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TWF/40/15 Add.
ORIGINAL: English
DATE: September 22, 2009

UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

TECHNICAL WORKING PARTY FOR FRUIT CROPS

Fortieth Session
Angers, France, September 21 to 25, 2009

ADDENDUM TO

PROPOSAL FOR A PARTIAL REVISION OF THE TEST GUIDELINES FOR MANDARIN (CITRUS GROUP 1)

Document prepared by an expert from Spain

At the fortieth session of the Technical Working Party for Fruit Crops (TWF), Mr. Guillermo Soler Fayos (Spain) made a presentation based on document TWF/40/15. A copy of that presentation follows:

The citrus fruit seedless tendency

The new international research programmes are focused on to get seedless varieties.
One of the methods more used to obtain citrus seedless varieties is the irradiation with gamma rays or neutrons.
This method is very widespread because let to the researcher to correct one or a few negative characteristics without altering the rest of the variety genotype, so we can obtain a variety equal to the initial variety but with the seedless characteristic.
Actually, we can find this kind of research programs all around the world and some of them have been successful. For example we can find five varieties of citrus fruits with commercial interest. Three of these five varieties were treated with gamma rays, whereas in the other two it were used thermal neutrons.

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Which is the problem with the self-incompatible parthenocarpy citrus fruits?

Nowdays, the most citrus varieties cultivated in the world are self-incompatible, so they do not produce seeds by themselves. So we consider that a self-incompatible variety is a seedless variety. But when we grow very close two varieties that can be cross-pollinated both varieties will produce seeds.
The quantity of seeds that we will find in the fruits will depend on the pollen viability and the ovule fertility.
At the moment, in the UPOV test guideline to test the ovule fertility we can find only the character nº 99 “Fruit: number of seeds (open pollination)”, but the quantifying of this character will depend on around varieties, the presence of pollinators and the weather.
Regarding to the pollen viability, in the UPOV test guideline we can find the character nº 25 “Anther: viable pollen” with only two options “absent or present”, but as we have said before the number of seeds that we will find inside another variaty cross-pollinated by this one will depend on the pollen viability.

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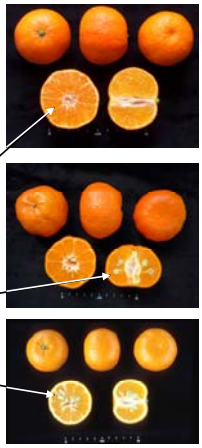
Our proposal

We suggests the incorporation of a new character in the table of characters of DUS to evaluate the ovule fertility by cross pollination with another variety or specie.
The character to add might stay as follows:

CPVO Nº	UPOV Nº	English	español	Example Varieties Variedades ejemplo	Note/ Nota
67 bis. (+)	**98 bis.**	**Fruit: number of seeds (controlled manual cross-pollination)**	**Fruto: número de semillas (polinización manual cruzada controlada)**		
QN		absent or very few	ausentes o muy bajo	Nulessin (CLE), Tango (HMA)	1
		few	bajo		3
		medium	medio	Marisol (CLE)	5
		many	alto		7
		very many	muy alto	Clemenules (CLE)	9



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Our proposal

In our experience, the fertilization of other varieties by manual controlled pollination with the Fortune pollen is a trustworthy, repetitive, reproducible and with sufficient expression variability method. In the next table we can see the Fortune pollination capability.

CUADRO DE POLINIZACIONES – 2004
POLLINATION TABLE-2004

♀ \ ♂	NADORCOTT	ARRUFATINA	BEATRIZ DE ANNA	CLEMENPONS	CLEMENTINA FINA	CLEMENTINA DE NULES	ELLENDALE	MONCADA	ESBAL	FORTUNE	HERNANDINA	LIMON FINO	LIMON VERNA	LORETINA	MARISOL	NOVA	ORITSU	ORONULES	OROVAL	ORTANIQUE	PRIMOSOLE	SALUSTIANA	STAR RUBY	VALENCIA LATE
NADORCOTT						** 6-42,,21		* 9-20,,15		* 6-14,,9	* 9-20,,13	* 7-12,,8	* 6-9,,8		* 5-18,,9	* 5-14,,10				* 0-16,,7				* 5-12,,8
ARRUFATINA							** 0-14,,6	** 4-22,,12		** 8-21,,4		* 4-14,,8	* 1-13,,5			** 8-22,,15				** 0-13,,4				* 0-4,,1
BEATRIZ DE ANNA	** 9-31,,19						* 0-20,,6			* 12-28,,20		** 2-21,,12	* 5-18,,9			* 6-29,,18				* 0-3,,1				
CLEMENPONS	* 4-29,,20							* 0-36,,25		** 0-39,,21		* 3-21,,12	* 0-20,,8			** 1-21,,13				* 0-5,,0	* 0-46,,11			* 0-11,6
CLEMENTINA FINA																* 3-26,,13								
CLEMENTINA DE NULES	* 5-35,,27						* 3-30,,11	*** 8-41,,30		** 11-35,,24		** 7-32,19	* 2-8,,3			*** 0-27,,11				*** 0-15,,2	* 9-41,,26		* 2-21,,10	*** 0-29,,16
ELLENDALE							* 11-21,,14			* 20-40,,32	* 20-40,,30	* 3-5,,4				** 14-50,,36		* 1-18,,10					* 5-32,,16	* 9-40,,21
MONCADA						*** 0-25,,17				** 1-22,,13	* 12-29,,18	* 2-11,,6				** 3-13,6				* 4-7,,6				
ESBAL	* 5-16,,11						* 0-1,,0	* 4-15,,9		** 4-21,,11		** 0-13,,7	* 1-8,,4			* 4-19,,10							* 5-12,,7	* 1-10,,4
FORTUNE	** 18-38,,26	* 1-5,,3	* 13-33,,22			* 18-28,,26	* 16-32,,24	** 10-36,,27	* 23-39,,31		** 12-43,,28	** 3-28,,19	* 1-11,,6	* 5-14,,9	* 13-21,,19	* 1-32,,19		** 21-42,,30	* 7-28,,19	** 7-34,,13	* 13-38,,27	* 2-23,,9	* 9-24,,13	** 13-35,,25
HERNANDINA	*** 2-37,,19						** 0-3,,1	* 23-43,,30		** 10-32,,23		* 9-23,,17	* 3-17,,9			** 7-36,,21				* 0-13,,3				* 3-24,,16
LIMON FINO	** 2-28,,19	* 0-0,,0	* 1-19,,11	** 0-26,,8		* 16-44,,28	* 4-28,,13	* 3-29,,11	* 7-24,,12	** 3-36,,18	* 3-19,,12	** 1-22,,9	* 9-14,,12	* 3-6,,5		** 5-23,,15		* 10-21,,14		** 5-33,,16		* 0-7,,4	** 0-25,10	** 2-26,,14
LIMON VERNA	** 4-16,,12		* 3-18,,10	* 4-14,,9		* 11-25,,17	** 3-13,,6		** 2-10,,5	** 0-17,,8	* 1-15,,8	** 2-10,,5			* 2-17,,8	** 3-17,,9		* 1-13,,5		** 4-14,,8			* 2-13,,7	* 3-9,,6
LORETINA	* 12-23,,17							* 10-33,21		** 2-22,,15		** 0-17,,7	* 0-4,,2			* 12-23,,17				* 3-6,,4				
MARISOL	* 5-15,,9						* 0-6,,2	** 2-15,,8		** 2-15,,8		** 1-13,,5	** 0-17,,4			* 2-9,,6				* 0-0,,0		** 0-0,,0		* 0-2,,2
NOVA	*** 2-40,,30	** 3-25,,9	* 24-45,,36	* 3-26,,9		* 4-40,,23	* 9-32,,22	*** 4-40,,27	*** 13-41,,28	* 17-30,,23	** 11-43,,29	* 11-29,,21	*** 1-32,,13	* 6-45,,32	* 20-36,,30			* 9-42,,26	* 13-37,,29	*** 4-29,,17		* 0-17,,7	* 4-19,,13	** 0-21,,9
ORITSU	** 0-7,,3						** 0-0,,0			** 0-7,,2		* 0-1,,0	* 0-0,,0			** 0-8,,2				* 0-2,,1				
ORONULES	* 7-16,,10						*** 0-18,,6	* 4-21,,13		*** 2-26,,13		** 0-4,,2	* 1-8,,4			*** 4-22,,16				*** 0-17,,8				* 2-8,,5
OROVAL	* 17-34,,25						* 6-14,,9			** 0-15,,10						** 0-16,,7								
ORTANIQUE	* 17-34,,25		* 4-19,,13	** 16-38,,25		** 0-15,,4	* 1-7,,2	*** 7-29,,22		** 15-39,,26	* 10-29,,21	** 2-22,17	** 4-21,,16	** 0-0,,0		*** 11-32,,23		* 0-17,,6				* 3-5,,4	* 2-11,,6	* 6-25,,16
PRIMOSOLE						* 0-25,,16				* 11-26,,18						* 0-26,,12				* 0-24,,12				
SALUSTIANA	* 2-7,,4			* 0-0,,0		* 1-11,,4				** 0-8,,3		** 1-4,,2	* 0-3,,2		* 0-2,,1	** 1-6,,4		* 0-2,,1		* 1-5,,3				
STAR RUBY						** 0-15,,3	** 0-6,,2		* 1-5,,3	* 0-7,,3	* 0-5,,2	* 0-2,,0	* 0-11,,2		* 0-4,,2	** 0-3,,2				** 0-5,,2				
DELTA SEEDLESS										* 0-2,,0														
VALENCIA LATE	* 1-13,,5			* 2-10,,5		* 1-10,,4		* 2-8,,5	* 4-9,,7	*** 2-10,,5	* 3-12,,6	** 1-5,,4		* 3-4,,3	* 2-6,,3	** 0-10,,4		* 3-8,,6		*** 2-11,,5				
WINKNIGHT										** 0-3,,1						* 0-1,,0								

♂ Variedades polinizadoras MALE (POLLEN DONOR VARIETY)
♀ Variedades polinizadas FEMALE (POLLINATED VARIETY)
Las dos primeras cifras de las casillas indican el menor y mayor número de semillas encontradas; la tercera cifra corresponde a la media.
* Datos de un año. ** Media de dos años. *** Media de tres años
Sin semillas (cruzamiento incompatible) Seedless (no compatible crossing)
Sin datos No data available

1st and 2º Figures are minimum and maximum number of seeds per fruit; 3rd figure is the average.
* 1 year data ** 2 years average ** 3 years average

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Our proposal

On the other hand, we suggest the modification of the character UPOV nº 25 based on studies realized in the Instituto Valenciano de Investigaciones Agrarias (IVIA) that shows the capability of pollen germination of a great number of varieties of citrus fruits, with trustworthy, repetitive, reproducible and with sufficient expression variability differences.

	Pollen germination percentage		
	Año 2003	Año 2004	Año 2005
CLEMENTINES			
Arrufatina	45,2	2,7	50,4
Capola	74,2	59,7	-
Clemenpons	72,8	41,2	-
Clemenules	74,0	61,0	-
Esbal	61,5	48,1	68,8
Fina	79,7	70,0	-
Hernandina	71,9	77,4	-
Loretina	57,4	48,3	-
Marisol	35,5	18,2	42,2
Orogrande	75,5	44,0	-
Oronules	74,4	55,4	-
Oroval	45,5	42,7	55,6
MANDARINS			
Ellendale	84,9	86,3	-
Fortune	84,6	74,5	-
Mandarino Comun	73,5	67,9	-
Moncada	49,0	58,1	82,6
Murcott	43,9	49,8	51,8
Murcott sd	36,3	28,1	48,7
Nadorcott	74,5	69,3	83,6
Nova	74,9	62,8	-
Ortanique	76,1	58,4	-
Primosole	40,6	36,9	-
SATSUMAS			
Okitsu	3,8	5,9	-
Owari	5,1	4,6	-
ORANGES			
Barberina	6,8	19,5	9,4
Bernalina	-	7,6	2,6
Delta	1,8	1,5	-
Midknight	-	-	2,4
Salustiana	14,4	6,9	-
Sanguinelli	22,8	21,8	-
Sucreña	46,4	41,1	60,2
Valencia Late	15,5	15,1	-
GRAPEFRUITS			
Marsh	20,6	8,7	17,2
Rio Red	22,0	3,0	24,5
Star Ruby	3,1	0,6	-
LEMONS			
Bétera	13,3	4,8	10,2
Eureka	21,6	18,6	-
Fino	53,4	44,4	40,2
Verna	20,1	23,4	-

- No data available

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So we propose the substitution of the currently classification (absent or present), by a more detailed classification. An example of the modified character can be seen in the table below:

CPVO Nº	UPOV Nº	English	español	Example Varieties Variedades ejemplo	Note/ Nota
18.	**25.**	**Anther: viable pollen**	**Antera: polen viable**		
QL		absent or very few	ausentes o muy bajo	Owari (SAT)	1
		medium	medio	Marisol (CLE)	5
		many	alto	Fortune (HMA)	9

Methodology

Determination of the ovule fertility

The methodology for the evaluation of the ovule fertility consists of pollinating manually at least 10 flowers per tree for each new variety under examination and another 10 flowers per tree of the similar variety. The flowers pollinated are protected by an individual mesh.
In our opinion the pollen from the Fortune variety is one of the best to realize the fertilization but it can be chosen the pollen from any other variety with a high fertilization capability (see Anex I).
Afterwards, it is necessary to count the number of seeds of each fruit.

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Methodology

Determination of the pollen viability

The flowers must be collected when begins the petal opening but when anthers are closed. The anthers are introduced into a Petri dish and placed inside the silica gel dryer at room temperature, about 20-48 hours of darkness. When the anthers are opened they must be put during an hour into a 8 ºC chamber with a 70-80 % Relative Humidity. Afterwards, the pollen must be sowed with a brush onto a microscope slide with 2 ml of Brewbacker medium (Brewbaker and Kwack. 1963). Finally the microscope slide must be put during 20 hours into a 24 ºC chamber with a 75 % RH.
The percentage of pollen fertilization is calculated by getting the average of pollen grains germinated observed with a binocular in 15 visual fields from 2 different microscope slides.

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