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TECHNICAL WORKING PARTY FOR FRUIT CROPS

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REVISION TO ADDENDUM 2 TO DOCUMENT TWF/45/31

PARTIAL REVISION OF THE TEST GUIDELINES FOR MANDARINS (CITRUS L.-GROUP 1) (DOCUMENT TG/201/1)

Document prepared by experts from Morocco

The annex to this document contains the presentation made by experts from Morocco at the forty-fifth session of the Technical Working Party for Fruit Crops (TWF), in relation to the ring test concerning the proposed new characteristic “Fruit: number of seeds (controlled manual cross-pollination)” being considered in relation to the partial revision of the Test Guidelines for Mandarins (Citrus L.-Group 1) (document TG/201/1).

[Annex follows]

Report of the results of the study conducted in Morocco relating to variability in the number of seeds in the mandarin tree group in manual pollination conditions

2013-2014

Introduction

Following the decisions of the Technical Working Party for Fruit Crops concerning the conduct of a study on the fertility of the ovule in certain varieties of citrus fruits through the estimation of the number of seeds produced in manual pollination conditions, an experimental protocol has been prepared in consultation between Morocco, Spain and South Africa, and coordinated by the European Union.

This report includes:

- details of the experimental protocol adopted;
- the main results obtained;
- the conclusions drawn from the results.

Overall aim

The study of the fertility of the ovule in a number of varieties of mandarin tree through the estimation of the number of seeds produced in manual pollination conditions.

Specific aims

- Estimation of the percentage of *in vitro* germination of pollen grains.
- Estimation of the receptivity of the female variety stigma through the estimation of the percentage of germination of pollen grains for the stigmas based on the pollination date.
- Estimation of the number of seeds produced in manual pollination conditions based on the pollination date, the pollinator male, the concentration of the pollen grain used and the region.
- Estimation of the number of seeds produced in open pollination conditions.

Experimental protocol

1. Region

The study was conducted in Morocco according to the protocol adopted by the countries participating in the ring test. The experiment was carried out in Haouz region. The tests were conducted on trees of 10 to 15 years old.

2. Pollinators and females used

The varieties used in the experiment are shown in Table1

Table 1: Varieties male and female used

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

Conditions of the pollen of the donor

- The pollen of the pollinator variety is extracted at the moment of maximum development of the flower, before the anther dehiscence.
- Open flowers at a growth stage of 61–62 on the Citrus BBCH phenological scale [Agustı and al., 1997] will be randomly selected for pollen collection at least the day before pollination.
- Anthers are put in petri-dishes at room temperature with a dehydrator (e.g. a piece of paper) until they open.

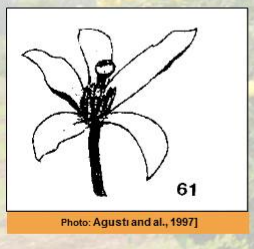


Photo: Agustı and al., 1997]

Stage of 61–62 on the Citrus BBCH phenological scale


Photo: INRA-ONSSA, 2012

Flowers at stage of 61–62 on the Citrus BBCH phenological scale


Photo: INRA-ONSSA, 2012

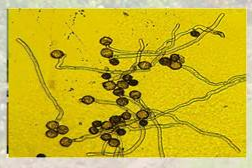
Anthers of donors at room temperature

Pollen Viability

- Verification of the germination capacity of the pollen and utilization of pollen with at least 50% germination capacity (assessment after pollen culture).
- 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 ºC 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 ºC 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. Pollen from male varieties will be at its optimal fertility

Pollen of donors

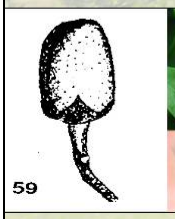
Pollen incubation in Brewbacker medium



germinated pollen grains observed with a binocular

Conditions of the female flowers

- 50 terminal flowers from each female variety will be used, if possible 10 flowers from 5 trees.
- Flowers will be fully developed but not yet opened: one day before anthesis (stage 59 on the Citrus BBCH phenological scale [Agust and al., 1997]), 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.
- Other flowers at the same stage (stage 59) at the time of emasculation must be marked to indicate the number of days after anthesis and to mark the flowers emasculated at the time of anthesis. (see additional explanation in Annex) Trees will be at the phenologic state of full bloom



stage 59 on the Citrus BBCH phenological scale

Emasculated flowers

bagged flowers to avoid self and free pollination

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
- Dissecting microscope is used in the field to evaluate the quantity of grains of pollen which must be more than 100. Those flowers will be compared with the ones with massive amounts of freshly pollen
- 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

Dissecting microscope is used in the field to evaluate the quantity of grains of pollen

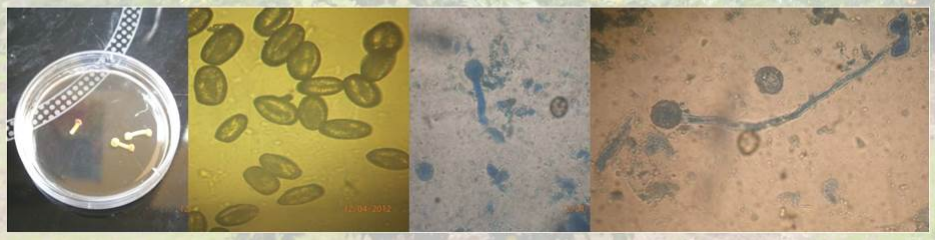
Pollen be applied by a brush

Pollinated flowers

Pollinated flowers isolated from the environment with an individual

Stigmat receptivity

- For each pollinating date, 10 additional flowers per variety will be removed 48 h after pollination and fixed with FPA (10% formaldehyde, 10% propionic acid, 80% ethanol at 70%).
- The percentage of pollen germination on the stigma will be calculated by counting at least 100 grains per pollinated flower.
- A pollen grain was considered to be germinated when the pollen tube length is longer than the pollen grain diameter. This is done by extracting the germinated pollen grain in the laboratory using the microscope.



Stigmat fixation in FPA

Not germinated pollen in vivo (in the stigmat after pollination

Germinated pollen in the stigmate after pollination

Hand pollination 2013

Results

1- Number of seeds in the fruits of four varieties under conditions of manual pollination

Number of fruits produced at each crossing

Male parent	Femeal parent	Date	Concentration	Fruits number
NADORCOTT	NADORCOTT	1	1	82
NADORCOTT	NADORCOTT	1	2	62
NADORCOTT	NADORCOTT	2	1	60
NADORCOTT	NADORCOTT	2	2	68
NOVA	NADORCOTT	1	1	65
NOVA	NADORCOTT	1	2	49
NOVA	NADORCOTT	2	1	55
NOVA	NADORCOTT	2	2	69
NOVA	Nules	1	1	48
NOVA	Nules	1	2	27
NOVA	Nules	2	1	29
NOVA	Nules	2	2	24
NADORCOTT	Nules	1	1	212
NADORCOTT	Nules	1	2	106
NADORCOTT	Nules	2	1	38
NADORCOTT	Nules	2	2	42
NADORCOTT	NOVA	1	1	9
NADORCOTT	NOVA	1	1	12
NADORCOTT	NOVA	1	2	2
NADORCOTT	NOVA	1	2	4
NADORCOTT	NOVA	2	1	2
NADORCOTT	NOVA	2	1	3
NADORCOTT	NOVA	2	2	1
NADORCOTT	NOVA	2	2	2
NADORCOTT	MARISOL	1	1	16
NADORCOTT	MARISOL	2	2	3
NADORCOTT	MARISOL	2	1	1
NOVA	MARISOL	2	2	2
NOVA	MARISOL	1	1	1
NOVA	MARISOL	1	1	13
NADORCOTT	MARISOL	1	2	26

Table 2: Number of seeds produced in fruits of four citrus varieties depending on the male pollinator varieties, date of pollination and the concentration of pollen grains

Male: NADORCOTT

	NADORCOTT	MARISOL	NULES	NOVA
D1/ C1: 1 day-100gp	0,5	11,81	10	16,63
D1/ C2: 1 day saturation	0.1	13.36	12.4	19
D1/ C1: 6 day 100 gp	0,1	9	0	0
D2/ C2 6 day-Saturation	0,5	7,33	*	*

Male: Nova

	NADORCOTT	MARISOL	NULES	NOVA
D1/ C1 : 1 day-100gp	0,22	6,84	1,62	*
D1/ C2 : 1 day-saturation	0.27	10	0	*
D2/ C1 : 6 day-100gp	0.84	3.8	0	*
D2/ C2: 6 day-Saturation	0,59	5,5	0	*

Table 2 show that :

- The number of seeds produced in fruits of four varieties varies with the male parent, the date of pollination and the pollen grain concentration. The number of the seeds is higher in the presence of the Nadorcott as male parent, for one days after anthesis pollination and at saturated concentration of pollen grains.

- In the presence of Nadorcott as male parent a distinction between NOVA and other mandarin varieties is observed one day after anthesis at saturation and 100 grains of pollen but a weak distinction is noted between Marisol and Clementine Nules.

NADORCOTT X NADORCOTT
D1 C1

NADORCOTT X NADORCOTT
D2 C1

NADORCOTT X NADORCOTT
D1 C2

NADORCOTT X NADORCOTT
D2 C2

NOVA X NADORCOTT
D1 C1

NOVA X NADORCOTT
D2 C1

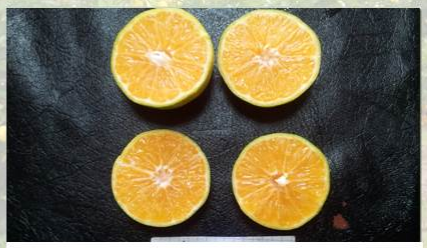
NOVA X NADORCOTT
D1 C2

NOVA X NADORCOTT
D2 C2

NADORCOTT X NOVA
D1 C1

NADORCOTT X NOVA
D1 C2

NADORCOTT X NULES
D1 C1



NADORCOTT X NULES
D2 C1

Table 3: Comparison between the number of seeds produced in fruits of four citrus varieties depending on the male pollinator varieties, date of pollination and pollen grains concentration for two years 2012 and 2013.

Male: NADORCOTT

2013

	NADORCOTT	MARISOL	NULES	NOVA
D1/ C1 : 1 day-100gp	0,5	11,81	10	16,63
D1/ C2 : 1 day saturation	0.1	13.36	12.4	19
D2/ C1 : 6 day 100 gp	0,1	9	0	0
D2/ C2 : 6 days saturation	0,5	7,33	*	*

Male: NADORCOTT

2012

	MARISOL	Nules
D1C1 : 1 day-100gp	13,33	29,33
D1 C2: 1 day-saturation	13	35,33
D2C1: 6 day- 100 gp	9	6
D2C2: 6 days-saturation	2	9,66

Table 3 show that:
The number of seeds produced in fruits of four citrus varieties varies with the male parent, the date of pollination, the concentration of pollen grains used and the year. Indeed, the highest number of seeds is produced in the presence of Nadorcott as male parent for pollination made one day after anthesis and at saturated grains of pollen in the Nules Clementine during 2012 while it is of around 10 seeds during the year 2013.

- The distribution of varieties also varies from year to year based on the number of seed produced in fruits (case of Nules clementine and Marisol)

Table 4: Comparison between the number of seeds produced in fruits of four citrus varieties depending on the male pollinator varieties, date of pollination and pollen grain concentration for two years 2012 and 2013.

Male: NOVA

2013

	NADORCOTT	MARISOL	NULES	NOVA
D1/ C1 : 1 day-100gp	0,22	6,84	1,62	*
D1/ C2 : 1 day-saturation	0.27	10	0	*
D2/ C1 : 6 day-100gp	0.84	3.8	0	*
D2/ C2: 6 day-Saturation	0,59	5,5	0	*

Male: NOVA

2012

NOVA	Marisol	Nules
D1/ C1 : 1 day-100gp	6	26
D1/ C2 : 1 day-saturation	10	20
D2/ C1 : 6 day-100gp	4,3	5
C2D2 : 6 day-Saturation	5,6	3,6

Table 4 show that:
- The number of seeds produced in fruits of four citrus varieties varies with the date of pollination, the concentration of pollen grains used and year. Indeed, the higher number of the seeds is produced, at one day after anthesis and at saturated concentration of pollen grains.

- The distribution of varieties also varies from year to another based on the number of seeds produced

II- Number of seeds in fruit under conditions of open pollination

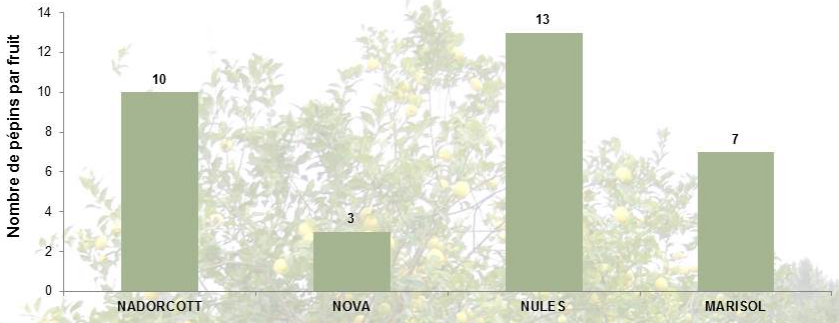


Figure 1: Number of seeds produced in fruits of four citrus varieties in condition of open-pollination during the year 2013.

Fig 1 show that::

The number of seeds in fruits at condition of open pollination varies between varieties used. It is clear from the figure a distinct repartition between the four varieties used:

The first group is represented by the clementine Nules. This variety has recorded the highest number of seeds in fruits (13 seeds).
The second group is represented by the mandarin Nadorcott 10 seeds per fruit.
The third group is represented by the clementine Marisol (7 seeds).
The fourth group is represented by the Nova mandarin (3 seeds).

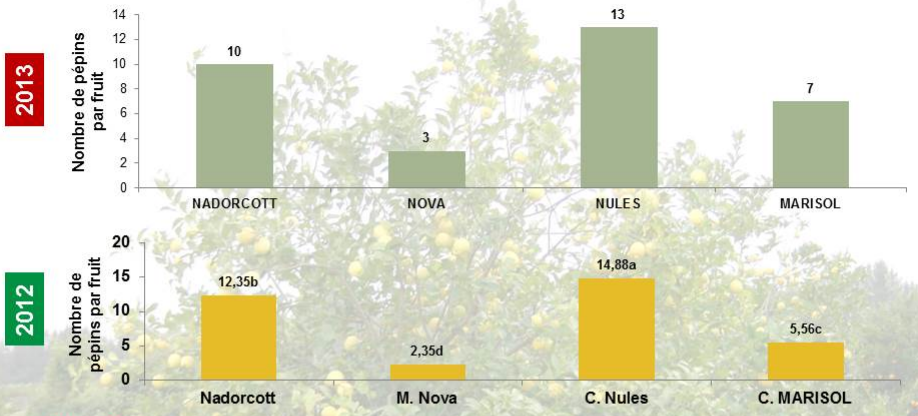


Figure 2: Number of seeds stored in four citrus varieties in condition of open pollination for two years 2012 and 2013

figure 2 show that:

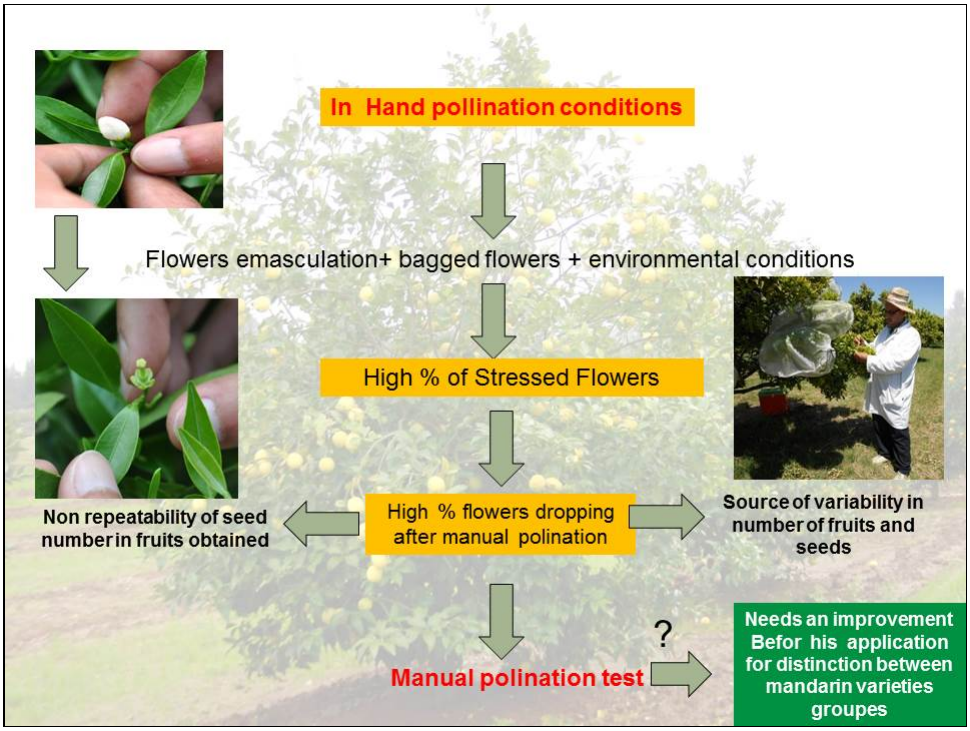
- The behavior of the four varieties in terms of the number of seeds produced under conditions of open pollination is similar in both years of the study
- The classification of four varieties is almost stable from one year to another

Conclusions

Based on the results obtained in this study we can conclude that :

- The number of seeds varies with male pollinator, the concentration of pollen grains , the date of pollination and year
- .Under conditions of open pollination, the varieties are divided into four statistically distinct groups according to the number of seeds per fruit produced by each variety.
- The distinction and classification of varieties based on the number of seeds product under conditions of manual pollination is **influenced by the year and the environmental conditions.**

The results obtained during the second year have confirmed the results of the first year on the importance of the use of open pollination for a clear distinction between varieties in terms of ovule fertility expressed by the number of seeds products . While under conditions of manual pollination degree of fertility is not stable but varied with year thing that could be due to experimental and environmental conditions.



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