
TSK x HTR051 (trifoliate hybrid – 051)	1
LCR x LAZ (sour orange)	104
LCR x CTARG	94
LCR x CTSW	41
LCR x LRF (‘Florida’ rough lemon)	34
LCR x CTC35	22
LCR x HTR116 (trifoliate hybrid – 116)	21
LCR x LVK	17
LCR x LHA	9
LCR x LPA	7
LCR x (LCR x TR)	7
LCR x CTTR	6
LCR x [TSK x TRSW (*P. trifoliata* Swingle)]	5
LCR x MCC (*Microcitrus*)	5
LCR x TSK	4
LCR x TRRB	3
LCR x (CLEO x TRSW)	2
LCR x CKRJ (*Citrus keraji*)	1
LCR x TRPO	1
LCR x CTCM	1
LCR x TRDP	1
LVK x CTSW	128
LVK x LCR	81
LVK x LPA	47
LVK x CTTR	42
LVK x [TSK x TRENG (*P. trifoliata* English)]	37
LVK x CTARG	22
LVK x CTCM	18
LVK x LVA (‘Valencia’ sweet orange)	16
LVK x CTC35	10
LVK x CTCG	8
LVK x TRPO	7
LVK x TRFD	4
LVK x TRBK	4
LVK x LAZ	3
LVK x (LCR x TR)	2
LVK x HTR116	1
LAZ x CTARG	124
LAZ x CTSW	124
LAZ x LHA	108

Continues

Continuation

Rootstock varieties	
Crossings	**N⁰ of hybrids**
LAZ x CTC35	90
LAZ x LVA	40
LAZ x CTTR	38
LAZ x LCR	28
LAZ x CTRK	18
LAZ x CTC32 (C-32 citrange)	17
LAZ x CTQT	13
LAZ x TRRD	10
LAZ x CTCM	8
LAZ x (TSK x TRENG)	4
LRF x LHA	10
LRF x LCR	6
LRF x (LCR x TR)	5
LRF x LPA	3
CLEO x LVK	5
CLEO x (LCR x TR)	5
CLEO x LPA	3
CLEO x LCR	2
CLEO x CTSW	1
OLD ('Orlando' tangelo) x CTTR	14
OLD x TRDP	2
OLD x (LCR x TR)	2
OLD x CTCG	1
LEE ('Lee' tangerine-tangelo) x CTARG	28
LEE x CTC25	22
LPA x LVK	12
LPA x LCR	1
TPL ('Temple' tangor) x CTTR	13
TPL x CTQT	4
TKG ('King' mandarin) x CTSW	21
TKG x TRDP	12
TKG x CTTR	2
MCPH (*Citrus macrophylla*) x TRBK	2
MCPH x CTC35	1
TDA ('Dancy' tangerine) x LVK	2
TKG x CTSW	21
CTYM ('Yuma' citrange) x LCR	6
TCD ('Chandler' pummelo) x [CLEO x CTCZ ('Carrizo' citrange)]	20
JDN (*Fortunella* 'Jin Dan') x LAZ	7
LCR x CKN (*Citrus karna*)	2
SSJC (Shane Shou Jin-Chen) x LAZ	1
[TCL x MCT ('Murcott' tangor)] x CTSW	5
HTR (trifoliate hybrids)	208
Total of hybrids	**4.010**

Continues

Continuation

Scion varieties	
Crossings	**N⁰ of hybrids**
TCL x LPB ('Parson Brown' sweet orange)	204
TCL x LHA	148
TCL x OLD	134
TCL x LVA	121
TCL x LMS ('Midsweet' sweet orange)	104
TCL x LST ('Sunstar' sweet orange)	79
TCL x LJF ('Jaffa' sweet orange)	65
TCL x TCR ('Cravo' mandarin)	60
TCL x LPE	54
TCL x LBD ('Biondo' sweet orange)	50
TCL x LPN ('Pineapple' sweet orange)	46
TCL x LSL ('Seleta' sweet orange)	24
TCL x MXC ('Mediterranean'mandarin)	22
TCL x LKN ('Kona' sweet orange)	22
TCL x TPG ('Page' tangerine-tangelo)	17
TCL x LSN ('Salustiana' sweet orange)	7
TCL x LFM ('Folha Murcha' sweet orange)	6
LEE x LHA	454
LEE x OLD	94
LEE x LPB	75
LEE x LVA	62
LEE x TMN ('Minneola' tangelo)	18
LEE x LNA ('Natal' sweet orange)	13
TRB ('Robinson' tangerine-tangelo) x LNA	61
TRB x LPN	45
TRB x LST	28
TRB x LHA	22
TRB x LDI ('Diva' sweet orange)	1
MXC x LHA	5
MXC x LPA	2
MXC x LPE	2
MXC x LLT ('Laboratory' sweet orange)	1
MXC x LCA ('Caipira' sweet orange)	1
OLD x LPE	3
OLD x LVA	2
OLD x LHA	1
TPG x TCR	2
TPG x MXC	1
TPG x LHA	1
TPL x LSN	27
TPL x LBD	19
TKG x LSL	4
TKG x LRB ('Rubi' sweet orange)	1

Continues

Continuation

Scion varieties	
Crossings	**N⁰ of hybrids**
TMN x LNA	2
TMN x LVA	1
KNW ('Kinnow' mandarin) x LPE	4
TBS ('Big of Sicily' mandarin) x LPE	2
MCT x LVA	2
TCR x LPA	1
TST ('Satsuma' mandarin) x LWT ('Westin' sweet orange)	1
LLB ('Lisboa' lemon) x LSL	1
Total of hybrids	**2,122**

Rootstock/Scion varieties	
Natural hybrids[1]	**N⁰ of hybrids**
LCR	64
LAZ	35
LVK	21
LGL ('Galego' lime)	18
CLEO	10
CTSW	8
COBV (*Citrus obovoidea*)	7
HTR001 (trifoliate hybrid – 001)	4
CTCZ	3
LVK x LCR 010	2
CTWN (*Citrus taiwanica*)	2
TRFD	2
TRRD	2
TSC ('Sun Cha' mandarin)	2
HTR070 (trifoliate hybrid – 070)	1
HTR166 (trifoliate hybrid – 166)	1
HTR103 (trifoliate hybrid – 103)	1
TSK x TRENG	1
CLEO x TRSW	1
LRU ('de Russas' sweet orange)	1
Total of hybrids	**186**

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