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ORIGINAL ARTICLE

Selection of CTV-tolerant citrus hybrids for ornamental use

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Abstract – Introduction. Ornamental citrus is well established in Europe and North America, but is still incipient in Brazil, despite the fact that the country is among the world's leading citrus fruit producers. However, the potential for innovation in this agribusiness segment is increasingly recognized. The Citrus Breeding Program of Embrapa Cassava & Fruits, among other objectives, aims to create plants suitable for ornamental use. **Materials and methods.** Ten hybrids were characterized by application of 39 morphological descriptors to identify their ornamental potential and to classify them in use categories. Additionally, these hybrids were evaluated regarding their susceptibility to the citrus tristeza virus (CTV). **Results and discussion.** The hybrids [(RPL x YMCT-005) × MCP-011], [(RPL × YMCT-005) × MCP-015], [GLINL × MCP-002], [GLINL × FTNL-001], [RPL × MCSH-002] and [CSM × MCP-002] can be recommended for use as potted plants, minifruit, landscaping and hedges; [(RPL × YMCT-005) × MCP-016], [CSM × MCP-003] are suitable as potted plants, landscaping and hedges; [(RPL × YMCT-005) × SMM-014] fits in the potted plant, landscaping and ornamental minifruit; and [(RPL × YMCT-005) × GLINL-001] is suitable for use as potted plants and in landscaping. According to the results on the reaction to the CVT pathogen, four hybrids were classified as resistant, four were classified as highly tolerant and two were classified as highly intolerant, indicating the need for their protection with weak CTV strains **Conclusion.** The hybrids evaluated presented substantial morphological variability that distinguished them from each other. Their traits qualify them for use as ornamental plants.

Keywords: Brazil / *Citrus* spp. / citrus tristeza virus / landscaping / minifruit / species selection

Résumé – Sélection d'hybrides d'agrumes tolérants au CVT à fin d'ornement. Introduction. La production d'agrumes ornementaux, bien établie en Europe et en Amérique du Nord, en est encore à ses débuts au Brésil, en dépit du fait que le pays figure parmi les principaux producteurs d'agrumes au monde. Cependant, le potentiel d'innovation dans ce segment de l'agro-industrie est de plus en plus reconnu. Le programme de sélection d'agrumes de la division Manioc & Fruits de l'Embrapa vise à créer, entre autres, des plantes adaptées à des fins ornementales. **Matériel et méthodes.** Dix hybrides ont été caractérisés par application de 39 descripteurs morphologiques, dans le but d'identifier leur potentiel ornemental et de les classer par catégorie d'utilisation. En outre, ces hybrides ont été évalués quant à leur sensibilité au virus de la Tristeza des agrumes (CTV). **Résultats et discussion.** Les hybrides [(RPL × YMCT-005) × MCP-011], [(RPL × YMCT-005) × MCP-015], [GLINL × MCP-002], [GLINL × FTNL-001], [RPL × MCSH -002] et [CSM × MCP-002] peuvent être recommandés pour une utilisation en tant que plantes en pot, pour la production de mini fruits, l'aménagement paysager et les haies ; [(RPL × YMCT-005) × MCP-016] et [CSM × MCP-003] conviendraient plutôt comme plantes en pot, pour l'aménagement paysager et les haies ; [(RPL × YMCT-005) × SMM-014] conviendrait comme plante en pot, en aménagement paysager et production de mini fruits ornementaux ; et [(RPL × YMCT-005) × GLINL-001] est adapté à une utilisation en plantes en pot et en aménagement paysager. Selon leur sensibilité au CVT, quatre hybrides ont été classés comme résistants, quatre ont été classés comme très tolérants et deux ont été classés comme très intolérants, ce qui indique la nécessité d'une protection par des souches de CTV atténuées. **Conclusion.** Les hybrides évalués présentent une variabilité morphologique importante qui les distingue les uns des autres. Leurs caractéristiques les qualifient comme plantes ornementales.

Mots clés : Brésil / *Citrus* spp. / virus de la tristeza des agrumes / aménagement paysager / mini fruits / sélection variétale

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1 Introduction

Citrus growing is an important economic activity in Brazil and the rest of the world, not only for the high value of the fruits produced, but also the large number of direct and indirect jobs generated. In 2013, global citrus fruit output reached 1,378 Mt, of this 53% being sweet oranges (*Citrus sinensis* (L.) Osbeck). Brazil stands out as a producer of citrus, with output of 197 Mt, mostly consisting of sweet oranges, making citrus the leading fruit growing segment in the country [1].

The genus *Citrus* L. belongs to the family Rutaceae and together with *Poncirus* L., *Fortunella* Swingle, *Microcitrus* Swingle and *Eremocitrus* Swingle forms the true citrus group, because of bearing fruits similar to the sweet orange or lemon [2].

The genetic variability of these genera is extensive, notably in *Citrus*, enabling obtaining new varieties, rootstocks and scions with the ability to promote sustainability of the citrus agribusiness, not only for production of fruits for food, but also for other purposes, such as making drugs, cosmetics and perfumes [3–5]. Additionally, because of the beauty and originality of plant shapes, they are increasingly being used for ornamental purposes [6–8].

Floriculture generates revenues of US$ 107 billion annually and has a great capacity to create employment and income, mainly for small-scale farmers [9]. In addition to this social aspect, the activity is also important for keeping people in the field. Thus, the ornamental citrus production emerges as an activity that can add social and economic value to the conventional citrus production.

Landscapers and gardeners are always in search of new innovations. In this context, tropical and subtropical fruit-bearing plants have great potential for ornamental use, in competition with traditional plants from temperate climates [1]. Among these fruiting plants, citrus species are highly attractive due to their pleasant-smelling flowers and fruits with varied sizes, shapes and colors. Depending on the variety, citrus plants can produce over almost the entire year.

In Brazil, research to obtain citrus varieties with ornamental potential, although still scant, has been intensifying in recent years. In this respect, the Citrus Breeding Program of Embrapa Cassava & Fruits (CBP) started developing hybrids for this purpose, based on the genetic variability existing in the Citrus Active Germplasm Bank of Embrapa Cassava & Fruits (CAGB), which contains more than 750 accessions [8], the most diversified in the world in tropical regions. This diversity supports the identification of promising varieties for ornamental uses and the creation of hybrids for this purpose.

Accessions of the CAGB have been crossed for the past five years aiming to develop hybrid varieties suitable as ornamental plants. Among the species of *Citrus* and related genera involved in this cross-breeding are: *C. amblycarpa* (Hassk.) Ochse, *C. aurantiifolia* (Christm.) Swingle, *C. medica* L., *C. depressa* Hayata, *C. sunki* (Hayata) hort. ex Tanaka, *C. sinensis*, *C. jambhiri* Lush., *C. limonia* Osbeck, *C. myrtifolia* Raf., *C. madurensis* auct, *C. webberi* Wester var. *montana* Wester, *C. maxima* (Burm.) Merr., *Poncirus trifoliata* (L.) Raf., *Fortunella* spp. and *Microcitrus* spp. The results of this hybridization program have been promising.

However, for citrus to become established in the ornamental plant market, besides having attributes valued by consumers, promising individuals must be selected considering a wide range of assessments, including phytosanitary considerations. Among the diseases that affect these plants, that caused by the citrus tristeza virus (CTV) stands out. This virus is transmitted by aphids mainly the brown citrus aphid (*Toxoptera citricida* Kirkaldy), and contaminated propagation materials [11]. Considerable harm is done by some strains of this pathogen, inducing stem pitting and visible grooves in the branches and trunk, impairing the plant's vigor and diminishing the productivity and size of fruits from intolerant scions. The disease's advance can cause collapse of the phloem and root structure, killing the plant [12,13]. The most effective way to control the disease is the use of resistant or tolerant varieties and pre-immunization with mild strains of the virus Therefore, knowledge on this virus tolerance is critical for the selection of new ornamental citrus hybrids.

Therefore, the aim of this study was to characterize and evaluate hybrid seedlings from the crosses carried out by the CBP to identify individuals with ornamental potential and to classify them in use categories, as potted plants, hedges, for landscaping or production of miniature fruit. The hybrids were also assessed in relation to CTV resistance.

2 Material and methods

2.1 Experimental design and plant materials

The study was conducted in an open area of the experimental farm of the CBP, located in the municipality of Cruz das Almas, Bahia, Brazil (12°40′19″ S latitude and 39°06′22″ W longitude, 220 m altitude).

According to the Köppen classification, the climate in Cruz das Almas is a transition between the Am and Aw zones, with average annual rainfall of 1,143 mm, average temperature of 24.4 °C and relative humidity of 81%. The soil of the experimental area is a typical dystrophic Yellow Latosol, a moderate, sandy clay loam texture, kaolinite, hypoferric, transition zone between sub-perennial and semi-deciduous rainforest, with slope of 0–3%.

Ten hybrid seedlings were evaluated:

RYM011 = [(RPL × YMCT-005) × MCP-011],

RYM015 = [(RPL × YMCT-005) × MCP-015],

RYM016 = [(RPL × YMCT-005) × MCP-016],

GM002 = [GLINL × MCP-002],

GF001 = [GLINL × FTNL-001],

RYG001 = [(RPL × YMCT-005) × GLINL-001],

CM002 = [CSM × MCP-002],

CM003 = [CSM × MCP-003],

RYS014 = [(RPL × YMCT-005) × SMM-014], and

RM002 = [RPL × MCSH-002].

In the latter case, the seeds came from a 12 year old plant, while in the other cases the parental plants were 3 to 4 years old. The abbreviations used here are: RPL = 'Rangpur' lime (*C. limonia*), YMCT = 'Yuma' citrange (*C. sinensis* × *P. trifoliata*), MCP = *M. papuana* Winters, GLINL = 'Galego Inerme Key' lime (*C. aurantiifolia*), FTNL = *Fortunella*, CSM = common 'Sunki' mandarin (*C. sunki*), SMM = 'Sunki Maravilha' mandarin, and MCSH = *Microcitrus* Sydney hybrid, a hybrid from crossing *M. australis* (A. Cunn. ex Mudie) Swingle × *M. australasica* (F. Muell.) Swingle.

2.2 Morphological descriptors

The morphology of these hybrids and their parentage was characterized using a list of adjusted morphological descriptors from the Descriptors for Citrus list of the International Plant Genetic Resources Institute [14], according to the needs of this work. A total of 39 descriptors were used, 11 quantitative and 28 qualitative. Of the quantitative descriptors, three were related to plant traits [plant height (PLH), trunk diameter (TRD) and crown diameter (CRD)], four to leaf traits [leaf length (LFL), leaf width (LFW), phyllode length (PHL) and phyllode width (PHW)], and four to fruit traits [fruit length (FRL), fruit diameter (FRD), number of fruits per bunch (NFB) and average number of fruits per bunch (AFB)]. In turn, of the qualitative descriptors, five referred to attributes of the plants [crown shape (CRS), branch density (DRB), spine density (SPD), spine length (SPC) and spine shape (SPS)], nine to leaf aspects [leaf division (LFD), leaf variegation (LVA), leaf color (LCO), leaf lamina insertion (LLI), leaf lamina shape (LLS), leaf lamina margin (LLM), leaf apex (LAP), phyllode (PHY) and phyllode shape (PHS)], nine to fruit appearance [fruit shape (FRS), shape of fruit base (FSB), shape of fruit apex (FSA), fruit variegation (FRV), immature fruit color (IFC), ripe fruit color (RFC), fruit surface texture (FST), albedo color (ALC) and pulp color (PUC)], and five to flower traits [color of open flowers (COF), arrangement of flowers (AFL), position of flower or inflorescence (PFI), aroma (ARO) and aroma persistence (ARP)]. The colors were compared with the color chart of the Royal Horticulture Society (RHS). In relation to the leaf descriptors, an average was obtained by observing 20 completely expanded and mature leaves, located in the middle third of the youngest branches from spring growth that did not show signs of active growth. The flowers and fruits were characterized based on 20 replications, with the flowers observed on terminal branches at the time of full flowering and the fruits at the harvest point, located on the periphery of the crown

The traits considered for classification of the hybrids in different categories for ornamental use followed the method described by Santos *et al.* [8], as summarized below:

- *Potted plants*: Plant height below 170 cm, crown diameter smaller than 150 cm, moderate or dense branching, preferably with few or no spines. Accessions with larger height and crown diameter could be considered, if associated with dwarf rootstocks or plants manageable by topiary to keep them small.
- *Minifruit plants*: Fruit diameter (or length for elongated fruits) varying from 2.5 cm to 4.5 cm.
- *Hedge plants*: Dense branching.
- *Landscaping plants*: Broad category, possibly including potted plant, minifruit and hedges. One desirable common feature of plants in this category is absence or low density of spines.

2.3 Virus testing

The assessment of susceptibility of the hybrids to CTV was carried out in two steps. Initially, the presence and severity of disease symptoms were evaluated on the branches. Ten branches, 20 cm long, were removed from each quadrant of the plant. These branches were autoclaved for 10 min to facilitate removal of the bark, after which they were evaluated by three examiners, using the scoring scale described by Meissner Filho *et al.* [15], where 1 = absence of grooves, 2 = presence of sparse grooves, 3 = intermediate number of grooves, 4 = more numerous superficial grooves and/or a few deep grooves; and 5 = entire surface of the branch covered with superficial and/or deep grooves. Then the sample branches were analyzed by the indirect ELISA test [16], utilizing the polyclonal antiserum against CTV. As negative control, bark samples from branches of *P. trifoliata* were used, while the positive control consisted of samples from *C. aurantiifolia*. Ten replications were employed for the negative control and three for the positive control. The absorbance was read by using ELISA plates (Elx 800 Universal Microplate Reader), after reaction for 10 min with the substrate buffer (0.87 mg L^{-1} p-nitrophenyl phosphate). To determine the absorbance limit value, which allows diagnosing infected and healthy samples, the mean of the negative control plus the standard deviation in each test was used

Based on the results on the presence and severity of grooves and the ELISA test, the hybrids were classified regarding susceptibility to the CTV, as: resistant (absence of virus in tissues and no grooves on branches), very tolerant (presence of virus in tissues but absence of grooves on branches), tolerant (maximum score of 2), intolerant (maximum score of 3), very intolerant (maximum score of 4), and extremely intolerant (maximum score of 5).

3 Results and discussion

3.1 Morphological characterization

A wide phenotypic variability was observed, even between individuals with one or both parents in common. The variability was most evident in the fruits and leaves, which presented considerable diversity of shapes, sizes and colors (*tables I–IV; figure 1*).

Among the hybrids selected, three came from crosses of [RPL × YMCT-005] (female parental) and MCP: RYM011, RYM015 and RYM016 (*figures 1a–1d*).

The hybrid RYM011 (*figure 1a*) has medium size (height of 230 cm). Its crown is ellipsoid and considerably dense, with a large number of small spines (*table I*). Although the presence

Table I. Morphological traits of 10 hybrid citrus seedlings with potential for ornamental use and their parentals. Values for quantitative descriptors are the mean of 3 replications followed by the standard errors.

Hybrids	PLH	TRD	CRD	CRS	DRB	SPD	SPC	SPS
RYM011	230.0 ± 12.5	7.0 ± 1.2	180.0 ± 10.2	ELI	DEN	HIG	6–15 mm	STR
RYM015	184.0 ± 10.3	7.5 ± 0.8	160.0 ± 8.7	ELI	DEN	HIG	6–15 mm	STR
RYM016	170.0 ± 8.7	4.3 ± 0.5	150.0 ± 9.2	OVO	DEN	HIG	6–15 mm	STR
GM002	230.0 ± 14.3	7.2 ± 1.0	200.0 ± 11.2	ELI	DEN	HIG	6–15 mm	STR
GF001	190.0 ± 6.3	7.6 ± 1.1	135.0 ± 7.2	ELI	DEN	HIG	6–15 mm	STR
RYG001	160.0 ± 4.4	7.9 ± 0.9	148.0 ± 5.2	SPH	DEN	HIG	6–15 mm	STR
RM002	150.0 ± 3.5	6.3 ± 0.8	141.0 ± 4.3	SPH	DEN	HIG	<5 mm	STR
CM002	337.0 ± 11.3	7.9 ± 0.5	239.0 ± 12.7	ELI	DEN	HIG	16–40 mm	STR
CM003	247.0 ± 9.3	6.7 ± 0.7	185.0 ± 6.8	ELI	DEN	HIG	16–40 mm	STR
RYS014	222.0 ± 8.7	8.8 ± 1.2	292.0 ± 9.2	SPH	DEN	MED	16–40 mm	STR
Parentals	PLH	TRD	CRD	CRS	DRB	SPD	SPC	SPS
RY005	279.0 ± 12.5	11.0 ± 1.0	339.0 ± 41.3	OVO	MED	ABS	ABS	ABS
MCP	140.0 ± 8.12	3.0 ± 0.3	85.0 ± 8.3	ELI	DEN	HIG	6–15 mm	STR
GLINL	330.0 ± 22.3	11.2 ± 0.9	540.0 ± 68.1	OVO	DEN	MED	<5 mm	STR
FTNL	235.0 ± 12.3	6.6 ± 0.8	140.0 ± 10.7	ELI	MED	ESC	6–15 mm	STR
RPL	325.0 ± 21.2	12.3 ± 0.7	332.0 ± 32.8	OVO	DEN	ABS	ABS	ABS
CSM	375.0 ± 52.3	11.2 ± 0.9	445.0 ± 42.3	SPH	DEN	ABS	ABS	ABS
SMM	394.0 ± 62.1	13.0 ± 1.1	450.0 ± 61.9	OVO	DEN	ABS	ABS	ABS
MCSH	155.0 ± 32.2	3.3 ± 0.3	73.0 ± 23.3	ELI	DEN	HIG	6–15 mm	STR

PLH = plant height (cm); TRD = trunk diameter (cm); CRD = crown diameter (cm); CRS = crown shape; DRB = density of branches; SPD = spine density; SPC = spine length; SPS = spine shape; OVO = ovoid; ELI = ellipsoid; SPH = spheroid; DEN = dense; MED = medium; ABS = absent; HIG = high; STR = straight; RYM011 = (RPL × YMCT- 005) × MCP-011; RYM015 = (RPL × YMCT-005) × MCP-015; RYM016 = (RPL x YMCT-005) × MCP-016; GM002 = GLINL × MCP-002; GF001 = GLINL × FTNL-001; RYG001 = (RPL × YMCT-005) × GLINL-001; RM002 = RPL × MCSH 002; CM002 = CSM × MCP-002; CM003 = CSM × MCP-003; RYS014 = (RPL × YMCT-005) × SMM-014; RY005 = RPL × YMCT-005; MCP = *Microcitrus papuana* Winters; GLINL = 'Galego Inerme Key' lime [*Citrus aurantiifolia* (Christm.) Swingle]; FTNL = *Fortunella* sp.; RPL= 'Rangpur' lime (*C. limonia* Osbeck); CSM = common 'Sunki' mandarin [*C. sunki* (Hayata) hort. ex Tanaka]; SMM = 'Sunki Maravilha' mandarin; YMCT = 'Yuma' citrange [*C. sinensis* (L.) Osbeck × *Poncirus trifoliata* (L.) Raf.]; MCSH = *Microcitrus* Sydney hybrid [*M. australis* (A. Cunn. ex Mudie) Swingle × *M. australasica* (F. Muell.) Swingle].

of spines is not a determinant for use as hedges, their existence along with dense crown are good attributes for this category, mainly for the purpose of protection and/or security [8]. The leaves of this hybrid are green and small (average length of 38 cm and width of 1.5 cm – *table II*), the fruits are spherical, like those of the female parent, on average measuring 3.8 cm in length and 3.6 cm in diameter, and were judged highly attractive (*table III*), especially because they are produced in large quantity throughout the year. These traits qualify this genotype for use as potted plants, for production of minifruits and for landscaping.

The hybrid RYM015 (*figures 1b* and *1c*) has characteristics related to structure similar to those of the previous hybrid. The plants also have medium size, with average height of 184 cm and dense ellipsoid crown, with a large number of spines on the branches (*table I*). Hence, it is also appropriate for use as hedges. Its leaves are green, small and elliptical (*table II*). The fruits are elongated, similar to the male parent, *M. papuana*. They measure approximately 9.0 cm long and 3.2 cm in diameter (*table III*). This hybrid is also distinct regarding its flowers (*table IV*), typically with three, four or five petals (*figure 1c* – detail). All these traits make it suitable as a potted plant, for production of minifruits and for landscaping.

The hybrid RYM016 (*figure 1d*) has small to medium size, with height of 170 cm. Its crown is very dense and ovoid and its branches have high thorn density (*table I*). The leaves are very small, measuring only 2.9 cm in length and 1.5 cm in diameter. They are obovate and dark green (*table II*). The fruits are spherical, with a neck at the apex, and measure 3.8 cm in length and 3.3 cm in diameter. When mature, they have a pleasing light yellow color (*table III*), which is an advantage for ornamentation. This hybrid is considered suitable for use as hedges, potted plants, landscaping and production of minifruits.

These three hybrids can be used for ornamental purposes due to their beauty and originality, and also considering their good resistance to the tristeza virus. The crown shape and density are adequate for topiary size control and the fruits are highly attractive besides being produced in large quantities.

It is important to stress that the hybrids were evaluated in the form of seedlings (ungrafted plants), so their height and crown diameter can be reduced by grafting on dwarfing rootstocks. The practice of grafting can also promote faster flowering and consequently fruit production. With respect to spines, if this is considered undesirable, it can be prevented by using buds for propagation taken from branches related to more advanced vegetative generations, *i.e.*, ontogenetically

Table II. Morphological traits of the leaves of 10 hybrid citrus seedlings with potential for ornamental use and their parentals. Values for quantitative descriptors are the mean of 20 replications followed by the standard errors.

Hybrids	LFL	LFW	LFD	LVA	LCO	LLI	LLS	LLM	LAP	PHY	PHL	PHW	PHS
RYM011	3.78 ± 0.18	1.55 ± 0.08	SIM	ABS	MED	SES	ELP	DEN	ATT	ABS	ABS	ABS	ABS
RYM015	4.62 ± 0.16	2.17 ± 0.10	SIM	ABS	MED	SES	OBO	DEN	ATT	ABS	ABS	ABS	ABS
RYM016	2.89 ± 0.10	1.53 ± 0.11	SIM	ABS	DAR	SES	OBO	DEN	ATT	PRE	0.46 ± 0.05	0.10 ± 0.03	OBO
GM002	2.92 ± 0.12	1.36 ± 0.09	SIM	ABS	MED	SES	ELP	DEN	ATT	ABS	ABS	ABS	ABS
GF001	3.60 ± 0.15	1.90 ± 0.07	SIM	ABS	MED	SES	ELP	CRE	ATT	ABS	ABS	ABS	ABS
RYG001	5.98 ± 0.28	4.11 ± 0.15	SIM	ABS	LIG	SES	ORB	CRE	ROU	PRE	0.40 ± 0.03	0.20 ± 0.02	OBC
RM002	3.80 ± 0.23	1.30 ± 0.06	SIM	ABS	DAR	SES	ELP	CRE	ATT	ABS	ABS	ABS	ABS
CM002	4.40 ± 0.16	2.80 ± 0.09	SIM	ABS	DAR	SES	ELP	DEN	ATT	PRE	0.40 ± 0.02	0.10 ± 0.02	OBC
CM003	4.90 ± 0.19	2.20 ± 0.10	SIM	ABS	DAR	SES	ELP	WAV	ATT	ABS	ABS	ABS	ABS
RYS014	10.2 ± 0.38	3.70 ± 0.12	SIM	ABS	MED	BRE	ELP	WAV	ATT	PRE	1.20 ± 0.08	0.30 ± 0.03	OBD
Parentals	LFL	LFW	LFD	LVA	LCO	LLI	LLS	LLM	LAP	PHY	PHL	PHW	PHS
RY005	6.30 ± 0.20	3.20 ± 0.18	SIM	ABS	MED	BRE	OVA	CRE	ATT	PRE	1.00 ± 0.06	0.30 ± 0.02	OBO
MCP	2.28 ± 0.10	0.96 ± 0.08	SIM	ABS	MED	SES	OVA	CRE	ATT	ABS	ABS	ABS	ABS
GLINL	9.12 ± 0.23	4.49 ± 0.12	SIM	ABS	LIG	SES	OVA	WAV	ATT	PRE	1.09 ± 0.06	0.36 ± 0.02	OBD
FTNL	8.28 ± 0.18	2.88 ± 0.07	SIM	ABS	MED	SES	ELP	CRE	ACM	ABS	ABS	ABS	ABS
RPL	10.10 ± 0.35	5.70 ± 0.09	SIM	ABS	MED	SES	ELP	CRE	ATT	PRE	1.00 ± 0.05	0.40 ± 0.03	OBO
CSM	6.34 ± 0.18	3.39 ± 0.10	SIM	ABS	DAR	SES	ELP	WAV	ATT	ABS	ABS	ABS	ABS
SMM	6.81 ± 0.15	3.00 ± 0.08	SIM	ABS	DAR	SES	OVA	CRE	ATT	ABS	ABS	ABS	ABS
MCSH	2.15 ± 0.10	0.88 ± 0.06	SIM	ABS	MED	SES	OVA	CRE	ATT	ABS	ABS	ABS	ABS

LFL = leaf length (cm); LFW = leaf width (cm); LFD = leaf division; LVA = leaf variegation; LCO = leaf color; LLI = leaf lamina insertion; LLS = leaf lamina shape; LLM = leaf lamina margin; LAP = leaf apex; PHY = phyllode; PHL = phyllode length (cm); PHW = phyllode width (cm); PHS = phyllode shape; SIM = simple; ABS = absent; PRE = present; LIG = light; MED = medium; DAR = dark; SES = sessile; BRE = brevipetiolate; ELP = elliptic; OBO = obovate; OVA = ovate; ORB = orbicular; WAV = wavy; CRE = crenada; DEN = dentada; ATT = attenuate; ROU = rounded; ACM = acuminate; OBO = obovate; OBD = obdeltate; OBC = obcordate; RYM011 = (RPL × YMCT-005) × MCP- 011, RYM015 = (RPL × YMCT-005) × MCP-015, RYM016 = (RPL × YMCT-005) × MCP-016, GM002 = GLINL × MCP-002, GF001 = GLINL × FTNL-001, RYG001 = (RPL × YMCT-005) × GLINL-001, CM002 = CSM × MCP-002, CM003 = CSM × MCP-003, RYS014 = (RPL × YMCT-005) × SMM-014 and RM002 = RPL × MCSH 002; MCP = *Microcitrus papuana* Winters; GLINL = 'Galego Inerme Key' lime [*Citrus aurantiifolia* (Christm.) Swingle]; FTNL = *Fortunella* sp.; RPL= 'Rangpur' lime (*C. limonia* Osbeck); CSM = common 'Sunki' mandarin [*C. sunki* (Hayata) hort. ex Tanaka]; SMM = 'Sunki Maravilha' mandarin; YMCT = 'Yuma' citrange [*C. sinensis* (L.) Osbeck × *Poncirus trifoliata* (L.) Raf.]; MCSH = *Microcitrus* Sydney hybrid [*M. australis* (A. Cunn. ex Mudie) Swingle × *M. australasica* (F. Muell.) Swingle].

adult branches, on which spines are very sparse or absent in citrus in general [17,18]. Studies of compatibility between varieties for grafting and thorn characteristics need to be carried out to confirm this assertion.

The hybrid GM002 (*figure 1e*) is the result of crossing GLINL ['Galego Inerme Key' lime (*C. aurantiifolia*)] and *M. papuana*. It has a height of 230 cm and a very dense crown with many spines (*table I*), traits inherited from *M. papuana.* Its leaves are small, approximately 2.9 cm long and 1.4 cm wide (*table II*), also related to the influence of its parentage of the genus *Microcitrus*. Its fruits have rough peels and are elongated like those of *M. papuana*. When ripe, they have a pleasing yellow color (*table III*), making them adequate to use for producing minifruits. This genotype can also be used to form hedges, as potted plants and for landscaping. As is the case of the 10 other hybrids evaluated, this one can be used as a potted plant even though the height and crown diameter exceed the standards established for this purpose, because of its outstanding decorative features, as long as it is propagated from dwarfing rootstocks or is pruned regularly.

The hybrid GF001 (*figures 1f* and *1g*) comes from crossing 'Galego Inerme Key' lime and *Fortunella* sp. (FTNL). The plant is short to medium in height, at 190 cm, and has a dense ellipsoid crown (*table I*). Its leaves are elliptical and small (3.6 cm long by 1.9 cm wide) (*table III*). The fruits are also ellipsoid with a neck, and are light yellow when ripe (*table III*). It also has good decorative potential, indicated in the potted plant, landscaping and ornamental minifruit plant categories This hybrid results from crossing genotypes that have ornamental potential, the 'Galego Inerme Key' lime, which has a dense crown, small fruits and no spines, and *Fortunella* spp., which has small size and produces small yellowish fruits with smooth peel [8]. The ornamental use of fortunella plants is widespread, especially in Europe [19,20].

The hybrid RYG001 (*figures 1h* and *1i*) has small size, reaching only 160 cm in height. The crown is very voluminous and the thorn density is low (*table I*). During the entire

Table III. Morphological traits of the fruits of 10 hybrid citrus seedlings with potential for ornamental use and their parentals. Values for quantitative descriptors are the mean of 20 replications followed by the standard errors.

Hybrids	FR	FR	FR	FS	FS	FR	IF	RF	FS	AL	PU	NF	AF
RYM011	3.77 ± 0.21	3.61 ± 0.18	SP	CO	RO	AB	GR	LY	SM	WH	GR	3.40 ± 0.50	2–4
RYM015	8.98 ± 0.33	3.24 ± 0.11	OT	MA	NE	AB	GR	LY	RG	WH	YE	1.30 ± 0.22	2–4
RYM016	3.93 ± 0.18	3.28 ± 0.21	SP	CO	NE	AB	GR	LY	SM	WH	YE	1.40 ± 0.18	2–4
GM002	7.40 ± 0.24	3.10 ± 0.16	OT	MA	NE	AB	GR	YE	RG	GR	GR	1.30 ± 0.25	2–4
GF001	6.30 ± 0.29	3.70 ± 0.26	EL	MA	NE	AB	GR	LY	SM	WH	GR	1.20 ± 0.33	2–4
RYG001	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
RM002	3.50 ± 0.15	2.70 ± 0.10	EL	AC	TR	AB	GR	DY	SM	GR	GR	1.20 ± 0.12	2–4
CM002	4.70 ± 0.19	2.90 ± 0.18	EL	MA	NE	AB	GR	LY	SM	WH	YE	1.00 ± 0.10	2–4
CM003	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
RYS014	3.30 ± 0.11	3.90 ± 0.21	OB	TR	TR	AB	GR	OR	SM	OR	OR	1.60 ± 0.15	2–4
Parentals	FR	FR	FR	FS	FS	FR	IF	RF	FS	AL	PU	NF	AF
RY005	4.10 ± 0.25	3.80 ± 0.10	SP	TR	TR	AB	GR	DY	SM	WH	YE	1.20 ± 0.15	2–4
MCP	5.45 ± 0.38	1.90 ± 0.08	OT	OT	OT	AB	GR	YE	RG	GR	GR	1.25 ± 0.20	2–4
GLINL	3.46 ± 0.18	3.94 ± 0.12	SP	RO	CO	AB	GR	LY	SM	WH	GR	1.80 ± 0.20	2–4
FTNL	2.65 ± 0.12	2.50 ± 0.20	EL	RO	CO	AB	GR	OR	SM	WH	YE	1.25 ± 0.25	2–4
RPL	5.80 ± 0.22	6.30 ± 0.30	SP	TR	CO	AB	GR	OR	SM	WH	OR	1.40 ± 0.10	2–4
CSM	3.35 ± 0.20	4.20 ± 0.18	OB	OT	TR	AB	GR	OR	SM	OR	OR	9.55 ± 0.50	+6
SMM	3.42 ± 0.16	4.30 ± 0.30	OB	TR	TR	AB	GR	OR	SM	OR	OR	9.20 ± 0.75	+6
MCSH	4.75 ± 0.28	1.73 ± 0.18	OT	OT	OT	AB	GR	YE	RG	GR	GR	1.20 ± 0.25	2–4

FRL = fruit length (cm; FRD = fruit diameter (cm); FRS = fruit shape; FSB = shape of fruit base; FSA = shape of fruit apex; FRV = fruit variegation; IFC = immature fruit colo; RFC = ripe fruit colo; FST = fruit surface texture; ALC = albedo colo; PUC = pulp colo; NFB = number of fruits per bunc; AFB = average amount of fruits per bunc; ELL = ellipsoid; SPH = spheroi; OBL = obloi; OTH = other; TRU = truncat; ROU = rounded; MAM = mammiform; ACU = acute; CON = conve; NEC = necke; ABS = absen; GRE = green; YEL= yellow; LYE = light yello; DYE = dark yellow; ORA = orang; RGH = rough; SMO = smoot; WHI = white; GRS = greenis; NOB = not observe; RYM011 = (RPL × YMCT- 005) × MCP-011; RYM015 = (RPL × YMCT-005) × MCP-015; RYM016 = (RPL × YMCT-005) × MCP-016; GM002 = GLINL × MCP-002; GF001 = GLINL × FTNL-001; RYG001 = (RPL × YMCT-005) × GLINL-001; RM002 = RPL × MCSH-002; CM002 = CSM × MCP-002; CM003 = CSM × MCP-003; RYS014 = (RPL × YMCT-005) × SMM-014; RY005 = RPL × YMCT 005; MCP = *Microcitrus papuana* Winter; GLINL =‘Galego Inerme Ke’ lime *[Citrus aurantiifolia* (Christm.) Swingle; FTNL = *Fortunella* sp.; RPL= ‘Rangpu’ lime *(C. limonia* Osbeck; CSM = common ‘Sunk’ mandarin *[C. sunk*i (Hayata) hort. ex Tanaka; SMM = ‘Sunki Maravilh’ mandari; YMCT = ‘Yuma’ citrange *[C. sinensis* (L.) Osbeck x *Poncirus trifoliata* (L.) Raf.; MCSH = *Microcitru*s Sydney hybrid *[M. australi*s (A. Cunn. ex Mudie) Swingle × *M. australasic*a (F. Muell.) Swingle].

evaluation period, this hybrid did not flower or produce fruits, an advantageous trait for planting in pots and for landscaping, mainly using the topiary technique.

The hybrid CM002 (*figure 1j*) is also a hybrid with strong ornamental potential. It has a short stature, reaching only 150 cm in height. The crown is very dense and has a large number of small spines (*table I*). It can be used to form hedges, and mainly in the construction of ornamental screens, because it responds well to topiary. Its leaves are small, measuring about 3.8 cm in length by 1.3 cm in width (*table II*). The fruits are small and ellipsoid, with mean width of 3.5 cm and diameter of 27 cm, with attractive dark yellow color (*table III*). This genotype, together with the dwarfing rootstock HTR-010, a trifoliate hybrid developed by the CBP, is very small, measuring only 25 cm in height in its adult phase [21]. This behavior indicates that the association of ornamental scions with dwarfing rootstocks can enhance the decorative characteristics. This will be the subject of future study of the genotypes presented here.

We tested two crosses between CSM and MCP: CM002 (*figures 1k* and *1l*) and CM003 (*figure 1m*). Both are tall, having reached respective heights of 337 cm and 247 cm. They both have dense ellipsoid crowns with branches starting at the base, giving them a cylindrical shape. They have long spines on the branches (*table II* and the leaves have similar size, shape and color (*table II*).

The hybrid CM002 produces small ellipsoidal fruits with neck, measuring 4.7 cm in length and 2.9 cm in diameter. When ripe, the fruits are light yellow, giving them high ornamental value. This hybrid can be used for the production of ornamental minifruits, as well as in the landscaping and hedge categories CM003 did not produce fruits or flowers during the study period, and is indicated for landscaping and hedges. The failure to produce fruits is probably due to the relatively long juvenile period of this genotype.

The hybrid RYS014 was the last of the 10 hybrids characterized (*figures 1n* and *1o*). It presented a height of 222 cm, with a spheroid crown and branches with dense and long spines

Table IV. Morphological traits of the flowers of 10 hybrid citrus seedlings with potential for ornamental use and their parentals.

Hybrids	COF	AFL	PFI	ARO	ARP
RYM011	WHI	SOL, INF	AXI TER	FRU	PER
RYM015	WHI	SOL, INF	AXI, TER	FRU, FLO	PER
RYM016	WHI	SOL, INF	AXI, TER	FRU, FLO	PER
GM002	WHI	SOL, INF	AXI, TER	FRU	PER
GF001	WHI	SOL, INF	AXI, TER	FRU, FLO	NPE
RYG001	NOB	NOB	NOB	NOB	NOB
RM002	WHI	SOL, INF	AXI, TER	FRU, FLO	PER
CM002	WHI	SOL, INF	AXI, TER	FRU, FLO	PER
CM003	WHI	SOL, INF	AXI, TER	FRU	PER
RYS014	WHI	SOL, INF	AXI, TER	FRU, FLO	PER
Parentals	COF	AFL	PFI	ARO	ARP
RY005	WHI	SOL, INF	AXI, TER	FRU, FLO	NPE
MCP	WHI	SOL, INF	AXI, TER	FRU	NPE
GLINL	WHI	SOL, INF	AXI, TER	FRU, FLO	NPE
FTNL	WHI	SOL, INF	AXI, TER	FRU, FLO	NPE
RPL	WHI	SOL, INF	AXI, TER	FRU, FLO	PER
CSM	WHI	SOL, INF	AXI, TER	FRU	NPE
SMM	WHI	SOL, INF	AXI, TER	FRU, FLO	NPE
MCSH	WHI	SOL, INF	AXI, TER	FRU	NPE

COF = colour of open flower; AFL = arrangement of flowers; PFI = position of flower or inflorescence; ARO = aroma; ARP = aroma persistence; WHI = white; NOB = not observed; SOL = solitary; INF = inflorescence; AXI = axillary; TER = terminal; FRU = fruits; FLO = flowers; NPE = not persistent; PER = persistent; RYM011 = (RPL × YMCT-005) × MCP-011; RYM015 = (RPL × YMCT- 005) × MCP-015; RYM016 = (RPL × YMCT-005) × MCP-016; GM002 = GLINL x MCP-002; GF001 = GLINL × FTNL-001; RYG001 = (RPL × YMCT-005) × GLINL-001; RM002 = RPL × MCSH-002; CM002 = CSM × MCP-002; CM003 = CSM × MCP-003; RYS014 = (RPL × YMCT-005) × SMM-014; RY005 = RPL × YMCT-005; MCP = *Microcitrus papuana* Winters; GLINL = 'Galego Inerme Key' lime [*Citrus aurantiifolia* (Christm.) Swingle]; FTNL = *Fortunella* sp.; RPL= 'Rangpur' lime (*C. limonia* Osbeck); CSM = common 'Sunki' mandarin [*C. sunki* (Hayata) hort. ex Tanaka]; SMM = 'Sunki Maravilha' mandarin; YMCT = 'Yuma' citrange [*C. sinensis* (L.) Osbeck × *Poncirus trifoliata* (L.) Raf.]; MCSH = *Microcitrus* Sydney hybrid [*M. australis* (A. Cunn. ex Mudie) Swingle × *M. australasica* (F. Muell.) Swingle].

(*table I*). It is the most unique of the hybrids studied, with elliptical leaves measuring 10.2 cm in length and 3.7 cm in width (*table II*). Its fruits are micro-tangerines, very similar to the male parent, 'Sunki Maravilha' mandarin. They have dark orange color, with average length of 3.3 cm and diameter of 39 and are highly decorative (*table III*). It has good ornamental potential, for planting in pots, landscaping and production of minifruits.

Except for the hybrid RM002, about which no information was obtained about flowering, and hybrids RYG001 and CM003, the other genotypes studied showed precocity regarding start of flowering and hence fruit production, which occurred at ages between three and four years, in comparison with most other citrus seedlings, which take at least seven years to reach maturity [22]. The early start of flowering is a very important trait, because flower and fruit production are desirable qualities of ornamental plants. The practice of grafting using buds obtained from mature branches in the matrix of each ornamental hybrid is especially favored if using dwarfing rootstocks that induce early flowering, because this enhances the agronomic manifestation of this attribute besides enabling substantial reduction of the size of the scion-rootstock combination.

3.2 CTV-tolerance evaluation

In regard to CTV tolerance, the results were promising with 40% of the hybrids showing resistance, 40% very tolerant and only 20% were very intolerant (*table V*). This evaluation was performed based on ELISA test and the density of grooves in the branches.

Despite the presence of the virus in the hybrids RYM016, RYG001, CM002 and RYS014, they were classified as very tolerant to CTV as the plants were free of grooves (*table V*).

In the hybrid GF001 (*figures 1f* and *1g*) the ELISA tests detected the presence of CTV in the tissues and very dense grooves on the branches, indicating high intolerance to the virus, a characteristic also found in its female parent, *C. aurantiifolia*, a species notably sensitive to the CTV [23]. The hybrid RM002 was also highly intolerant to CTV, having high intensity of grooves in its branches. This behavior of both hybrids indicates the need for protection with mild CTV strains.

This kind of study is fundamental for the adoption of these new hybrids by growers since this disease is really important to the citrus crop. Therefore, the most effective way to control the disease is the use of resistant or tolerant varieties and pre-immunization with mild strains of the virus.

Figure 1. Plants and details of the branches, fruits, leaves and flowers of 10 hybrid seedlings with potential for ornamental use. Citrus Breeding Program of Embrapa Cassava & Fruits. (a) RYM011 = (RPL × YMCT-005) × MCP-011. (b)–(c) RYM015 = (RPL × YMCT-005) × MCP-015. (d) RYM016 = (RPL × YMCT-005) × MCP-016. (e) GM002 = GLINL × MCP-002. (f)–(g) GF001 = GLINL × FTNL-001. (h)–(i) RYG001 = (RPL × YMCT-005) × GLINL-001. (j) RM002 = RPL × MCSH-002. (k)–(l) CM002 = CSM × MCP-002. (m) CM003 = CSM × MCP-003. n-o RYS014 = (RPL × YMCT-005) × SMM-014.

4 Conclusion

The hybrids evaluated presented substantial morphological variability that distinguished them from each other. Their traits qualify them for use as ornamental plants The genus *Microcitrus* has potential for use in crosses aimed at obtaining hybrids with potential ornamental use. The 10 hybrids assessed can be recommended for use for landscaping, and in association with dwarfing rootstocks, as potted plants. Eight can be indicated for use to form hedges and eight for use as ornamental minifruit plants. Of the 10 hybrids evaluated for resistance to the citrus tristeza virus (CTV), four were resistant, three very tolerant and two very intolerant.

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Table V. Presence and intensity of grooves and indirect ELISA test results for citrus tristeza virus (CTV) in 10 hybrid seedlings with potential for ornamental use.

Hybrids	Detection of CTV via indirect ELISA	Mean presence and intensity of grooves caused by CTV[1]	Reaction to CTV
RYM011		1.03 ± 0.03	Resistant
RYM015		1.20 ± 0.10	Resistant
RYM016	+	1.00 ± 0.00	Very tolerant
GM002		1.00 ± 0.00	Resistant
GF001	+	3.90 ± 0.10	Very intolerant
RYG001	+	1.20 ± 0.10	Very tolerant
RM002	+	3.50 ± 0.50	Very intolerant
CM002	+	1.10 ± 0.05	Very tolerant
CM003		1.10 ± 0.05	Resistant
RYS014	+	1.00 ± 0.00	Very tolerant

RYM011 = (RPL × YMCT-005) × MCP-011; RYM015 = (RPL × YMCT- 005) × MCP-015; RYM016 = (RPL × YMCT-005) × MCP-016; GM002 = GLINL x MCP-002; GF001 = GLINL × FTNL-001; RYG001 = (RPL × YMCT-005) × GLINL-001; RM002 = RPL × MCSH 002; CM002 = CSM × MCP-002; CM003 = CSM × MCP-003; RYS014 = (RPL × YMCT-005) × SMM-014; MCP = *Microcitrus papuana* Winters; GLINL = 'Galego Inerme Key' lime [*Citrus aurantiifolia* (Christm.) Swingle]; FTNL = *Fortunella* sp.; RPL= 'Rangpur' lime (*C. limonia* Osbeck); CSM = common 'Sunki' mandarin [*C. sunki* (Hayata) hort. ex Tanaka]; SMM = 'Sunki Maravilha' mandarin; YMCT = 'Yuma' citrange [*C. sinensis* (L.) Osbeck × *Poncirus trifoliata* (L.) Raf.]; MCSH = *Microcitrus* Sydney hybrid [*M. australis* (A. Cunn. ex Mudie) Swingle × *M. australasica* (F. Muell.) Swingle].

[1] Means obtained from analysis of ten branches by three evaluators based on rating scale described by Meissner *et al.* [16].

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