

THE SECRETARY-GENERAL  
OF THE INTERNATIONAL UNION FOR THE  
PROTECTION OF NEW VARIETIES OF PLANTS

Subject: Draft amendment of the examination guidelines for Mediterranean mandarin.

In response to the comments made by the Spanish experts on the report submitted by Moroccan researchers, at the forty-first session of the Technical Working Party for Fruit Crops (TWF) and in relation to the draft amendment of the guidelines for the Mediterranean mandarin, in particular the study of the fertility of the ovule by controlled manual pollination, I have the honor to send you herewith a note detailing the scientific and technical arguments which support the Moroccan position.

I would therefore be grateful if you would distribute this note among the members of the Technical Working Party for Fruit Crops, with a view to it being discussed at the forty-second session of the TWF.

Yours sincerely,  
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Le Directeur Général de l'Office  
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## **RESPONSE TO THE COMMENTS MADE BY SPANISH EXPERTS RELATING TO THE STUDY OF FERTILITY OF THE OVULE BY MANUAL POLLINATION**

In response to the comments made by the Spanish experts on the report submitted by Moroccan researchers, at the forty-first session of the Technical Working Party for Fruit Crops (TWF) and in relation to the draft amendment of the guidelines for the Mediterranean mandarin, in particular the study of fertility of the ovule by controlled manual pollination, the following should be noted:

### **1- Choice of technique: Open or Hand pollination**

According to Masashi *et al.* (1995), seed formation in citrus fruits is affected by both genetic and environmental factors. The genetic factors influencing the presence of seeds in citrus fruits include the fertility of the ovule. These researchers demonstrated a highly significant correlation between seed number of hand pollination and that of open pollination. Consequently, the degree of female fertility or ovule fertility could be estimated from the seed number of **open pollination** or **free pollination**.

According to the comments by the Spanish experts, Masashi *et al.* (1995) obtained a higher number of seeds in conditions of hand pollination than with open pollination. However, for these authors the phenomenon was observed only **in the male sterile and self-incompatible** varieties. In conditions of open pollination a lower order of pollination occurs. The male sterile and self-incompatibles varieties have a smaller chance of fertilization than have the self compatible varieties. Consequently, the increase in the number of seeds in conditions of hand pollination, in comparison with open pollination, is simply a case of certain varieties.

### **2- The concentration of pollen seeds.**

Brown and Krezdorn (1969) compared the effect of three **pollination** techniques on the number of seeds produced in certain varieties of citrus fruits:

- **free pollination;**
- **pollination with different concentrations** of pollen seeds;
- **pollination with unknown concentrations** of pollen seeds.

These authors rejected the technique of pollination with unknown concentrations of pollen grains (technique proposed by the Spanish experts). The authors showed that the number of

seeds increases with the concentration of the pollen grains: The number of seed per fruit increased with each increase in quantity of pollen and the differences in seed content between the 50 and 100 grains and saturation treatments were significantly different.

According to these authors, the hand pollination tests such as that proposed by the Spanish experts do not take account of environmental conditions, concentrations of pollen grains and preferences of varieties for bees. These researchers showed that the technique closest to these conditions consists in using clearly determined concentrations of pollen grains.

Furthermore, Glenn Wright (2007) reported that the hand pollination technique with unknown concentrations of pollen grains in the mandarin does not take account of the effect of bees, the concentration of pollen grains transported by bees to receptive flowers and the attractiveness of flowers. The amount of pollen that the bees deliver to the receptive flower, and the attractiveness of that flower would certainly affect the degree of seediness of the mandarin fruit. This author adds that many pollination researchers cover the stigma with pollen, such as that reported by the Spanish experts, which may or may not simulate the effect of actual bee pollination, and recommended the use of more controlled levels of pollen. Ngo *et al.* (2010) therefore used a clearly determined low concentration of pollen grains, as recommended by Glenn Wright (2007).

Therefore, on the basis of the results obtained by Brown and Krezdorn (1969), the technique proposed by the Spanish experts, based on the use of an unknown concentration of pollen grains and the distinction between the varieties on the basis of the number of seeds produced does not allow reproducible results to be obtained. A variation in the quantity of pollen grains could lead to unreliable results for a clear distinction between varieties.

### **3-Physiological stage of flowers**

The pollination technique proposed by the Spanish experts does not provide any information on the physiological stage of the flowers used, nor on the actual stage of pollination or the receptiveness of the stigma (Sanzol *et al.*, 2003).

#### **3-1: Period of receptiveness:**

Mesejo *et al.* (2007) showed that the Satsuma Owari flowers have a short period of receptiveness demonstrated by the formation of seeds two days after the anthesis, compared with Valencia and Clemenules, where the formation of seeds begins eight days after the anthesis. The difference between the period of receptiveness of the flowers

between species and cultivars in other genuses (*Pyrus and Malus*) was reported by Sanzol and Herrero (2001).

### **3-2: Stage of pollination:**

Mesejo *et al.* (2007) showed that the number of seeds varies based on the stage of pollination. In the Clemenules variety, the fruits resulting from pollinated flowers one, two and four days after the anthesis give an average number of seeds of about 25 per fruit. By contrast, in the fruits resulting from flowers pollinated eight days after the anthesis, the number of seeds is significantly reduced to seven.

The absence of this information in terms of the methodology proposed by the Spanish experts does not therefore allow a reproducible estimate of the number of seeds produced by a given variety and consequently the distinction between the varieties.

### **Conclusion:**

From the above, it is clear that the study of fertility of the ovule must take account of the number of pollen grains, the stage of receptiveness of the stigma and the stage of pollination. In conditions of hand pollination, as proposed by the Spanish experts, these parameters have by contrast been ignored.

With a view to elucidating this issue concerning the study of the fertility of the ovule on scientific and technical grounds, we propose the preparation, within the citrus fruits subgroup, a protocol which determines the conditions for conducting cross tests in two or three countries, taking into consideration the following parameters:

- the origin of the pollen;
- the concentration of pollen grains;
- the period of receptiveness of the flowers;
- the stage of pollination.

Morocco is willing to participate in this sub-working party for the preparation of such a protocol and for the conduct of tests.

**Bibliographical references:**

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