

The Cutting Edge

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Using Biotechnology For Citrus Cultivar Improvement – Sectorial Chimeras

Karin Hannweg

ARC-Institute for Tropical and Subtropical Crops,
Private Bag X11208, Nelspruit 1200
E-Mail: karin-h@itsc.agric.za

How can we improve on cultivars we cannot breed in the conventional way?

Focused selection of genetic material from sectorial chimeras can be used to the southern African citrus industry's advantage. A high percentage of the region's fruit mutations can be screened, selected out, and those with potential, cloned, without going back to the original tree.

- Sectorial chimeras on fruit occur frequently in citrus plantings but since the fruit can not be sold they are discarded at the packhouse.
- Each discarded fruit is an important source of genetic variation, especially where the genetic base of a particular cultivar is extremely small, for example, in the case of the sweet orange.

What are the advantages of using sectorial chimeras?

- Tree characteristics will remain, i.e., a navel tree will remain a navel tree having the same growth form, vigour, etc., even though the fruit is potentially of a higher quality.
- Other fruit quality characteristics are not radically changed as in conventional breeding methods where unpredictable gene combinations could result in the expression of disadvantageous traits making the 'new' cultivar unmarketable.
- For cultivars having a very small degree of variation (e.g., navels and Valencias), these methods provide the most rapid and cost effective method for introducing new cultivars for which specific traits (e.g., altered maturation date, increased rind/flesh colour, increased Brix level) have been selected.
- The time taken from selecting an advantageous chimera to when the resulting plant can be planted out for evaluation trials is 6-9 months.

Which traits/marketable characteristics can be improved on?

Various types of chimeras exist and by identification of the appropriate chimera, the following characteristics can be selected for:

- Early ripening
- Late ripening
- Enhanced rind colour
- Enhanced flesh colour
- Improved juice colour
- Enhanced TSS (TSS/Acid ratios)
- Improved rind texture
- Eliminating of creasing
- Improved rind thickness
- Pest resistance
- Citrus Greening Disease resistance
- Altered sensitivity to rind blemish agents
- Tetraploids

How are improved cultivars developed?

- Chimera characteristics including external and internal fruit qualities are recorded to determine their potential over the existing cultivar.
- Undeveloped ovules from the chimeric sector of interest are dissected out from fruit and cultured under tissue culture conditions on a specific nutrient-rich formulation.
- Plants are germinated and hardened-off for evaluation less than 1 year after chimeric sectors are identified.
- Cultivars are evaluated 4 years after topworking.

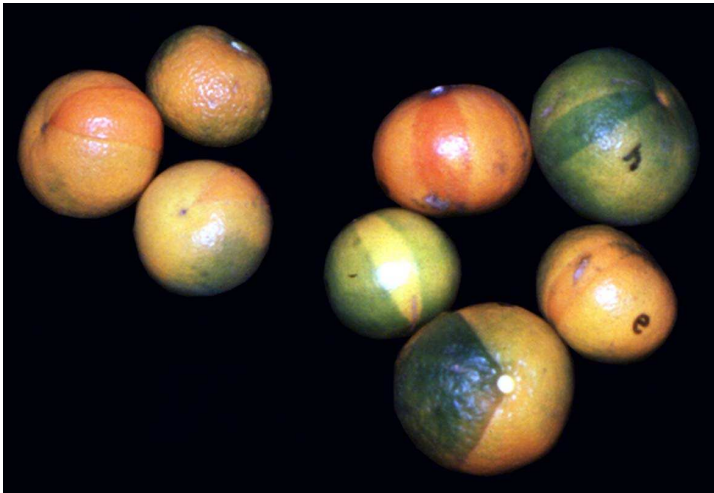
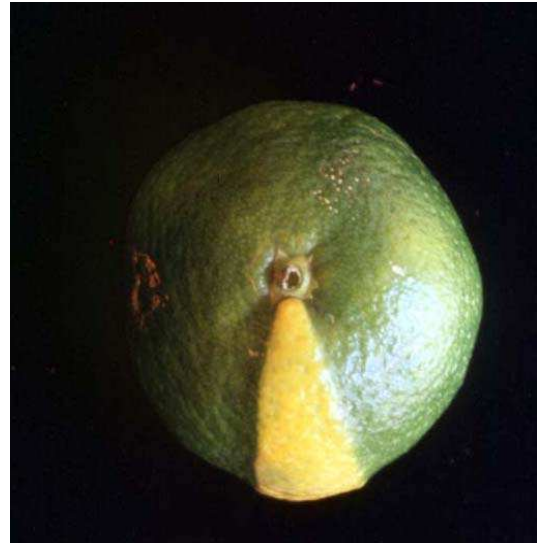
How does southern Africa benefit from this research?

- High quality fruit with improved fruit characteristics and altered maturity dates will ensure that high prices are obtained, particularly on the international market.
- Maximum benefit of improved trait selection as a large percentage of the country's citrus harvest can be screened.
- Time to cultivar release is far shorter as a specific trait can be directly selected without dramatically changing other characteristics.

- Difficult-to-breed cultivars (e.g., sweet oranges)

can be improved for specific traits.

SOME EXAMPLES OF CHIMERAS



Use of Citrus Waxes in the SA Citrus Industry

Keith Lesar
CRI, Nelspruit

Every grower who exports citrus, funds research conducted by the Citrus Research International (CRI) Group. Before changing established practices, citrus growers are accustomed to referring to CRI research results and in order to provide this service, CRI remains abreast of all new developments affecting citrus production.

CRI conducts post-harvest research to improve the overall waste control of export fruit as well as optimising the shelf life and quality of all citrus

cultivars, thus ultimately maximizing the benefits to the producer. Part of this research involves evaluation of new chemical products such as sanitizing agents, new fungicides, waxes, etc. for possible approval and recommendation by CRI for use in the Southern African Citrus Industry.

There are currently five suppliers of citrus waxes that are being used in the South African citrus industry and one supplier awaiting CRI recommendation for this citrus season.

A number of factors need to be considered when a citrus packhouse changes from one type of wax to another, as well as changing from one method of wax application to another. These include the type of wax to be used and the market to which the fruit is to be shipped. Uniformity of coverage as well as

the quantity of wax on the fruit can both determine the condition of fruit on arrival at the market.

Over-waxed fruit may develop off-flavours and under-waxed fruit will shrink excessively due to weight loss. The application method used is the single most important factor in uniformly applying the wax. Improved and efficient waxing will only be achieved if the following aspects in the packhouse are given the necessary attention: the correct application of wax at the recommended rate; application to dry and clean fruit; allowance for uneven fruit throughput due to variable production loads, and proper drying of the waxed fruit.

It has been brought to our attention that certain impurities have been detected in certain citrus waxes that are being used on South African export citrus cultivars destined for Europe and other markets. These impurities are listed as “not permitted”. If wax suppliers are attempting to place cheaper waxes into the marketplace by reformulating waxes with these impurities, this practice will not only be to the detriment of the supplier but also to the producer and the entire Southern African citrus industry.

In the light of this information, CRI will be conducting random sampling of waxes throughout the industry during the coming season and thereafter every new production season. The samples will be submitted for evaluation and if any impurities listed as “not permitted” are detected, CRI will forthwith inform all producers of the findings and will advise that the use of the said wax be discontinued immediately.

CRI appeals to all suppliers of citrus waxes not to succumb to the temptation of producing inferior quality waxes that may jeopardise our exports.

Ultracure – a new Post-Harvest Fungicide for Citrus

Keith Lesar
CRI, Nelspruit

A new formulation of the post-harvest fungicide guazatine was submitted to CRI during 2002 for evaluation. The product will be known as UltraCure and contains the same active ingredient as Deccotine and CitriCure.

The compound was screened against the post-harvest citrus pathogens, *Penicillium digitatum* (green mould) and *Geotrichum candidum* (sour rot) for waste control. The evaluation was done by artificially inoculating lemons and Valencia oranges with both pathogens and then treating them with the compound. A 100% inhibition of both pathogens was achieved. Fruit samples were submitted thereafter for residue analyses.

The product has subsequently been registered for the post-harvest treatment of green and blue mould and sour rot on citrus.

UltraCure 210 SL is compatible with Imazalil Sulphate 750 WSP, Imazalil base 800 EC, Tecto 500 SC and 2,4-D Sodium salt (Deccomone). UltraCure is not compatible with SOPP SL, calcium hypochlorite or other active chlorine compounds and cannot be mixed with citrus waxes in the packhouse.

The product is recommended at a concentration of

- 4.8 litres per 1000 litres water (1000 ppm) in a fungicide bath, and
- 2.4 litres per 1000 litres water (500 ppm) in a pre-degreening fungicide drench.

Please note that when the product is used in a pre-degreening drench, the fruit must be dry prior to degreening, as subjecting wet fungicide-treated fruit to ethylene gas could result in phytotoxicity (burn) to sensitive rinds. The following additional precaution is recommended. Prior to pre-degreening or packhouse treatment with guazatine, dip a few fruit (green to well coloured) in a solution of the compound at the recommended concentration and allow the fruit to stand at ambient temperature for a few days to determine the status of the sensitivity of the rinds.

The use of 2,4-D Amine and Ester Formulations for Post-harvest Treatment of Citrus

Keith Lesar
CRI, Nelspruit

Take note that the amine and ester formulations of 2,4-D (2,4-dichlorophenoxyacetic acid) are not registered for post-harvest use on citrus. The only registered formulation is the sodium salt (Deccomone).

The amine and ester formulations are not recommended for use in fungicide hot water baths in citrus packhouses. However, some packhouses are using these products. Research has demonstrated that both the amine and ester formulations reduce the efficacy of the fungicide Imazalil. Incorrect use of these two formulations of 2,4-D can also lead to serious “burn” (phytotoxicity) to sensitive citrus rinds as has been previously reported in a pre-degreening drench situation.

Editor: Tim G Grout, tg@cri.co.za,
Tel +27 13 759 8000.



Die Gebruik Van Biotegnologie Vir Sitruskultivarverbetering – Sektoriale Chimera's

Karin Hannweg

LNR-Instituut vir Tropiese en Subtropiese Gewasse, Privaatsak X11208, Nelspruit 1200
E-pos: karin-h@itsc.agric.za

Hoe kan ons kultivars verbeter wat nie op die konvensionele manier geteel kan word nie?

Doelgerigte keuring van genetiese materiaal van sektoriale chimera's kan tot die suidelike-Afrikaanse sitrusbedryf se voordeel gebruik word. 'n Groot persentasie van die gebied se vrugmutasies kan uitgesoek en gekeur word en dié met potensiaal gekloon word sonder om die oorspronklike boom te gebruik.

- Sektoriale chimera's op vrugte kom dikwels in sitrusaanplantings voor, maar omdat die vrugte nie bemerkbaar is nie, word hulle by die pakhuis weggegooi.
- Elke weggegooide vrug is 'n belangrike bron van genetiese verskeidenheid, veral wanneer die genetiese basis van 'n besondere kultivar baie klein is, byvoorbeeld by die soetlemoen.

Wat is die voordele om sektoriale chimera's te gebruik?

- Boomeienskappe sal behoue bly, bv. 'n nawelboom sal 'n nawelboom bly wat dieselfde groeivorm, lewenskragtigheid, ens, het, alhoewel die vrug potensieel van 'n beter kwaliteit kan wees.
- Ander vrugkwaliteiteienskappe word nie ingrypend verander nie, soos met konvensionele teeltgnieke waar onvoorspelbare geenkombinasies tot die uiting van nadelige eienskappe kan lei wat die "nuwe" kultivar onbemarkbaar maak.
- Vir kultivars met min variasie (bv. nawels en Valencias), voorsien dié tegnieke die vinnigste en kostedoeltreffendste metode om nuwe kultivars bekend te stel wat vir besondere eienskappe (soos veranderde rypwordingsdatum, verhoogde skil/vleiskleur, verhoogde Brixvlak) uitgesoek is.
- Die tyd vanaf die uitsoek van 'n voordelige chimera totdat die voortspruitende plant uitgeplant kan word, is 6-9 maande.

Watter karaktertrekke/bemerkbare eienskappe kan verbeter word?

Verskillende tipes chimera's bestaan en wanneer die toepaslike chimera geïdentifiseer word, kan die volgende eienskappe gekeur word:

- Vroeë rypwording
- Laat rypwording
- Verhoogde skilkleur
- Verhoogde vleiskleur
- Verhoogde sapkleur
- Verbeterde TOV (TOV/Suurverhouding)
- Verbeterde skiltekstuur
- Uitskakeling van kraakskil
- Verbeterde skildikte
- Plaagbestandheid
- Weerstand teen Sitrusvergroeningsiekte
- Veranderde sensitiwiteit vir oorsake van skilletsels
- Tetraploïede

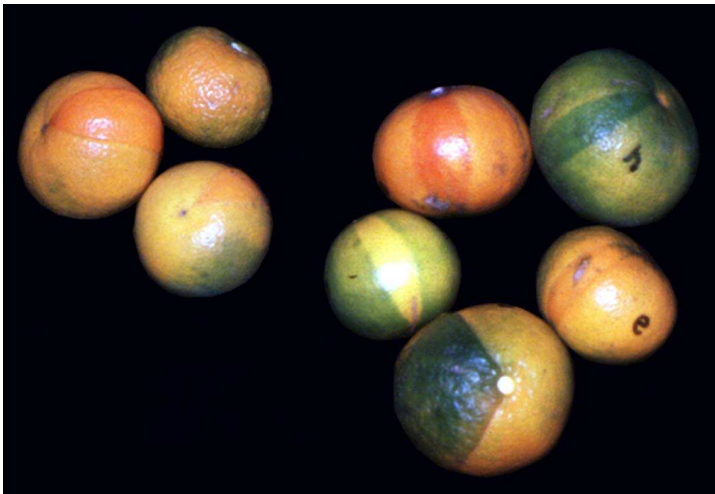
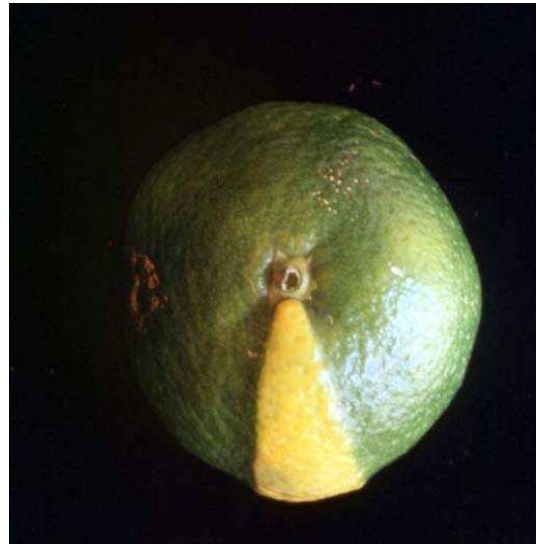
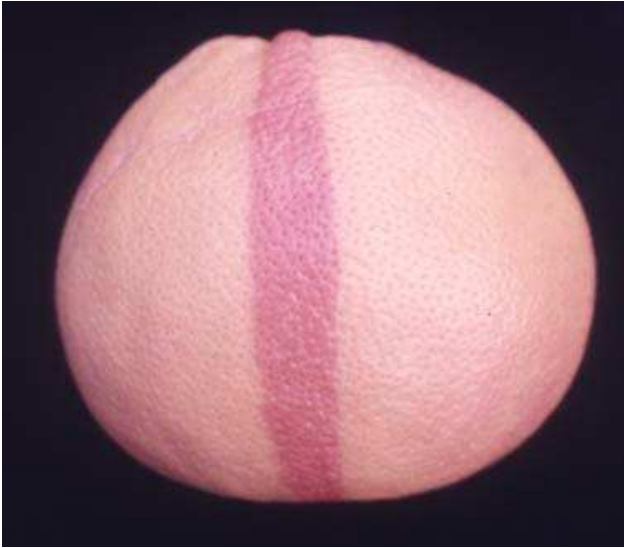
Hoe word kultivars verbeter?

- Chimera-eienskappe, wat uitwendige en inwendige vrugkwaliteit insluit, word aangeteken om hul potensiaal met die bestaande kultivar te vergelyk.
- Onontwikkelde kiemselle van die verlangde chimeriese sektor word uitgedissekteer en onder weefselkultuurtoestande op 'n voedingsryke medium gekweek.
- Plante word ontkiem en verhard vir evaluasie binne een jaar nadat chimeriese sektore geïdentifiseer was.
- Kultivars word 4 jaar na oorwerking geëvalueer.

Hoe trek suidelike-Afrika voordeel uit dié navorsing?

- Goeie kwaliteit vrugte met verbeterde vrugkwaliteiteienskappe en veranderde vrugrypwordingstye sal verseker dat hoë pryse veral in internasionale markte verkry word.
- Maksimum voordeel kan van verbeterde eienskapkeuring verkry word, omdat 'n groot persentasie van die land se sitrusoes gesif kan word.
- Tydsverloop tot kultivarvrystelling is baie korter, omdat 'n besonder eienskap direk gekeur kan word sonder om ander eienskappe opvallend te verander.
- Besondere eienskappe in moeilik-teelbare kultivars (soos soetlemoene) kan verbeter word.

VOORBEELDE VAN CHIMERAS



Die gebruik van Sitruswaks in the SA Sitrusbedryf

Keith Lesar
CRI, Nelspruit

Elke produsent wat vrugte uitvoer befonds navorsing wat deur die Citrus Research International (CRI) Groep gedoen word. Voordat vasgestelde praktyke verander word, is sitrusprodusente gewoon daaraan om CRI-navorsingsresultate te raadpleeg. Om dië diens te lewer, bly CRI op hoogte met die jongste verwikkelinge wat sitrusproduksie beïnvloed.

CRI doen na-oesnavorsing om die algehele bederfbeheer op uitvoervrugte te verbeter, sowel as om die rակlewe en kwaliteit van alle vrugkultivars te optimaliseer, om sodoende uiteindelik tot maksimum voordeel van die

produsent te wees. Gedeelte van die navorsing behels die evaluasie van nuwe chemiese produkte soos ontsmettingsmiddels, swamdoders, wakse, ens., vir moontlike goedkeuring en aanbeveling deur CRI vir gebruik in die Suider-Afrikaanse sitrusbedryf.

Daar is huidiglik vyf verskaffers van sitruswakse wat in die Suider-Afrikaanse sitrusbedryf gebruik word, terwyl een verskaffer vir CRI goedkeuring vir die komende seisoen wag.

Verskeie faktore moet in gedagte gehou word wanneer 'n sitruspakhuis van een tipe waks na 'n ander oorskakel, sowel as wanneer die metode van waksaanwending verander word. Dit sluit in die tipe waks wat gebruik gaan word, sowel as die mark waarheen die vrugte verskeep gaan word. Die eenvormigheid van waksoedekking, asook die hoeveelheid waks op die vrug, kan 'n invloed op die houvermoë van die vrug op pad na die mark hê.

Oorwakste vrugte kan wansmaak ontwikkel en te liggewakste vrugte sal oormatig krimp as gevolg van water verlies. Die toedieningsmetode is die enkele belangrikste faktor vir eenvormige waksbedekking. Verbeterde en doeltreffende waksing kan slegs verkry word indien waksaanwending in die pakhuis die nodige aandag kry, te wete die regte aanwending van die waks teen die aanbevole hoeveelheid, aanwending op skoon en droë vrugte, toelating vir die onegalige toevoer van vrugte as gevolg van onbestendige produksie en behoorlik droogmaak van die gewakste vrugte.

Dit het onder CRI se aandag gekom dat sekere onsuierhede in sekere wakse gevind is op Suider-Afrikaanse uitvoersitrus na Europa en ander markte. Dié onsuierhede is as “ontoelaatbaar” geklassifiseer. Indien waksverskaffers probeer om goedkoper wakse te verkoop deur dit met dié onsuierhede te herformuleer, sal die praktyk nie slegs nadelig vir die verskaffer wees nie, maar ook vir die produsent en die hele Suidelike Afrika sitrusbedryf.

CRI sal gevolglik verteenwoordigende monsters neem van wakse in die hele bedryf gedurende die komende seisoen, asook in die toekoms. Die monsters sal geëvalueer word en indien enige ontoelaatbare onsuierhede opgespoor word, sal CRI alle produsente onverwyld daarvan in kennis stel en aanbeveel dat gebruik van die betrokke waks onmiddellik gestaak word.

CRI versoek alle verskaffers van sitruswaks om nie toe te gee aan die versoeking om waks van minderwaardige kwaliteit te produseer wat nadelig vir uitvoere kan wees nie.

Ultracure ‘n Nuwe Na-oes Swamdoder op Sitrus

Keith Lesar
CRI, Nelspruit

‘n Nuwe formulasie van die na-oes swamdoder Guazatine is voorgelê vir evaluasie by CRI gedurende 2002. Die produk het dieselfde aktiewe bestanddeel as Deccotine en CitriCure, d.i. guazatine, en die produk sal bekend staan as UltraCure.

Die nuwe formulasie is getoets teen die na-oes sitruspatogene, *Penicillium digitatum* (groen skimmel) en *Geotrichum candidum* (suurvrot) vir bederfbeheer. Suurlemoene en Valencia lemoene is geïnkuleer met die twee bogenoemde patogene en dan behandel met die nuwe middel. Dié middel het albei patogene 100% inhibeer. Vrugte monsters van hierdie evaluasie is daarna gestuur vir residu ontleding.

Die produk is agterna geregistreer vir die naoes behandeling van groen en blou skimmel en suurvrot op sitrus.

UltraCure 210 SL is verenigbaar met Imazalil Sulfaat 750 WSP, Imazalil 800 EC, Tecto 500 SC en 2.4-D Natrium sout (Deccomone). UltraCure is nie verenigbaar met NOFF SL, Kalsium hypochloriet of ander aktiewe chloor middels en kan nie gemeng word met sitrus wakse in the pakhuis.

Die produk word aanbeveel teen ‘n konsentrasie van

- 4.8 liter in 1000 liter water (1000 dpm) in ‘n swamdoder bad; en
- 2.4 liter in 1000 liter water (500 dpm) in ‘n voorontgroenings swamdoder storting (drench)

Neem kennis van die feit dat wanneer dié produk in ‘n voorontgroenings storting gebruik word, die vrugte droog moet wees voor ontgroening. Blootstelling van die nat swamdoder behandelde vrugte aan etileengas ontgroening kan lei tot fitotoksiteit (brand) van sensitiewe vrugte.

Doop ‘n paar vrugte (groenerig en opgekleur), voor ontgroening of pakhuis behandeling, met guazatine, in ‘n oplossing van die produk teen die aanbevole konsentrasie. Laat die vrugte teen kamer temperatuur staan vir ‘n paar dae om die stand van die vrugsensitiwiteit te bepaal.

Die Gebruik van 2.4-D Amien en Ester Formulasies vir Na-oes Behandeling van Sitrus

Keith Lesar
CRI, Nelspruit

Let op dat die amien en ester formulasies van 2.4-D (2.4-dichloorfenoksi-asynsuur) nie geregistreer is vir naoes gebruik op sitrus nie.

Die amien en ester formulasies word nie aanbeveel vir gebruik in swamdoder warm-waterbaddens in sitrus pakhuis nie. Nietemin, van die pakhuis gebruik die produkte in swamdoderbaddens. Navorsing bewys dat albei die amien en ester formulasies belemmer die effektiwiteit van die swamdoder Imazalil. Verkeerde gebruik van die twee formulasies van 2.4-D kan ook ernstige “brand” (fitotoksiteit) op sensitiewe sitrus vrugte veroorsaak. So ‘n geval is al voorheen waargeneem in ‘n voorontgroeningsstorting (drench).