



TWF/42/19

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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

TECHNICAL WORKING PARTY FOR FRUIT CROPS

Forty-Second Session
Hiroshima, Japan
November 14 to 18, 2011

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

Document prepared by an expert from Spain

1. The Test Guidelines for Mandarin (document TG/201/1) were adopted in 2003. However, there have been applications for new types of mandarin varieties which are not adequately covered by the current Test Guidelines. To address those new types, it is proposed that there be a partial revision of the Test Guidelines in order to update characteristic 25 “Anther: viable pollen”, and to add a new characteristic.
2. At present, characteristic 25 “Anther: percentage of viable pollen” has two states of expression, “absent” or “present”. Differences observed in the pollen germination percentage cannot be reflected with only two states. This characteristic supposes one of the main targets in the new international improvement programs in citrus fruits. It is proposed that the states “absent” or “present” be substituted by a more detailed classification.
3. Given the use of the new methods to obtain new citrus varieties and as a consequence of the applications for new varieties with reduced fertility, it is necessary to introduce a new characteristic to evaluate the ovule fertility by cross-pollination with another variety or species.
4. At its fortieth session, held in Angers, France, from September 21 to 25, 2009, the Technical Working Party for Fruit Crops (TWF) considered a proposal for a partial revision of the Test Guidelines for Mandarin (Citrus Group 1) (document TG/201/1).

5. The subgroup for the Test Guidelines for Mandarin considered document TWF/40/15 and a presentation at the session, a copy of which is provided as document TWF/40/15 Add. (see http://www.upov.int/restrict/en/twf/index_twf40.html). It was agreed that, in order to provide all interested experts with additional time to check the proposed partial revision, the proposal should be circulated to the TWF for agreement by correspondence. It was agreed that the proposal should also include the explanation for the new characteristic, as it would be provided in Chapter 8. If no comments were received, it was proposed that the partial revision should be put forward for adoption by the Technical Committee in 2010 (see document TWF/40/17 “Report”, paragraphs 49 and 50).

6. On the above basis, Circular E-1143, was issued on November 30, 2009, containing the proposed partial revision concerning a change to the states of expression for characteristic 25 “Anther: viable pollen” and the introduction of a new characteristic “Fruit: number of seeds (controlled manual cross-pollination)”. It was also noted that the changes to the Test Guidelines for Mandarin would need to be reflected in the overall Table of Characteristics included in the Annex to documents TG/83/4, TG/201/1, TG/202/1, TG/203/1 and TG/204/1, by means of a partial revision to those Test Guidelines.

7. The proposal presented in Circular E-1143 is provided in Annex I to this document.

8. In response to Circular E-1143, comments were received from Australia, Morocco and the International Community of Breeders of Asexually Reproduced Ornamental and Fruit-Tree Varieties (CIOPORA), copies of which are reproduced as Annex II, Annex III and Annex IV to this document, respectively.

9. With regard to the comments of CIOPORA, a reply was sent to Mr. Krieger by Mrs. Bronislava Bátorová, Chairperson of the TWF, and Mr. Peter Button, Technical Director of UPOV, in which it was explained that the basis for considering characteristics in the Test Guidelines is set out in document TG/1/3 “General Introduction to the Examination of Distinctness, Uniformity and Stability and the Development of Harmonized Descriptions of New Varieties of Plants” (General Introduction), Chapter 4.2 “Selection of Characteristics” and Chapter 4.8, Table “Standard Test Guidelines Characteristic”. In addition, CIOPORA was informed that its concerns regarding the examination of distinctness would be reported to the Technical Committee (TC) for consideration at its forty-sixth session, to be held in Geneva from March 22 to 24, 2010. A copy of the reply to CIOPORA is provided in Annex V to this document.

10. At its forty-sixth session, held in Geneva from March 22 to 24, 2010, the Technical Committee concluded as follows (see document TC/46/15 “Report on the Conclusions”):

“9. The TC noted the comments made by the International Community of Breeders of Asexually Reproduced Ornamental and Fruit-Tree Varieties (CIOPORA) in relation to the use of characteristics in the examination of distinctness, as reported in document TC/46/3, paragraphs 7 to 13. The TC endorsed the clarification that had been provided to CIOPORA by the Office of the Union, as set out in document TC/46/3, paragraph 14 [see Annex V to this document], and recalled that CIOPORA were invited to attend the Technical Working Parties and the Technical Committee where they could receive further explanation of the basis for the selection of characteristics for inclusion of Test Guidelines. It was agreed that the adoption of the Test Guidelines for Mandarin should not be delayed on the basis of the comments by CIOPORA.

- “10. The TC noted that, with regard to the partial revision of the Test Guidelines for Mandarin (Citrus Group 1), as set out in Circular E-1143, dated November 30, 2009, in addition to the comments of CIOPORA, substantial comments had been received from Australia and Morocco. On the basis of those substantial comments, the Leading Expert, Mr. Chomé Fuster (Spain), had concluded that it would be appropriate for the partial revision of the Test Guidelines for Mandarin to be considered further by the TWF at its forty-first session, to be held in Cuernavaca, Morelos State, Mexico, from September 27 to October 1, 2010.”
11. On the basis of the discussions at the TC, Circular E-1187, dated February 12, 2010, was issued to the TWF explaining that “On the basis that substantial comments were received in response Circular E-1143, the Leading Expert, Mr. Chomé Fuster (Spain), has concluded that it would be appropriate for the partial revision of the Test Guidelines for Mandarin to be considered further at the TWF at its forty-first session, to be held in Cuernavaca, Morelos State, Mexico, from September 27 to October 1, 2010.”.
12. With regard to the comments from Morocco (see Annex III to this document) concerning the proposed new characteristic “Fruit: number of seeds (controlled manual cross-pollination)”, information was provided by experts from *Instituto Valenciano de Investigaciones Agrarias* (IVIA) and *Unidad de Examen Técnico de Identificación Varietal* (UETIV), a copy of which are reproduced in Annex VI to this document. Their guidance is summarized as follows:

Comment 1: expression depends of the origin of the pollen
(Vithanage (1991), Nadori *et al* (1996), etc.)

Explanation by IVIA and UETIV: the methodology for the determination of ovule fertility that we use for controlled manual cross-pollination requires that the same pollinator variety is used for all varieties examined. The pollinator variety is a variety that has good production of pollen: one example is variety ‘Fortune’, but other varieties could also be used, e.g. ‘Ellendale’, ‘Nadorcott’, ‘Mandarino Común’, etc.. The observation is made on the candidate variety and a reference variety. Therefore, the source of variability suggested by Nadori et al (1996), is removed.

Comment 2: expression depends of the agroclimatic conditions
(Nadori *et al* (1996))

Explanation by IVIA and UETIV: all candidate and reference varieties are grown in the same plot. Such an approach is a normal means of addressing potential variation due to different agroclimatic conditions.

Comment 3: expression depends of the conditions of the receiving variety , as
ovule longevity, compatibility, etc. (Mesejo *et Al* (2007), Pardo *et al* (2007))

Explanation by IVIA and UETIV: it is confirmed that the expression of controlled manual cross-pollination is influenced by the pollinated variety, which is the reason why it is an appropriate DUS characteristic.

13. At its forty-first session held in Cuernavaca, Morelos State, Mexico, from September 27 to October 1, 2010, the Technical Working Party for Fruit Crops (TWF) discussed document TWF/41/28, as presented by Mr. Pedro Miguel Chomé Fuster and Mr. Guillermo Soler Fayos (Spain).

14. The TWF agreed to propose to the Technical Committee to adopt the partial revision of the Test Guidelines for Mandarin on the basis of document TWF/41/28 (copy provided at http://www.upov.int/export/sites/upov/restrict/en/twf/41/twf_41_28.pdf) with the reservation of experts from Morocco with regard to the proposed new characteristic (after characteristic 98) “Fruit: number of seeds (controlled manual cross-pollination)”, for which the experts from Morocco explained that more time was needed for study of the new characteristic. The TWF agreed that the Technical Committee should be invited to consider the “Comments of Morocco concerning the new characteristics proposed ‘Fruit: number of seeds (controlled manual crosspollination) and pollen viability in the UPOV Test Guidelines for Mandarin”, as set out in Annex VII to this document, in conjunction with its consideration of the proposed partial revision of the Test Guidelines for Mandarin.

15. At its meeting on January 6, 2011, the Enlarged Editorial Committee (TC-EDC) concluded that there were technical issues to be resolved and recommended that those issues be referred back to the TWF for further consideration.

16. The TC, at its forty-seventh session, held in Geneva from April 4 to 6, 2011, agreed that, as explained in document TC/47/24, there were technical issues to be resolved concerning the proposed revision of the Test Guidelines for Mandarins (Citrus; Group 1) (document TG/201/1) and recommended that those issues be referred back to the TWF for further consideration.

17. The response of the Leading Expert to the comments of the experts from Morocco is provided in Annex VIII to this document.

PROPOSAL

18. The proposal for a partial revision of the Test Guidelines for Mandarin is set out in Annex IX to this document.

[Annexes follow]

ANNEX I

*Proposal for a partial revision of the Test Guidelines for Mandarin (Citrus Group 1)
(document TG/201/1) – as presented in Circular E-1143 of November 30, 2009*

prepared by an expert from Spain

7. Table of Characteristics/Tableau des caractères/Merkmalstabelle/Tabla de caracteres

Characteristic 25

Current wording

25.	Anther: viable pollen	Anthère: pollen viable	Anthere: keimfähiger Pollen	Antera: polen viable	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
QL	(b) absent	absent	fehlend	ausente	Owari (SAT)	1
[239]	present	présent	vorhanden	presente		9

Proposed new wording

25.	Anther: viable pollen	Anthère: pollen viable	Anthere: keimfähiger Pollen	Antera: polen viable	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
QN	(b) absent or very low	absent ou très faible	fehlend oder sehr gering	ausente o muy bajo	Owari (SAT)	1
	low	faible	gering	bajo		3
	medium	moyen	mittel	medio	Marisol (CLE)	5
	high	élevé	hoch	alto		7
[239]	very high	très élevé	sehr hoch	muy alto	Fortune (HMA)	9

Proposed New Characteristic (after characteristic 98)

NEW	Fruit: number of seeds (controlled manual cross-pollination)	Fruit: nombre de pépins (allogamie manuelle contrôlée)	Frucht: Anzahl Samen (manuelle kontrollierte Fremdbe-fruchtung)	Fruto: número de semillas (alógama manual controlada)	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
(+)						
QN	(c) absent or very few	absents ou très peu nombreux	fehlend oder sehr gering	ausentes o muy bajo	Nulesin (CLE)	1
	few	peu nombreux	gering	bajo		3
	medium	moyen	mittel	medio	Marisol (CLE)	5
	many	nombreux	groß	alto		7
	very many	très nombreux	sehr groß	muy alto	Clemenules (CLE)	9

8.2 Explanations for individual characteristics

Ad. NEW Fruit: number of seeds (controlled manual cross-pollination)

Method: 10 flowers should be observed on each tree. The 10 flowers should be protected from pollination by use of an individual mesh on each flower. Manual cross-pollination of the 10 flowers should be done with pollen from a variety with a high fertilization capability, such as 'Fortune'.

Observation: The number of seeds should be counted in each of the fruits.

Partial revision of Annex to documents TG/83/4, TG/201/1, TG/202/1, TG/203/1 and TG/204/1

The changes to the Test Guidelines for Mandarin would also be reflected in the overall Table of Characteristics included in the Annex to documents TG/83/4 (Trifoliate Orange (*Poncirus*) (*Citrus* L. - Group 5)), TG/201/1, TG/202/1 (Oranges (*Citrus* L. - Group 2)), TG/203/1 (Lemons and Limes (*Citrus* L. - Group 3)) and TG/204/1 (Grapefruit and Pummelo (*Citrus* L. - Group 4)) by means of a partial revision to those Test Guidelines.

[Annex II follows]

ANNEX II

From: Nik.Hulse@ipaaustralia.gov.au
Sent: jeudi, 17. décembre 2009 04:30
To: mail, Upov
Cc: Doug.Waterhouse@ipaaustralia.gov.au
Subject: Re: E-1143: Test Guidelines for Mandarin (Citrus Group 1): Proposal for a Partial Revision [SEC=UNCLASSIFIED]
Attachments: E_1143_Mandarin.pdf; disclaimer.txt

Dear Peter

I have consulted with the relevant crop experts and there is no substantial objection to the proposed partial revision. Generally it is considered that these changes are useful and will assist in the DUS testing of new Mandarin varieties. There are, however, some comments on additional information that may also be considered for inclusion to provide further guidance for the DUS examiner. It is also noted that these characteristics should remain unasterisked.

Characteristic 25

In the meeting in Angers there was some discussion on a method of determining pollen viability. This involved placing suitable anthers in a petri dish under controlled conditions of temperature and relative humidity before recording germinated pollen under a microscope.

Proposal: To provide guidance, perhaps the method and observation should be included in Chapter 8.2 similar to the method and observation proposed for determining the number of seeds. In addition, an indication of the percentage range for the states of expression could be included in Chapter 8.2. For example absent or very low may be 0 to 10%, medium 50-60% and very high >95%. (see GN19(4) in TGP 7/1). These ranges are only indicative and could be changed.

New Characteristic Fruit: number of seeds

The number of seeds will depend on the pollinator. If 'Fortune' is used then the example varieties may be a useful indicator of the state of expression. However, in many cases it will not be possible to use 'Fortune'. Consequently, in Chapter 8.2, inclusion of the range of seed counts for each state of expression would be useful.

Proposal: In chapter 8.2 after "Observation: The number of seeds should be counted in each of the fruits" an indication of the range of counts for each of the states of expression should be included. For example absent or very low may be 0 to 2, medium 5 to 7 and very high >20. (see GN19(4) in TGP 7/1). These ranges are only indicative and could be changed.

I will be on leave from today until 4th January.

Best wishes for Christmas and the New Year.

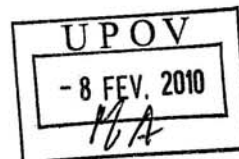
Kind regards

Nik

Nik Hulse

Senior examiner, Plant Breeder's Rights Office IP Australia
Tel 02 6283 7982 Fax 02 6283 7999

[Annex III follows]



N° 114 ONSSA/DCTP/CSP/BH

Rabat, le 04 FEV 2010

Objet : Projet d'amendement des Principes directeurs d'examen pour le Mandarinier

Faisant suite à nos courriers échangés en date du 15 octobre 2009 et 04 novembre 2009 et relatifs au projet de modification partielle des Principes directeurs d'examen pour le Mandarinier, proposé par l'Espagne et examiné lors de la réunion du Groupe Technique de Travail des espèces fruitières qui s'est tenue à Angers du 21 au 25 septembre 2009 (document TXF/40/15), j'ai l'honneur de vous faire parvenir ci-joint une note qui relate les arguments scientifiques et techniques qui appuient l'objection du Maroc quant à l'adoption de telles modifications. La note susvisée, démontre que ces modifications ne sont ni pertinentes ni nécessaires pour distinguer entre deux variétés de Mandarinier.

En outre, je vous saurais gré de bien vouloir transmettre la position du Maroc ainsi que la note jointe aux membres du Groupe Technique de Travail des espèces fruitières ainsi qu'aux membres et observateurs du Comité Technique.

Veuillez agréer, Monsieur le Secrétaire Général, l'expression de mes salutations distinguées.

**MONSIEUR LE SECRETAIRE GENERAL
DE L'UNION POUR LA PROTECTION
DES OBTENTIONS VEGETALES**

Le Directeur Général de l'Office
National de Sécurité Sanitaire
des Produits Alimentaires

Hamid BENAZZOU



NOTE

RELATIVE AU PROJET DE MODIFICATION DES PRINCIPES DIRECTEURS D'EXAMEN POUR LE MANDARINIER

Dans la version d'origine (Ch4.2 : Choix des caractères, Principes directeurs UPOV pour le mandarinier), le caractère 25 "Anthères : pollen viable", est noté par deux états d'expression : "absent ou présent". La subdivision de la notation de ce caractère en différents degrés (très faible, faible, moyen, élevé et très élevé), telle qu'elle est rapportée dans le document TWF/40/15, reste valable et répond toujours aux exigences des principes directeurs d'examen (Document TG/1/3 "Introduction générale à l'examen de la distinction, de l'homogénéité et la stabilité des descriptions des obtentions végétales", Chapitre 4.2 : "Choix des caractères"). En effet, dans ses différents niveaux d'expression, ce caractère est entièrement lié à la variété concernée.

Pour ce qui est du nombre de pépins issus d'une allopollinisation, celui-ci par contre peut varier selon l'origine du pollen ainsi que la variété qui reçoit le pollen. Ce constat est rapporté dans plusieurs travaux scientifiques anciens et récents (De Langc et al., 1974; Burger 1985; Vithanage 1991; Burdette 1993; Futch and Jackson 2003; Kahn et Chao 2004 ; Chao 2005; Mesejo et al. 2007 ; Papadakis et al. 2009 ; Schneider et al., 2009). Ainsi, Vithanage (1991) a étudié l'effet de l'origine du pollen sur le nombre de pépins produits par la variété mandarine 'Ellendale', en utilisant comme parents pollinisateurs les mandariniers 'Imperial', 'Siiverhill' satsuma, 'Emperor', 'Dancy', et 'Murcott' et l'oranger 'Valencia'. L'auteur a montré que le nombre de pépins produits par la variété 'Ellendale' varie significativement selon le parent utilisé. En effet, le pollen des variétés 'Murcott' et 'Emperor' a induit la formation d'un nombre élevé de pépins, par contre, celui de la variété 'Imperial' a engendré le nombre le plus bas de pépins.

Par ailleurs, une étude réalisée au Maroc pour évaluer le risque d'inter pollinisation entre diverses variétés de mandariniers a montré que le nombre de pépins dans les fruits d'une variété donnée diffère selon les variétés pollinisatrices. Aussi, pour une même variété, le nombre de pépins dans les fruits produits par des fleurs castrées et pollinisées manuellement varie selon les sites et donc les conditions agro-climatiques du verger (Nadori et al., 1996).

Office National de Sécurité Sanitaire des Produits Alimentaires

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الفاكس : 05 37 68 20 49

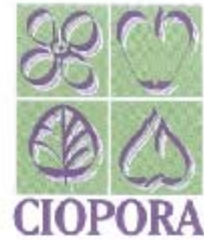
Les raisons de ces différences sont multiples et comprennent entre autre : la période et durée de réceptivité des stigmates, la qualité du pollen et la vitesse et degré de croissance du tube pollinique et sa durée de vie, et la longévité des ovules (Mesejo et al. 2007).

Il ressort de ces travaux que le nombre de pépins produits par fruit issus de l'allo pollinisation n'est pas un caractère spécifique pour caractériser une variété donnée. L'instabilité de ce critère le rend non fiable et inefficace pour distinguer entre différentes variétés. Donc, il ne pourra pas être pris en compte dans les descripteurs de l'UPOV pour la description et la distinction des variétés du groupe du mandarinier.

Références bibliographiques

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- Papadakis I.E., Protopapadakis E.E. et Therios I.N., 2009. Yield and fruit quality of 'Nova' hybrid [*Citrus clementina* hort. ex Tanaka x (*C. reticulata* Blanco x *C. paradisi* Macfaden)] and two Clementine varieties (*C. clementina* hort. ex Tanaka) as affected by self- and cross-pollination. Sci. Hort. 121: 38-41.
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**Communauté Internationale des Obtenteurs de Plantes
Ornementales et fruitières de Reproduction Asexuée**



Via e-mail
INTERNATIONAL UNION FOR THE PROTECTION OF
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Technical Working Party for Fruit Crops (TWF)

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Internet: www.ciopora.org

Hamburg, 04 January 2010

**E-1143: Test Guidelines for Mandarin (Citrus Group 1): Proposal for a Partial
Revision**

Dear members of the TWF,

Making reference to the E-mail of 30 November 2009 and the before mentioned proposal CIOPORA asks to postpone the decision to put forward the partial revision to the Technical Committee and instead to discuss the matter again in the TWF meeting in Cuernavaca in 2010.

CIOPORA was not yet able to finally evaluate the justification of the partial revision of the TG/201/1. However, members in particular have expressed concern that the proposed change could lead to too small minimum distances between varieties, which would make the exclusive right of the title holder of the first variety worthless.

Against the background of the potentially significant negative consequences of a change in the test guideline CIOPORA is of the opinion that the proposed revision should be studied further.

Respectfully yours,

CIOPORA

Dr. Edgar Krieger
Secretary General

From: mail, Upov
Sent: jeudi, 4. février 2010 12:10
To: 'edgar.krieger@ciopora.org'
Cc: 'chris.barnaby@pvr.govt.nz'; Button, Peter; 'brislava.batorova@uksup.sk'
Subject: RE: AW: E-1143: Test Guidelines for Mandarin (Citrus Group 1): Proposal for a Partial Revision

CIOPORA February 4, 2010

Dr. Edgar Krieger
Executive Secretary
CIOPORA
Düsternstrasse 3
20355 Hamburg
Germany
e-mail: edgar.krieger@ciopora.org

cc: Chris Barnaby (Chairman of the Technical Committee)
Email: chris.barnaby@pvr.govt.nz

Dear Dr. Krieger,

Thank you for your e-mail of January 4, 2010, concerning circular E-1143 "Test Guidelines for Mandarin (Citrus Group 1): Proposal for a Partial Revision".

The Office of the Union received comments from Australia and CIOPORA concerning circular E-1143. Copies of those comments will be circulated to the Technical Working Party for Fruit Crops (TWF) together with an amended proposal concerning a partial revision of the Test Guidelines for Mandarin (Citrus Group 1), for agreement by the TWF by correspondence.

With regard to your comment that the proposed change to the Test Guidelines for Mandarin could lead to too small minimum distances between varieties, it is recalled that the basis for considering characteristics for inclusion in the Test Guidelines are set out in document TG/1/3 "General Introduction to the Examination of Distinctness, Uniformity and Stability and the Development of Harmonized Descriptions of New Varieties of Plants" (General Introduction), Chapter 4.2 and Chapter 4.8 as follows:

"4.2 Selection of Characteristics

"4.2.1 The basic requirements that a characteristic should fulfill before it is used for DUS testing or producing a variety description are that its expression:

"(a) results from a given genotype or combination of genotypes
(this requirement is specified in Article 1(vi) of the 1991 Act of the UPOV Convention but is a basic requirement in all cases);

"(b) is sufficiently consistent and repeatable in a particular environment;

"(c) exhibits sufficient variation between varieties to be able to establish distinctness;

"(d) is capable of precise definition and recognition
(this requirement is specified in Article 6 of the 1961/1972 and 1978 Acts of the UPOV Convention, but is a basic requirement in all cases);

"(e) allows uniformity requirements to be fulfilled;

"(f) allows stability requirements to be fulfilled, meaning that it produces consistent and repeatable results after repeated propagation or, where appropriate, at the end of each cycle of propagation.

“4.2.2 It should be noted that there is *no* requirement for a characteristic to have any intrinsic commercial value or merit. However, if a characteristic that is of commercial value or merit satisfies all the criteria for inclusion it may be considered in the normal way.

“4.2.3 For inclusion in the Test Guidelines, further criteria are set out in section 4.8, “Functional Categorization of Characteristics” and in document TGP/7, “Development of Test Guidelines.” The characteristics included in the individual Test Guidelines are not necessarily exhaustive and may be expanded with additional characteristics if that proves to be useful and the characteristics meet the conditions set out above.”

[...]

“4.8 Functional Categorization of Characteristics

“The following table categorizes the way in which characteristics can be used in the examination and the appropriate criteria.

“TABLE. FUNCTIONAL CATEGORIES OF CHARACTERISTICS

Type	Function	Criteria
“Standard Test Guidelines Characteristic	1. Characteristics that are accepted by UPOV for examination of DUS and from which members of the Union can select those suitable for their particular circumstances.	1. Must satisfy the criteria for use of any characteristic for DUS as set out in Chapter 4, section 4.2. 2. Must have been used to develop a variety description by at least one member of the Union. 3. Where there is a long list of such characteristics and, where considered appropriate, there may be an indication of the extent of use of each characteristic.
[...]”		

CIOPORA’s general concerns regarding minimum distances between varieties will be reported to the Technical Committee (TC) at its forty-sixth session, to be held in Geneva from March 22 to 24, 2010, within document TC/46/3 “Matter arising from the Technical Working Parties”, for consideration under agenda item 5 of the TC agenda. In addition to your comments on the partial revision of the Test Guidelines for Mandarin, CIOPORA’s comments on worries with regard to minimum distances in the “Exercise on Color”, considered by the Technical Working Party for Ornamental Plants and Forest Trees (TWO) at its forty-second session, held in Angers, France, from September 14 to 18, 2009 (see document TWO/42/13, Annex VII, pages 3 and 4 and main document “Summary of the Comments”, paragraph 9) will also be reported in document TC/46/3.

Yours sincerely,

Bronislava Bátorová, Chairperson of the Technical Working Party for Fruit Crops

Peter Button
Technical Director

[Annex VI follows]

Information provided by experts from *instituto valenciano de investigaciones agrarias (IVIA)* and *Unidad de Examen Técnico de Identificación Varietal (UETIV)*



UETIV
Unidad de Examen Técnico
de Identificación Varietal
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REFERENTE A LA REVISIÓN PARCIAL DEL TEST GUIDE LINE PARA MANDARINOS, GROUP 1 OF CITRUS , UPOV TG 201/1

En respuesta a la carta remitida por Hamid Benazzou manifestamos lo siguiente:

Lo que se pretende con el carácter “fruto: número de semillas” es determinar la capacidad de una variedad para producir semillas cuando es polinizada por otra variedad que presenta una alta fertilidad del polen.

Antecedentes

Tal y como se ha descrito en los diferentes estudios realizados hasta el momento, el número de semillas que presenta una variedad en polinización cruzada depende principalmente de la variedad receptora del polen (Mesejo et al. 2007), lo que nos indica que entre las variedades receptoras de polen hay diferencias en la capacidad de producir semillas que se pueden evaluar.

Además de la variedad receptora del polen, el número de semillas que produce un fruto en polinización cruzada depende también del origen del polen, tal y como describe Vithanage (1991), por lo que para evitar esta variabilidad utilizamos polen de la misma variedad (por ejemplo: Fortune, Ellendale, Nadorcott y Mandarino común) tanto para la variedad de referencia como para la variedad candidata, haciendo la polinización manualmente. De esta manera se elimina la variabilidad sugerida por Nadori et al. (1996) y que es debida al polinizador.

Dado que, tal y como describe Nadori et al. (1996), las condiciones agroclimáticas influyen en el número de semillas, nosotros proponemos que tanto la variedad de referencia como la candidata estén en la misma parcela de ensayo.

Por otro lado, en los estudios realizados por Pardo et al. (2007) y Mesejo et al. (2007) se observa que al utilizar polen con un alto porcentaje de germinación la capacidad de producir semillas depende, tal y como se describe en su carta, de la variedad receptora del polen, bien por la compatibilidad del tubo polínico y el estigma o de la longevidad de los óvulos, lo cual es precisamente lo que se pretende evaluar con este carácter del número de semillas.

Metodología propuesta

A continuación se describe detalladamente la metodología de nuestra propuesta mediante la cual se minimizan los inconvenientes que según ustedes podrían surgir en la realización del ensayo.

La polinización cruzada se lleva acabo depositando, con un pincel, polen con un porcentaje de germinación superior al 50% (previa comprobación mediante un cultivo de polen) de la variedad polinizadora sobre el estigma de la variedad a polinizar. Esta operación se repite en 10 flores por cada uno de los cinco árboles disponibles para los exámenes de DHE

situados en una misma parcela de ensayo, y por tanto, bajo las mismas condiciones agroclimáticas.

El polen de la variedad polinizadora se extrae de flores en el momento de máximo desarrollo de la flor, antes de que se produzca la dehiscencia de las anteras, y habiendo sido seleccionadas en el estado fenológico de plena floración. Las anteras se colocan en cajas Petri e introducidas en un desecador que contiene gel de sílice de 20 a 48 horas, hasta que las anteras se abren. El desecador se mantiene en oscuridad y a temperatura ambiente (Pardo et al. 2007).

Al llegar la maduración del fruto, se cuentan las semillas en al menos 25 frutos.

Moncada, 15 de febrero de 2010

Fdo.

Dr. Vicent Cebolla

Director de la Unidad de Examen Técnico de Identificación
Varietal

Instituto Valenciano de Investigaciones Agrarias

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[Annex VII follows]

ANNEX VII

**COMMENTS OF MOROCCO CONCERNING THE NEW CHARACTERISTICS
PROPOSED “FRUIT: NUMBER OF SEEDS (CONTROLLED MANUAL
CROSSPOLLINATION) AND POLLEN VIABILITY IN THE UPOV TEST
GUIDELINE FOR MANDARIN
*October 1, 2010***

Presented at the forty-first session of the TWF in 2010
(see document TWF/41/30 Rev. “Revised Report”, Annex VI)

Comment 1: Ovule fertility

In the UPOV test guideline to test the ovule fertility we can find only the character n° 99 “Fruit: number of seeds (open pollination)”. This character corresponds to the study of female fertility under open-pollination conditions.

The Spanish proposal on this character, namely the female fertility involves the introduction of a new character 98 bis, which is the study of female fertility by cross-pollination.

However:

1- It was shown that the study of female fertility in citrus is very effective under open pollination conditions than hand pollination (**Masahi et al., 1995**).

2- Brown and Krezdorn. (1969), reported that standard pollination tests involving massive applications of pollen alone are not sufficient to delineate those varieties which are good pollinators and to distinguish the degree of female fertility. Indeed, They do not take into account species or variety preference by the bees, the amount of pollen carried by bees, the number of visits bees make to citrus flowers and the amount of pollen produced by flowers of given varieties. These factors ignored in the new proposed character are taken into account in the character 99 (UPOV test guideline) which corresponds to the study of female fertility.

Comment 2: Pollen viability

In the original version (Ch4.2: Choice of characters, UPOV Guidelines for the mandarin), the character 25 "Anthers: pollen viable," is noted by two states of expression: "absent or present. To change this character by the addition of different expression levels of pollen viability, the Spanish proposal was based on the fact that the number of seed in the fruit depends on the pollen viability.

However:

1-In a study it was reported that The reduced seediness in the Orlando tangelos set by Minneola pollen cannot be attributed to low viability of the pollen because Minneola pollen on King orange flowers produced the highest degree e of seediness of all the combinations tested, with an average of 30 seeds in King fruit (**Philip. et al. 1961**).

2- Masashi et al. (2006) in a study designed to investigate the compatibility and

incompatibility between the tangerine and the variety Ariake that pollen tube growth in styles of Ariake x clementine and reciprocal cross combination, Clementine x Ariake was inhibited, although both accessions could produce viable pollen.

The number of seed in the fruits depends on compatibility of the pollen with the stigma of the female variety, and pollen viability rather than only the degree of pollen viability.

Conclusions

Based on these arguments, the ovule fertility can be estimated by open pollination rather than hand pollination and the new character proposed by *expert from Spain* would not be added in the UPOV

The number of seed in the fruits depends on pollen compatibility with the stigma of the female variety, and pollen viability rather than only the degree of pollen viability.

On the other hand we support the remarks made by Australian delegation concerning the conditions of experimentation regarding hand pollination. This supposes that in experimentation we should use source of pollen which in practice is not practicable.

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[Annex VIII follows]

ANNEX VIII

**RESPONSE BY EXPERTS FROM SPAIN TO THE COMMENTS OF MOROCCO
OF OCTOBER 1, 2010**

Regarding last comments from Morocco experts concerning the proposed new characteristic “Fruit: number of seeds (controlled manual cross-pollination)” the point of view of experts from *Instituto Valenciano de Investigaciones Agrarias* (IVIA) and *Unidad de Exament Técnico de Identificación Varietal* (UETIV) is the following:

Comment 1: Ovule fertility.

1.- By no means at all, anyone can infer from the Masashi et al. (1995) study that open pollination is more effective than hand pollination. The results presented in the study by Masashi et al. (1995) shows that open pollination had a good efficiency *in relation* with hand pollination. But, on the contrary, Masashi et al. (1995) state that “*in all cultivars*, average number of seeds per fruit was greater by hand pollination than by open pollination”. Furthermore, Masashi et al. (1995) also concluded that “female fertility is directly related to seediness”. Therefore, when it is critical to precisely determine the level of female fertility of a certain variety without interferences from environmental hazardous restrictions, citrus researches have always used hand pollination instead of open pollination even with different goals. These are just two recent and illustrative examples: a) Ngo et al. (2010) to identify self-incompatibility S genotypes in citrus cultivars, and b) Mesejo et al. (2006) to study *in vivo* citrus pollen germination. In addition, it has also been recently demonstrated in two very closed related citrus lines (Usach et al., 2011) that hand pollination also produces more number of seeds than open pollination in these varieties and therefore hand pollination shows greater efficiency to discriminate with higher accuracy the degree of citrus female fertility also in very related varieties. These observations are of particular relevance because the study was conducted during 6 consecutive years with identical results.

Aside from research, and in the experimental field, hand pollination has also been the choice to determinate the pollination relationships between most important citrus commercial varieties in the Spanish Citrus Industry (see annex of our proposal). Moreover, Soler (1999) in a divulgative publication dealing with the field recognition of citrus varieties also reported on hand pollination relationships rather than upon open pollination.

2.- The goal of the other work (Brown and Krezdorn. 1969) cited by the Moroccan experts was to evaluate the capacity of pollinators in citrus. This aim is not directly related to the determination of the female fertility of a variety, but rather to its capability as pollinator. Since female fertility is the core of our proposal, this study has very little to add to the argument that open pollination is a more convenient method to test female fertility than hand pollination. Actually, the study clarifies that, on the contrary, hand pollination avoids all variability and inconsistencies arising in non-controlled environments that are inherent to open pollination, such as preferences of bees, the amount of pollen carried by bees, the number of bees visiting citrus flowers and the amount of pollen produced by unknown species or lines. All these parameters that may be relevant to identify good citrus pollinators are the major source of inconsistency when trying to determine with accuracy female fertility, and therefore should be avoided through the simple use of hand pollination.

Since the time of this publication, 40 years ago, good citrus pollinators have been identified, are well established in the Citrus Industry and they have been tested and used infinite folds as excellent candidates for hand pollination.

3.- **In conclusion**, the aim of our proposal is to distinguish between closed related varieties that exhibit such a different female fertility ability that can offer significant advantages or disadvantages regarding the presence of seeds in fruit, and therefore to condition the agronomical value of the yield.

To test female fertility in citrus and according to what has been exposed above, hand pollination is: a) more trouble-free, b) more effortless, c) more precise and d) more convenient than open pollination.

- a) Hand pollination is more trouble-free because is not subjected to hazardous environmental restrictions, insect preferences, and climatic conditions, than open pollination.
- b) Hand pollination is more effortless because with pollen from a single pollinator, female fertility can be precisely determined, while in open pollination complicated field designs involving pollinators and reference and candidate varieties should be first established for reproducibility.
- c) Hand pollination is more accurate and precise because produces higher number of seeds than open pollination, increasing in this way the differences between both varieties for adequate analysis.
- d) Hand pollination is more convenient because regarding synchrony of pollen maturity and stigma receptivity, reproducibility of results, availability of designed fields, number of tests and personal involved is inexpensive in comparison with the requirements of open pollination

Thus, hand pollination is simply standardised by using pollen of the same good pollinator to saturate the receptivity of the stigma in candidate and reference varieties under the same climatic and environmental conditions.

Comment 2: Pollen viability

1.- Regarding to pollen viability, differences in pollen germination between varieties are widely reported in many international publications such as: Brewbaker et al. (1963), Mesejo et al. (2006), Kumari et al. (2009) and Chaudary et al (2010). This point is well addressed in the character 25 as was accepted, in the last Technical Working Party held in Cuernavaca, Mexico.

2.- The Moroccan experts claim that the proposal is solely based on the fact that the number of seeds depends upon pollen viability. They, then, comment on two examples (Philips et al. 1961 and Masashi et al. 2006) showing that seed number is also dependent on the compatibility of the pollen with the female style. This is a misinterpretation that we would like to make clear. It is very obvious that for a certain female variety, this is, for a certain female fertility, number of seeds in fruit is then dependent of male pollen fertility. The two examples noted by the Moroccan experts are particular cases of cross-incompatibility, but in nature we can find four kinds of varieties: self-compatible, self-incompatible, cross-

compatible and cross-incompatible (Agustí et al. 2003, Mashashi et al. 2006). The aim of this new character is to differentiate between the cross-compatible and cross-incompatible varieties.

After cross pollination on a certain variety with a good pollinator, fruits may still produce a reduced number of seeds because two main reasons:

- 1- Reduced female fertility.
- 2- Gametophytic incompatibility (McClure et al. 1990, Nasrallah et al. 1994, Ngo et al. 2010,) that may be responsible of both self- and cross-incompatibilities and is 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.

In both cases, there will be differences in the seed number of fruit between the reference and the candidate variety.

Conclusions

- a) Based on the above facts, two closed related varieties differing in ovule fertility can be more conveniently differentiated by hand pollination than by open pollination under mayor economical and scientific points of view.
- b)
- c) The proposed methods (hand pollination) are inexpensive, accurate and very easy to perform in experimentation, much more than open pollination.

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[Annex IX follows]

ANNEX IX

Proposal for a partial revision of the Test Guidelines for Mandarin (Citrus Group 1)
(document TG/201/1)

prepared by an expert from Spain

7. Table of Characteristics/Tableau des caractères/Merkmalstabelle/Tabla de caracteres

Characteristic 25

Current wording

25.	Anther: viable pollen	Anthère: pollen viable	Anthere: keimfähiger Pollen	Antera: polen viable	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
QL (b)	absent	absent	fehlend	ausente	Owari (SAT)	1
[239]	present	présent	vorhanden	presente		9

Proposed new wording

25.	Anther: viable pollen	Anthère: pollen viable	Anthere: keimfähiger Pollen	Antera: polen viable	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
QN (b)	absent or very low	absent ou très faible	fehlend oder sehr gering	ausente o muy bajo	Owari (SAT)	1
	low	faible	gering	bajo		3
	medium	moyen	mittel	medio	Marisol (CLE)	5
	high	élevé	hoch	alto	Murcott (HMA)	7
[239]	very high	très élevé	sehr hoch	muy alto	Fortune (HMA)	9

Proposed New Characteristic (after characteristic 98)

NEW	Fruit: number of seeds (controlled manual cross-pollination)	Fruit: nombre de pépins (allogamie manuelle contrôlée)	Frucht: Anzahl Samen (manuelle kontrollierte Fremdbe-fruchtung)	Fruto: número de semillas (alógama manual controlada)	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
(+)						
QN (c)	absent or very few	absents ou très peu nombreux	fehlend oder sehr gering	ausentes o muy bajo	Okitsu (SAT)	1
	few	peu nombreux	gering	bajo	Nulesin (CLE)	3
	medium	moyen	mittel	medio	Esbal (CLE)	5
	many	nombreux	groß	alto	Primsole (HMA)	7
	very many	très nombreux	sehr groß	muy alto	Clemenules (CLE)	9

8.2 Explanations for individual characteristics

Ad. 25: Anther: percentage of viable pollen

Method: The pollen should be collected when the petals begin to open (but with the anthers closed). The anthers should be introduced into a Petri dish and placed inside a silica gel dryer at room temperature, for 20-48 hours of darkness. When the anthers are open they should be moved to a 8 °C chamber with a 70-80 % Relative Humidity for one hour. Afterwards, the pollen should be brushed onto a microscope slide with 2 ml of Brewbacker medium (Brewbaker and Kwack. 1963). Finally, the microscope slide should be placed in a 24 °C chamber with a 75 % RH for 20 hours.

The percentage of pollen fertilization is calculated as the average of germinated pollen grains observed with a binocular in 15 visual fields from 2 different microscope slides.

(Brewbaker, J.L. and Kwack, B.H. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. *Amer. Jour. Botany*. 50: 859-865.)

Percentage range indication for the states of expression:

Example varieties	Note	Range
Owari (SAT)	1	$\leq 7\%$
	2	$> 7 \leq 14\%$
	3	$> 14 \leq 21\%$
	4	$> 21 \leq 28\%$
Marisol (CLE)	5	$> 28 \leq 35\%$
	6	$> 35 \leq 45\%$
Murcott (HMA)	7	$> 45 < 55\%$
	8	$> 55 < 65\%$
Fortune (HMA)	9	$\geq 65\%$

Ad. NEW Fruit: number of seeds (controlled manual cross-pollination)

Method: 10 flowers should be observed on each tree. The 10 flowers should be protected from pollination by use of an individual mesh on each flower. Manual cross-pollination of the 10 flowers should be done with pollen from a variety with a high fertilization capability, such as 'Fortune', 'Ellendale', 'Nadorcott', 'Nova' or 'Common mandarin (Willowleaf, Mediterranean)'.

Range of counts for each of the states when pollinator is “Fortune”:

Example varieties	Note	Range
Okitsu (SAT)	1	≤ 3
	2	$> 3 \leq 6$
Nulessin (CLE)	3	$> 6 \leq 9$
	4	$> 9 \leq 11$
Esbal (CLE)	5	$> 11 \leq 15$
	6	$> 15 \leq 17$
Primosole (HMA)	7	$> 17 < 20$
	8	$> 20 < 23$
Clemenules (HMA)	9	≥ 23

Partial revision of Annex to documents TG/83/4, TG/201/1, TG/202/1, TG/203/1 and TG/204/1

The changes to the Test Guidelines for Mandarin would also be reflected in the overall Table of Characteristics included in the Annex to documents TG/83/4 (Trifoliate Orange (*Poncirus*) (*Citrus* L. - Group 5)), TG/201/1, TG/202/1 (Oranges (*Citrus* L. - Group 2)), TG/203/1 (Lemons and Limes (*Citrus* L. - Group 3)) and TG/204/1 (Grapefruit and Pummelo (*Citrus* L. - Group 4)) by means of a partial revision to those Test Guidelines.

[End of Annex IX and of document]