Breeding for resistance to greening disease in citrus

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Abstract

Attention has been given to sexual breeding as well as preliminary studies directed towards in vitro selection of callus and protoplasts for resistance to greening disease in Citrus. Approximately 2000 hybrids have recently been infested with the vector, citrus psylla (Triozae oryzae Dell Guercio) from trees heavily infected with greening. Emphasis has been placed on lime (C aurantiifolia) parentage. Tahiti lime is considered highly tolerant to greening. Due to its triploidy, a small number of seeds resulting in only 86 hybrid seedlings, were obtained from a total of approximately 20000 fruits.

Nucellar callus of several citrus cultivars were initiated for intended studies on in vitro selection for resistance to greening.

Introduction

There are different approaches to a possible solution of the greening problem. Treatments of trees with antibiosis and other chemicals constitute the pathological viewpoint. The entomological approach is aimed at effective control of the insect vector. In addition is selection and breeding for cultivars resistant to greening. The latter project, however, is long-term and presently without any indications of the possibility of success.

The economic importance of the disease, however, necessitates attention to all possible and especially curative approaches. Antibacterial treatments of the tree and measures to control citrus psylla, might reasonably be considered as palliatives.

As the present study is of long-term nature, only preliminary results are presented. The paper should be considered a progress report and an indication of objectives in the project.

Selection of parents in breeding

The absence of proven sweet orange, mandarin, grapefruit and lemon cultivars resistant to greening poses a problem in resistance breeding. According to Aubert (B. pers. comm. 1983) the sweet orange cultivar, Gold Seal, seems to be tolerant to greening. This cultivar was imported to South Africa from Reunion. Greening-like symptoms appeared on a few of the plants being kept in a glasshouse in Nelspruit. Tests are underway to determine the presence of greening in these plants.

Selection of greening resistance trees in commercial orchards, even in severely affected ones, does not seem to be a feasible approach due to the erratic occurrence of greening.

The susceptibility of different cultivars of several citrus species was studied locally (McCLean & Schwarz, 1970; Schwarz, 1968). Experimentally infected seedlings and field trees of sweet orange, mandarin and grapefruit were found to be generally stunted and to show foliar symptoms. A certain degree of differential susceptibility, however, was noted between different cultivars of a species.

Before conclusions are drawn from observations of field trees it should be borne in mind that all trees do not flush simultaneously. Healthy trees may have flushed during a period of low psylla incidence and are thus escapees, rather than resistant to the disease.

Generally, true lemon, rough lemon, lime and trifoliolate orange and its hybrids were found to show milder symptoms. Based on deductions by McClean and Schwarz, (1970) and also our own field observations, the lime-group is more resistant to greening.

Fig. 1 Leaf shapes of limes, a few Microcitrus species, a Microcitrus x sweet orange hybrid and 25 of the 86 Tahiti hybrids available.
Horticulturally there are three
groups of limes viz the sweet limes
(such as Palestine sweet lime), the
small-fruited acid limes (such as West
Indian lime) and the large-fruited acid
limes (such as Bearss and Tahiti). This
latter group seems to be highly tolerant
to greening (de Lange, 1976).
The lime group therefore, seems to
merit special attention as a possible
source of greening tolerance in citrus
breeding. Breeding for tolerance to
greening in sweet orange, mandarin
and grapefruit by using a lime parent,
would require many generations of
back crossing to obtain a com-
mercially acceptable fruit.
Barrett and Rhodes (1976) studied
affinity relationships of many Citrus
cultivars and relatives by using a total of
of 146 characters. They speculated
that lime is probably a trihybrid inter-
generic cross involving citron (C. medica),
shaddock, (C. grandis) and a species of
Microcitrus.
Reece and Childs (1962) succeeded
in raising 77 hybrids of the sexually
almost-sterile Tahiti lime from open
pollination. These hybrids were classi-
ified in 10 different citrus groups based
on fruit characteristics. The citron
group was the largest. Natural cross-
pollination from surrounding trees
could have been responsible for
occurrence of the sweet orange and
grapefruit-type seedlings but not for
the citron-type. They concluded that
Tahiti lime may have originated from
a chance cross between citron (C. medica) and lime (C. aurantiifolia).
One of these postulated parents, citron
and shaddock both show clear green-
ing symptoms. It could be possible,
however, that genes for tolerance to
greening, are masked in these geno-
types. The resistance of Microcitrus
to greening is unknown. A few Micro-
citrus species as well as Microcitrus x
sweet orange hybrids have been estab-
lished at the Friedenheim Experi-
mental Farm near Nelspruit.
It was decided to concentrate on
Tahiti lime, Palestine sweet lime (being
a low acid fruit), citron and shaddock
with the emphasis on Tahiti lime.
For in vitro selection breeding it was
decided to employ nucellar callus of
commercial sweet orange, mandarin,
grapefruit and lemon cultivars.

Methods
TAHITI LIME HYBRIDS
HAND CROSS-POLLINATION:
A total of 2,600 Tahiti lime flowers
were cross-pollinated during the 1975
season with pollen of Eureka lemon,
Tomango sweet orange, Marsh grape-
fruit and a pollen-producing navel
mutant.
OPEN POLLINATION: All the
seeds were collected in the fruit of 13
mature Tahiti trees during the 1980
and 1981 seasons. A large percentage
of seeds was underdeveloped. The
seeds were aseptically germinated and
grown in culture tubes and trans-
planted into steam sterilised soil at
approximately the five leaf stage. For
the in vitro germination, the seed coats
were removed, the seeds sterilised for
10 min in 1% sodium hypochlorite
and placed on a 1% agar medium con-
taining the Murashige and Skoog (1962)
medium and 5% sucrose.

The resulting hybrids have been
kept in a glasshouse and 20 Troyer
citrange seedlings have been budded
from each of the hybrids. Half the
number of these budded hybrid trees
were exposed to infection to establish
their relative degrees of tolerance to
greening.

Ten trees of Minneola tanglo which
is highly susceptible to greening were
included as controls.
The trees are being exposed to
greening at Hazyskloof. Approximately
1,000 closely spaced citrus trees serve
as greening inoculum. These infected
trees were established in 1981 by
grafting 5 cm long shoots severely
infected with greening to surplus nursery
trees. A high population density of
citrus psylla is being maintained on
these trees. Infestation by psylla and
greening infection are obtained by
placing the hybrids to be evaluated
amongst the source trees.
The Tahiti hybrids have also been
established in a Valencia orchard,
heavily infected by greening, by top-
working of the mature trees. The aim
of this topworking is to get earlier
fruition as well as additional infor-
mation on resistance to greening.

OTHER HYBRIDS
During the 1982-83 season Clement-
ine flowers were cross-pollinated by
hand. The pollen parents were Pales-
tine sweet lime, citron, shaddock and
sour orange. The hybrids obtained
from this breeding, are also exposed to
greening infection at Burgershall Experi-
mental Farm.

IN VITRO SELECTION FOR
CALLUS AND PROTOPLASTS
RESISTANT TO GREENING
If any toxin(s) should be isolated
from cultures of greening bacteria, in
vitro selection for possible resistant
callus lines would receive high prior-
ity. It is therefore essential that suitable
callus cultures would be available for
such work.
Callus cultures were initiated by
using techniques developed by Koch-
ba, Spiegel-Roy and Safran (1972).
Cultures of ovules from 24 different
species and cultivars were made with
the object to develop embryogenic
callus from nucleolar tissue.

Results and discussion
TAHITI LIME
HAND CROSS-POLLINATION:
Despite the fact that a total of 2,600
Tahiti flowers were cross-polli-
nated, only five seeds were obtained and none
germinated.

According to Bacchi (1940) both the
large-fruited acid limes, Tahiti or
Persian lime and Bearss, are triploids.
Reece and Childs (1962), however,
found only 18 chromosomes, the
normal diploid number for the genus.
The pollen sterility and nearly total
seedlessness of both cultivars, how-
ever, support the observations of
Bacchi (1940). This phenomenon
could explain the failure in the local
hand-pollination experiment.

OPEN POLLINATION: Under
conditions of natural cross-pollina-
tion Tahiti seeds can be found although
at an extremely low frequency. In this
experiment only 150 seeds were re-
covered from approximately 20,000
fruits. From these seeds 86 seedlings
were obtained.

Eight of these seedlings had two em-
byros while the remainder were all
mono-embryonic. Morphologically the
two seedlings from a single seed were
in each case identical indicating the
single zygotic origin of the two
embryos. From a breeding point of
view, this mono-embryony — or at
least an extremely low percentage of
poly-embryony (Reece and Childs, 1962)
— is an advantage.

After multiplication of the hybrids
by budding onto Troyer citrange root-
stocks and exposure at Burgershall,
the trees were heavily infected with
citrus psylla. These trees were only
recently transferred to Burgershall. After
three months’ exposure these trees will
be brought back to a glasshouse in
Nelspruit and kept together with the
unexposed controls at a relatively low
temperature for maximum expression
of greening symptoms.
The 86 Tahiti hybrids were grafted onto mature Valencia trees heavily infected by greening. All the Valencia shoots that accidentally developed from the topworked trees are showing acute greening symptoms. Although some Tahiti hybrids do show greening symptoms, some have already grown vigorously to a height of approximately 3 m without any symptoms of greening.

An additional observation on these trees was that the majority of the hybrids also show vein clearing on the leaves similarly to that caused by tristeza in the Tahiti parent. Three of the hybrids do not show any such vein clearing symptoms.

Only a few hybrids have fruited so far. These fruits exhibited predominantly lime and lemon characteristics.

It is anticipated that in some hybrids fruits with some sweet orange, mandarin or grapefruit character might be produced as was found in Florida by Reece and Childs (1962). If any such hybrids also show resistance to greening, they would be valuable in further breeding.

A promising phenomenon is that of 21 hybrids that have already flowered, 20 were sexually fertile.

A wide range of leaf forms are evident amongst the 86 different Tahiti hybrids (Fig. 1). Much of this variation would be the result of natural cross-pollination.

The narrow leaf hybrids may substantiate the views of Barrett and Rhodes (1976) on the possible Microcitrus origin of lime.

Biochemical taxonomic studies might yield valuable information in this regard.

Microcitrus australis, M. australasica, M. inodora as well as different Microcitrus x sweet orange hybrids are available at the Friedenheim Experimental Farm. The reaction of these plants to infection with greening should be investigated.

OTHER HYBRIDS

Approximately 2000 hybrids were obtained from the cross-pollination involving Clementine as female parent. These plants have been exposed to psylla infestation at Burgershall. It is still too early to draw conclusions from this work.

*Fig. 2* Ovular development in West Indian and Tahiti lime on the 4th day prior to anthesis, at anthesis and on the 7th day following anthesis.
IN VITRO SELECTION FOR CALLUS AND PROTOPLAST RESISTANT TO GREENING

Suitable callus cultures have been established from the following cultivars:

Mandarins and mandarin hybrids: Cleopatra, Empress, Temple, Minneola, Ponciana, and Tangelo.

Sweet oranges: Midknights, Tomango, Rough lemon.

In the cultures of some other cultivars a problem is being experienced with excessive embryogenesis. In the case of other cultivars such as Tahiti lime no callus has initiated as yet.

While Tahiti lime is of no economic importance in South Africa, callus of this cultivar is required for comparative research. Any factor(s) isolated from cultures of green and bacteria which would affect callus and protoplasts of e.g. sweet orange more than those of Tahiti lime, could be important in the selection system.

To investigate the failure of Tahiti ovule cultures, anatomical sections were made of ovaries of Tahiti and West Indian lime on the fourth day prior to anthesis, at anthesis and the seventh day following anthesis (Fig. 2). These sections demonstrate some abnormalities in ovular development in Tahiti. Seven days after anthesis there were already indications of degeneration of Tahiti ovules. Four days prior to anthesis ovular development in Tahiti lags behind that of West Indian lime. The best material to initiate in vitro development from Tahiti ovules could possibly be that obtained at anthesis. Normally two- to six-week-old citrus fruitlets are used in ovule extraction for culture.

Tahiti is mono-embryonic, a further factor which complicates the establishment of nucellar callus cultures (Kobayashi et al., 1982). Callus developing in vitro at the stylar abscission zone has been subcultured but found to stop growing after a short period. A worthwhile alternative could be to initiate nucellar callus from a sexually fertile and green resistant Tahiti hybrid as soon as such a hybrid has been identified by the current tests.

Conclusions

Resistance is seen as the ultimate solution to the greening problem. Although the selection and breeding approach does not guarantee success, there are some positive indications. It will still require several seasons before any meaningful preliminary conclusions can be drawn.

The work with Tahiti lime hybrids could ultimately yield dividends. Indicators of success in this direction would be sexually fertile F1 progeny which have retained greening resistance. Their importance would be enhanced if they also exhibit some sweet orange, mandarin or grapefruit characteristics.

The proposed in vitro selection for resistance is dependant on progress in the in vitro culture of the greening pathogen and relevant biochemical studies.

Literature


