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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
Geneva

TECHNICAL WORKING PARTY FOR FRUIT CROPS

Forty-Third Session

Beijing, July 30 to August 3, 2012

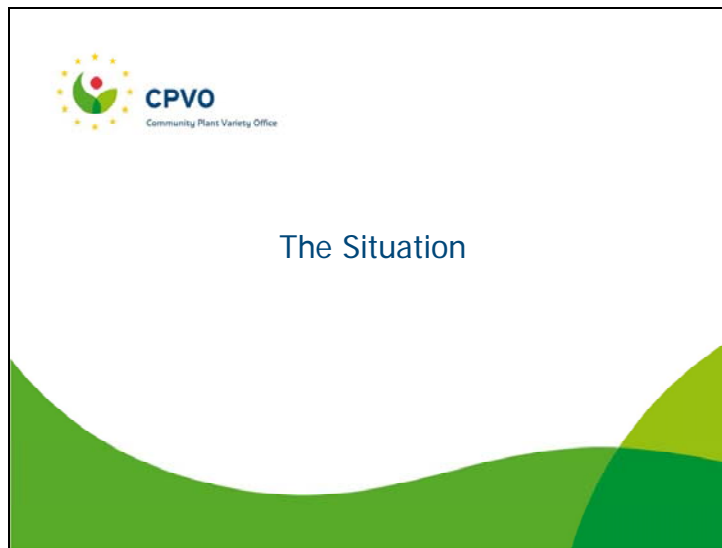
ADDENDUM

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

Document prepared by the Office of the Union

1. This document contains the presentations made in relation to the proposal for a partial revision of the test guidelines for mandarins, made at the Technical Working Party for Fruit Crops (TWF), at its forty-third session.
2. Annex I to this document contains a copy of the presentation prepared by an expert from the European Union.
3. Annex II to this document contains a copy of the presentation prepared by an expert from Morocco.
4. Annex III to this document contains a copy of the presentation prepared by an expert from Spain.

[Annex follows]



Revision of the mandarin guideline

- Mandarin test guidelines were adopted in 2003
- Since then, problems occurred in varieties supposed to be seedless with the presence of seeds derived from unwanted cross pollination with other neighboring plantations



Revision of the mandarin guideline

- Breeding programs have developed in order to improve the situation with 2 kinds of methodologies:
 - triploid varieties (sterile pollen)
 - irradiation of varieties to reduce their fertility.Irradiated varieties have a reduced ovule fertility and produce no seeds independently from pollinators that might be present in the vicinity



Revision of the mandarin guideline

- Proposal for a partial revision of the test guideline in order to address these new types:
 - update characteristic 25 '*Anther viable pollen*', currently with two states of expression, 'Absent' – 'Present' where differences observed in the pollen germination percentage cannot be reflected with only two states. Proposal to move to a quantitative characteristic.
 - Proposal not contested



Revision of the mandarin guideline

- Proposal for a partial revision of the test guideline in order to address these new types:
 - to introduce a new characteristic to evaluate the ovule fertility by cross pollination with another variety or species: '*Fruit: number of seeds (controlled manual cross-pollination)*'
 - Proposal that needs further investigations



Revision of the mandarin guideline

- The TWF agreed in 2011 that
 - The methodology for observing this characteristic should be well defined and tested
 - The wording of the characteristic might need to be reviewed



Revision of the mandarin guideline

- The TWF designed a subgroup
 - Morocco
 - South Africa
 - Spain
 - Coordination by the CPVO



Revision of the mandarin guideline

- The subgroup agreed on a protocol drafted on the basis of the Spanish proposal and after comments of other subgroup members in order to test the methodology for the assessment of the characteristic in question
- Work in the field started in March 2012



About the presence of seeds in Citrus varieties

Revision of the mandarin guideline

Incompatibility issues in Citrus are recognized to be gamete related: they are based on the unsuccessful growth of pollen tubes because the S allele carried by the haploid male pollen matches either of the two S alleles existing in the diploid maternal tissue of the pistil. It should be specified that clementines are self incompatible.



Revision of the mandarin guideline

- Consequence: the observation of the absence or the presence of few seeds for a variety can have different reasons (or combinations thereof)
 1. The pollen of the pollinator is not viable
 2. The pollen of the pollinator is incompatible with the stigma of the candidate variety
 3. Ovules of the candidate variety have a reduced fertility.



Revision of the mandarin guideline

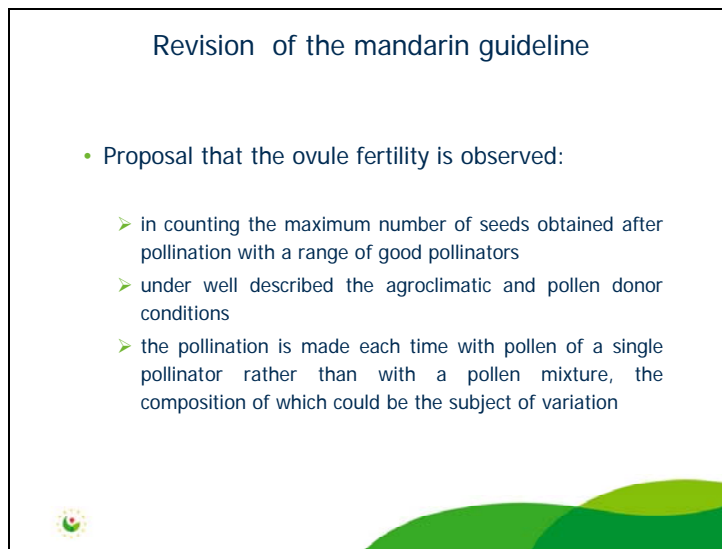
- It is important to define precisely what is intended to be observed and to define precisely the method of observation
 - Otherwise, a characteristic like 'Fruit : Number of seeds' might be the subject of very different observations depending on the pollination environment, might thus not be repeatable and finally not fulfill the definition of a UPOV characteristic.



Revision of the mandarin guideline

- Objective of the characteristic: to assess the ovule or female fertility of the variety
 - the pollinator and receiver should be fully compatible
 - No publication about groups of compatibility in Citrus
 - Molecular techniques to assess the compatibility of 2 Citrus varieties do not seem to be available
 - How to make sure that the absence of seeds is linked to weak ovule fertility and not to an incompatibility between the pollinator and the candidate variety?





Revision of the mandarin guideline

- For the purpose of the ring test, identification of varieties to be used as pollinators (male) and as receptors (female) available for all partners
 - Possibility to draw a pollination table
 - Comparison of the consistency of results
 - At the end of the ring test, conclusion whether the characteristic can be reliably assessed or not



Revision of the mandarin guideline

- The pollination table

		POLLINATOR	
		'Nova'	'Nadorcott'
Female	'Marisol'		
	'Clemenules' = 'Nules'		
	'Nadorcott'		
	'Nova'		





Revision of the mandarin guideline

- Hand pollination
- Conditions of the Pollen of the Donor
 - Viability : germination capacity at least 50%, assessment after pollen culture, detailed methodology is defined in the protocol
 - Pollen from male varieties will be at its optimal fertility

The pollen of the pollinator variety is extracted at the moment of maximum development of the flower, before the anther dehiscence, detailed methodology defined in the protocol

The image shows the CPVO logo in the bottom left corner and a decorative graphic of overlapping green and yellow shapes at the bottom right of the slide.

Revision of the mandarin guideline

- Conditions of the Female flowers
 - 50 terminal flowers from each female variety will be used, if possible 10 flowers from 5 trees. If less than 5 trees, the number of trees to be pollinated should be specified
 - Flowers will be fully developed but not yet opened: one day before anthesis



Revision of the mandarin guideline

- Conditions of the Female flowers
 - Flowers will be emasculated and bagged to avoid self and free pollination
 - Stigma of female varieties will be at its optimum stage of receptivity. Alternatively, 50 flowers a day will be cross-pollinated by hand respectively 1 and 6 days after anthesis.



Revision of the mandarin guideline

- Agroclimatic conditions
 - Wet days are not suitable and the temperature should be above 20°



Revision of the mandarin guideline

- Methodology of Hand pollination
 - Pollination is made each time with pollen of a single pollinator
 - Pollination should take place at the moment of maximum development of the flower
 - Quantity of pollen : to reach the saturation of the stigma, at least 100 grains of pollen, detailed methodology defined in the protocol



Revision of the mandarin guideline

- Methodology of Hand pollination
 - Pollen will be applied by a brush
 - After pollination, the pollinated flowers should be isolated from the environment with an individual mesh in order to avoid further pollination
 - If felt necessary, 48 hours after pollination, the germination of pollen on stigma will be checked, detailed methodology described in the protocol



Revision of the mandarin guideline

- Observation of results
 - Fruits produced from manual pollination should be identified
 - Seeds produced in each individual resulting fruit must be counted and registered when the fruits are at stage of fruit maturity or one month before to get an average of the number of seeds per fruit. The number of fruits observed for each variety should be reported.



Revision of the mandarin guideline

- First results expected for the spring 2013
- 2 years might be foreseen



Thank you for your attention



[Annex II follows]



Ring test Protocol Report of Morocco



1. Pollinators and females used

		POLLINATOR	
		'Nova'	'Nadorcott'
Female	'Marisol'		
	'Clemenules' = 'Nules'		
	'Nadorcott'		
	'Nova'		

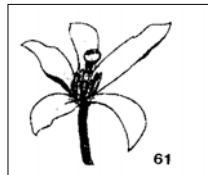


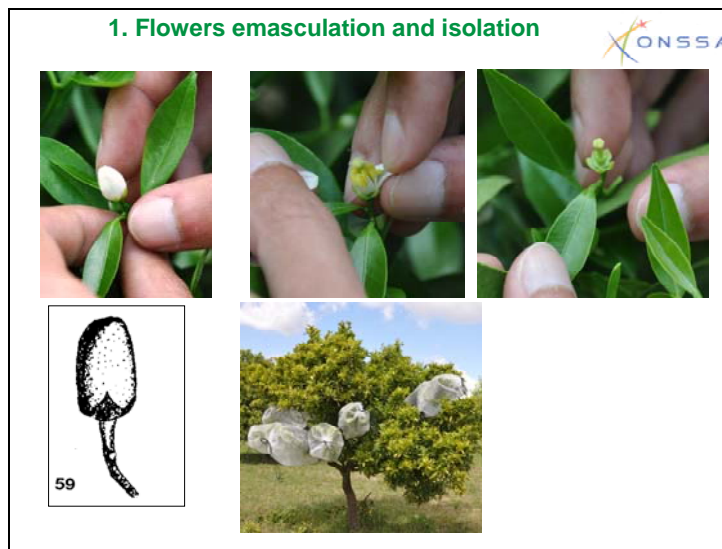
The ring test is carried out in two areas :

- SOUIHLA, Marrakech area.
- EL MENZH and BELKSSIRI, Gharb area.



Pollen preparation



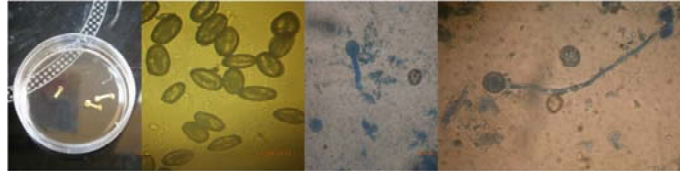








**In vivo test of pollen germination to determinate
stigate receptivity**



SOUHLA

	Marisol	Nules	NOVA	NADORCOTT
Number of flowers pollinated	767	1079	1458	705

EL MENZEH

Variétés	Marisol	Nules	NOVA	NADORCOTT
Number of flowers pollinated	886	927	-	357

BELKSIRI

Variétés	Marisol	Nules	NOVA	NADORCOTT
Number of flowers pollinated	-	-	685	-



Marisol pollinated with Afourer ('Nadorcott')



Nules pollinated with Afourer ('Nadorcott')







Nova pollinated with Afourer ('Nadorcott')



[Annex III follows]

	ivia instituto valenciano de investigaciones agrarias		UETIV Unidad de Examen Técnico de Identificación Varietal C/da. Moncada-Náquera Km 4.5, 46113 Moncada, Valencia, España. Tel: 96 384 8000
<p>UETIV (TECHNICAL EXAMINATION UNIT FOR VARIETY IDENTIFICATION)</p> <p>MANDARIN TRIAL FOR NEW CHARACTER: CONTROLLED MANUAL CROSS-POLLINATION</p> <p>Guillermo Soler Aurelio Buj</p> <p>Moncada 27th of July of 2012</p>			

	ivia instituto valenciano de investigaciones agrarias		UETIV Unidad de Examen Técnico de Identificación Varietal C/da. Moncada-Náquera Km 4.5, 46113 Moncada, Valencia, España. Tel: 96 384 8000
<p>SPANISH TRIAL PROCEDURE</p> <ul style="list-style-type: none">• On 7th of May 2012, more than 100 flowers per variety of the varieties Marisol and Clemenules were bagged, and more than 50 flowers per variety of the varieties Nadorcott and Nova. 			

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SPANISH TRIAL PROCEDURE

- On 10th of May 2012, 50 flowers per variety of the varieties Marisol and Clemenules were pollinated with pollen (collected the day before) of Nadorcott and Nova (25 flowers each one). Moreover 25 flowers per variety of the varieties Nadorcott and Nova were pollinated with pollen of Nova and Nadorcott respectively.


Nadorcott


Nova

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SPANISH TRIAL PROCEDURE

- On 16th of May 2012, 50 flowers per variety of the varieties Marisol and Clemenules were pollinated with pollen of Nadorcott and Nova (25 flowers each one). Moreover 25 flowers per variety of the varieties Nadorcott and Nova were pollinated with pollen of Nova and Nadorcott respectively.


Fruits appearance from pollinated flowers, nowadays



[End of Annex III and of document]