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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

TECHNICAL WORKING PARTY FOR FRUIT CROPS

Forty-Fifth Session
Marrakesh, Morocco, from May 26 to 30, 2014

ADDENDUM TO DOCUMENT TWF/45/31

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

Document prepared by South Africa

1. The annex to this document contains the presentation to be made by experts from South Africa, 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]

Mandarin Ring Test

Second season's report from South Africa

TECHNICAL WORKING PARTY FOR FRUIT CROPS
Forty-fifth Session
Marrakesh, Morocco, May 26 to 30, 2014

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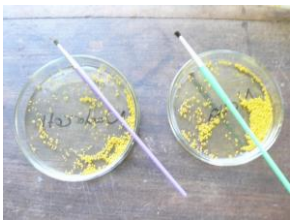
Background

- Proposal for a partial revision of test guidelines for mandarins for the introduction of new characteristic to evaluate ovule fertility by controlled manual cross pollination with another variety or species: 'Fruit: number of seeds (controlled manual cross pollination)'
- Objective of ring test is to reach an agreement on test methodology in order to have a reliable observation regarding the characteristic
- Two years of results were obtained for testing the methodology for the acceptability of the new characteristic for inclusion in the test guidelines

Materials & Methods for 2013-14

- Trial Sites:
  - Addo Research Station in Eastern Cape (for pollinations on Marisol, Nules, Nova)
  - Farm Sontule near Addo (for pollinations on Nadorcott)
  - Climate for both sites: temperate costal summer/winter rainfall area
- Pollen preparation as per methodology:
  - Pollen collection 2 days before pollination
  - Pollen germination test
  - Emasculation and flower bagging one day before anthesis



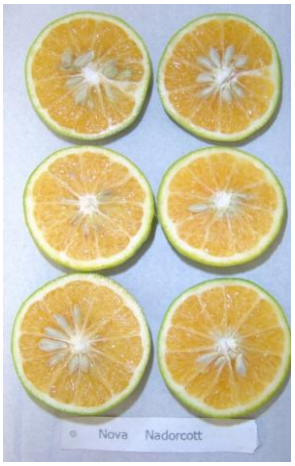

Materials & Methods cont.

- Pollinations :
  - At anthesis (26 September 2013)
  - 6 days after anthesis (2 October 2013)
  - 70 flowers per open and controlled manual cross pollination, and pollination date



- Evaluations (May 2014):
  - Number of seed per fruit (full, small and abortive seed) for Marisol, Nules, Nova and Nadorcott
  - Fruit set for Marisol, Nules, Nova and Nadorcott

Results



Pollen germination test

- 50% pollen germination capacity as per guideline was achieved except for Nova on second pollination date

	Germinated pollen (%) *(2012 values)	
Test date	**Nadorcott**	**Nova**
26-Sept-13	56 (44)*	50 (45)
02-Oct-13	62 (54)	48 (36)

- Pollen viability (aceto-carmine staining method) showed high pollen viability for male parents

	% Pollen viability *(2012 values)
Nadorcott	80 (73.2)*
Nova	83 (84.3)

Number of fruit evaluated

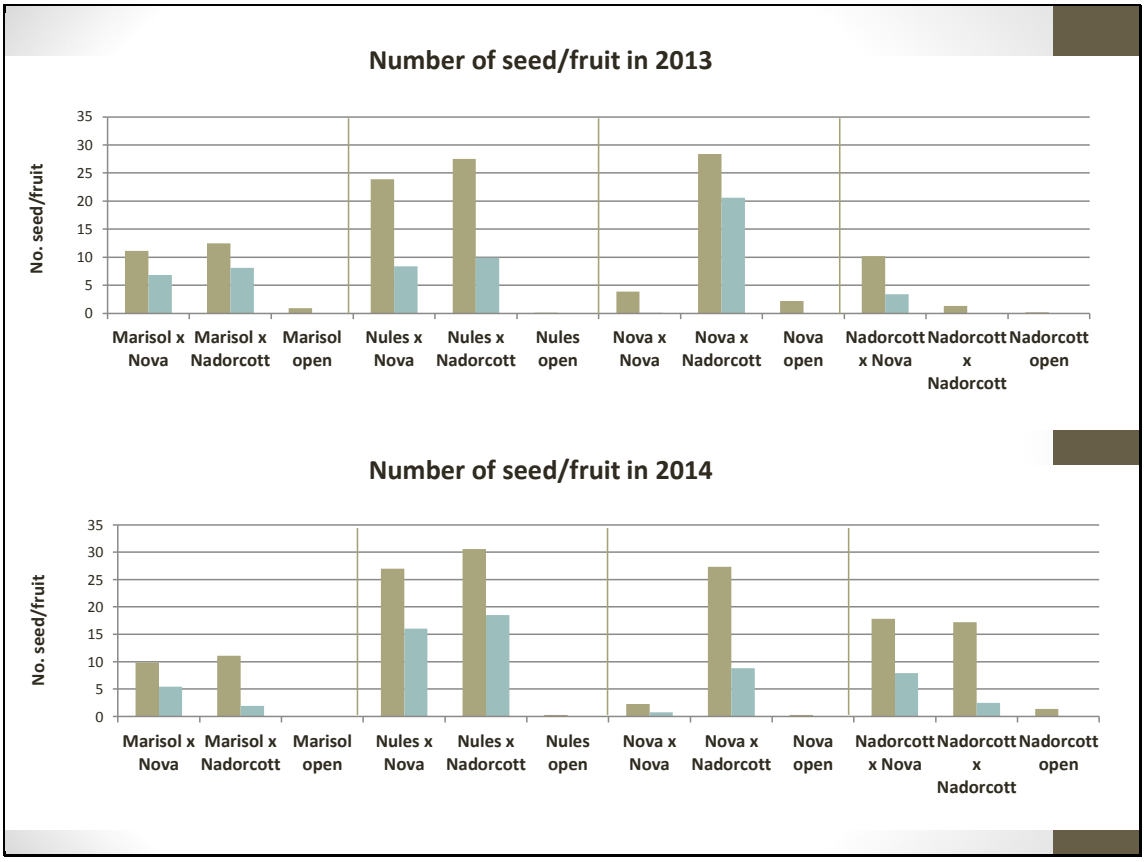
- Controlled manual cross pollination showed an increased fruit set potential compared to open pollination

		No. of fruit harvested in May 2014 and evaluated from 70 pollinated flowers				
		Pollinator Date 26/09/2013		*Pollinator* Date 02/10/2013		
		Nova	**Nadorcott**	**Nova**	**Nadorcott**	**Open pollinated**
Female	**Marisol**	24	17	11	14	11
	Nules	52	48	35	49	19
	Nova	21	44	20	27	33
	Nadorcott	49	38	14	8	8

Number of seed per fruit

		Pollinator at anthesis		*Pollinator at day 6 after anthesis*		
		Nova	**Nadorcott**	**Nova**	**Nadorcott**	**Open pollinated**
	Marisol 2013	11.1	12.5	6.8	8.1	0.9
Female	Marisol 2014	9.9	11.1	5.5	1.9	0.1
	Nules 2013	23.9	27.5	8.4	9.9	0.1
	Nules 2014	27.0	30.6	16.1	18.5	0.3
	Nadorcott 2013	10.2	1.3	3.4	0	0.2
	Nadorcott 2014	17.9	17.2	7.9	2.5	1.4
	Nova 2013	3.9	28.4	0.1	20.6	2.2
	Nova 2014	2.3	27.4	0.8	8.8	0.3

- Seed number varied with cultivars (male and female parent)
- Seed number reduced with later pollination date
- Open pollination showed lower seed number than controlled pollination and gives the least indication on female fertility
- Pollen parent and pollination time influence seed number and need to be considered when drawing conclusions about female fertility



Summary – both seasons

- Number of seed was higher in controlled manual cross pollinated flowers compared to open pollinated
- Number of seed reduced with later pollination date probably due to reduced stigma receptivity
- Fruit set potential was increased with controlled manual cross pollination
- Nadorcott as male parent caused higher number of seed in female parents compared to Nova as male parent
- Results over two seasons were repeatable

	Seed formation		
	Yes	**No**	**Suitability for testing female sterility**
Open pollination	• Female fertile • Male fertile	• Possibly female sterile • Possibly male sterile	• No
Self-pollination	• Female fertile • Male fertile	• Self-incompatibility • Male sterile	• No
Controlled cross pollination (with standard testers)	• Female fertile	• Highly possible female sterile (seedless under normal conditions; low seeded (0-1 seed) under high pollen pressure)	• Yes

Conclusion

- Seed formation is affected by genetic and environmental factors
- Female fertility is directly related to seediness
- Open pollination is too prone to environmental factors and is insufficient as test method alone
- Degree of female fertility can be estimated with higher accuracy/probability by controlled manual cross pollination
- Cross pollinations at anthesis alone are sufficient for reliable results

➢ **Two years' of testing the methodology has proven repeatability of the results and therefore controlled manual cross pollinations can be used for reliable testing of female fertility**

The Team

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(from left to right)

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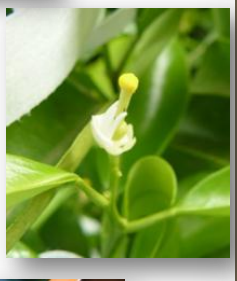
THANK YOU!



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