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Progress with Local Selections in the Quest for a More Tolerant/Resistant Avocado Rootstock than Duke 7

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ABSTRACT

The major objective of the ARC-ITSC's avocado rootstock breeding programme is the development of avocado rootstocks which are tolerant to Phytophthora cinnamomi*. Since the initiation of the programme in 1992 a total of 36 selections were made. For further testing of* Phytophthora *tolerance/resistance, these selections were clonally multiplied. Easy and effective clonal multiplication of rootstocks is an important nursery practice. Clonal multiplication is therefore, in addition to* Phytophthora*-tolerance/resistance, a selection criterion in the breeding programme. The selections earmarked for phase II evaluation and for clonal multiplication, were identified and would be discussed. Another 3 323 seedlings were screened for* Phytophthora *susceptibility in 1996.The seeds were collected at Levubu research station from a source not previously used. In the initial selection process, five per cent of these seedlings were selected, as being tolerant/resistant,*

UITTREKSEL

Die teel en seleksie van 'n Phytophthora*-verdraagsame/bestande onderstam is die hoofdoelwit in die LNR-ITSG se avokado onderstam teelprogram. 'n Totaal van 36 seleksies is gemaak sedert die aanvang van die program in 1992. Met die oog op verdere ondersoeke vir verdraagsaamheid/bestandheid is die seleksies klonaal vermeerder.Vinnige en maklike klonale vermeerdering is 'n essensiéle kwekery praktyk. Effektiewe klonale vermeerdering is dus naas* Phytophthora*-verdraagsaamheid/bestandheid 'n belangrike seleksie kriterium in die onderstam teelprogram. Die resultate van die klonale vermeerdering sowel as die vordering met die seleksie vir Fase II evaluering word bespreek. 'n Verdere 3 323 saailinge is die afgelope seisoen (1996) vir* Phytophthora*-verdraagsaamheid/bestandheid getoets. 'n Deel van die saad is versamel vanaf 'n bron wat nog nie in die verlede gebruik is nie en 'n hoe mate van oorlewing is waargeneem.Vyf persent van die saailinge is geselekteer.*

INTRODUCTION

Avocado root rot caused by *Phytophthora cinnamomi* has a large financial impact on the South African avocado industry. Development and selection of *Phytophthora*-tolerant avocado rootstocks is therefore one of the major research objectives of the ARC-ITSC's avocado research programmes. The ITSC's avocado rootstock programme was started

in 1992. Progress was reported by Bijzet *et al.,* (1993); Koekemoer *et al.,* (1994); Breedt *et al.,* (1995) and Bijzet *et al.,* (1996).

Since the initiation of the programme in 1992 a total of 36 selections were made. For further testing of *Phytophthora*-tolerance/resistance, these selections were clonally multiplied. Easy and effective clonal multiplication of rootstocks is an important nursery practice. Clonal multiplication is therefore, in addition to *Phytophthora* tolerance/resistance, a selection criterion in the breeding programme. The selections earmarked for phase II evaluation and for clonal multiplication, were identified and would be discussed. Another 3 323 seedlings were screened for *Phytophthora* susceptibility in 1996. The seeds were collected at Levubu research station from a source not previously used. In the initial selection process, 5% of these seedlings were selected as being tolerant/resistant.

MATERIAL AND METHODS

Various stages in the rootstock breeding programme were described by Bijzet *et al.,* (1993), and summarized in figure 1.

Rootstock breeding

Since 1992, open-pollinated seeds from avocado rootstocks and from other cultivars in the close proximity to the avocado rootstock material were germinated for screening. Detailed accounts of the results were given by Bijzet *et al.* (1993), Koekemoer *et al.* (1994), Breedt *et al.* (1995) and Bijzet *et al* (1996). Due to the recent drought only 25 to 33 % of the target number of 10 000 seeds to be screened annually could be obtained during the past two seasons (table 1).

Pollen derived from non-resistant sources detracts from the efficiency of the current procedure of producing seedlings. An isolated orchard consisting only of rootstock material, was necessary. In addressing this need a polycross nursery was established. The polycross nursery consists of six terraces, each three metres wide, 50 metres long and with 15 well-drained plant pots 1,25m in diameter and spaced three metres apart, giving 90 pots in total. This area is covered with shade cloth which is supported with treated poles. The result is an area of approximately 1000 m^2 that can be isolated from other avocado plantings, enclosing pollinators and only rootstock material with potential resistance to *Phytophthora* root rot (figure 2).

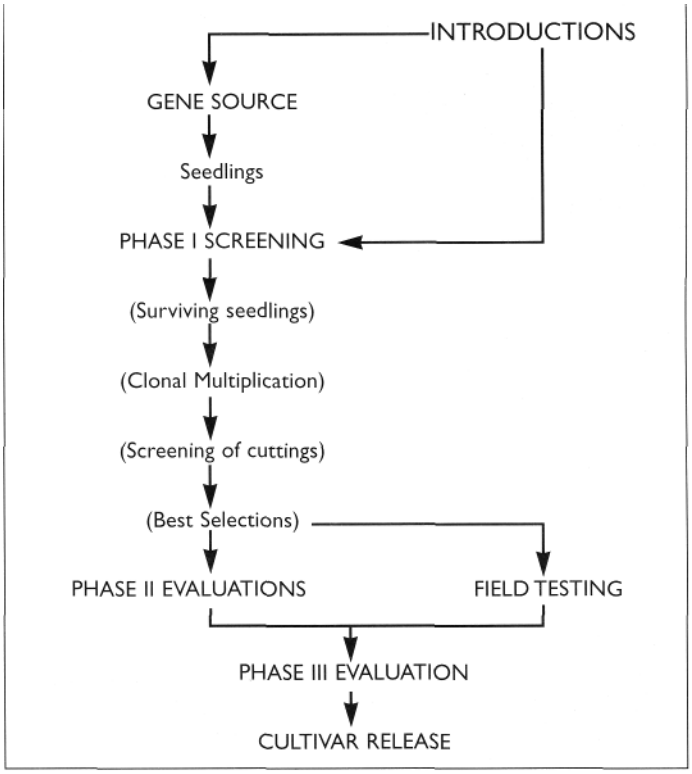

Figure 1
ITSC – rootstock breeding strategy

Table I
Screening of open-pollinated seedlings

Year	*Seeds*	*Selections*	*Survival*
1992	5 717	12 (0,21 %)	7
1993	2 799	15 (0,54 %)	2
1994	16 381	26 (0,16 %)	9
1995	2 437	4 (0,16 %)	0
1996	3 323	190 (5 %)	Still under observation

Figure 2
Renovated shade cloth structure

Phase I screening

The protocol has been finalized to screen Phase I seedlings. This is done by planting seed directly in bins filled with *Phytophthora cinnamomi* soil (figure 3).

The seeds are left to germinate and subsequently to die of *Phytophthora* root rot. Indicator plants show whether the disease pressure is correct and if not, a mycelium suspension is applied approximately 120 days after germination. Surviving seedlings are selected. If the percentage surviving selections are too high, further elimination is done after an inspection of the root systems. The surviving seedlings are then treated as described by Koekemoer *et al.* (1994) and transplanted to black 50 litre rubber dustbins that are filled with sterilized soil. This allows proper root expansion and subsequent top growth which is required for further multiplication of the selections.

Figure 3
Avocado seeds in large bins filled with *Phytophthora* infested soil

The second phase, a statistical screening of the surviving selections then follows. For this, 20 to 30 cuttings of each selection and of Duke 7 as control are made. These are then planted in bins filled with sterilized soil to which a known concentration of *Phytophthora* mycelium is added. Valid comparisons can be made and selections better than Duke 7 are included in field trials and in a phase II programme. The field trial will

be carried out at Burgershall, in an orchard known to have a high incidence of *Phytophthora.*

Clonal propagation

The main methods of propagating avocado clonally were described by Frolich & Platt (1972), Ernst (1978) and Moll & Wood (1980). The method of Moll & Wood (1980) was chosen to multiply selected *Phytophthora* tolerant seedlings since limited material was available for grafting and a large number of plants are required for additional *Phytophthora* tests as well as horticultural evaluations.

Seed of a sunblotch-free avocado tree, cv. Edranol, were planted in small plastic bags (70 mm diameter, 150 mm high) containing a well-drained sterile medium. After germination, the nurse seedlings were grafted to the required selections to be multiplied. At budburst the nurse plant was transferred to darkness for etiolation. Two methods were followed from this step, depending on the growth habit of the selection:

- When leaves developed during the etiolation process, cuttings were taken while still in the dark when the shoot stopped growing actively (approximately 300 400 mm). Cuttings were taken internode length and placed in a mist bed with bottom heating at 26°C and a mist blow of two seconds every minute.

- Selections, which did not develop leaves in the dark, were removed from the etiolation chamber when the etiolated shoots were approximately 20 30 mm long. The shoots were painted black with a bitumen-based tree sealing compound and the plants were left in daylight to develop normal green leaves. When one or two leaves have developed, the shoot was cut and placed in a mist bed as described above.

Roots developed approximately 4-8 weeks later. When the roots have developed, the cuttings were transplanted to the same size plastic bags with medium as the nurse seedlings. The transplanted cuttings were left under the mist spray for a week where after they were hardened off.

RESULTS AND DISCUSSION

Rootstock breeding

The polycross nursery was established with 80% of the mother trees already planted. It is expected that this facility will produce seed within the coming season.

Phase I screening of seedlings

From 1992 to 1995 a total of 27 334 seedlings were screened for resistance/tolerance to *Phytophthora* and 59 selections were initially made. During the subsequent multiplication period 29 of the weaker selections died. Twelve seedlings apparently showing strong root development and few signs of *Phytophthora* root rot also died after being transplanted with the seed lobes removed. It is postulated that survival of these seedlings was due to the reserves available in the seed lobes. The four seedlings that

were reported during 1996 as having strong root development and being promising were part of these 12 seedlings, thus leaving a total of 18 seedlings in the multiplication process. Another 3 323 seedlings were harvested and screened in 1996. Three quarters of the seed were taken from a gene source at Levubu research station that was not previously exploited. Although judging from the indicator plants in the bin, a high *Phytophthora* pressure was maintained, more than 50% of the seedlings still survived. The surviving seedlings were mainly from the new gene source. On close inspection of the roots, 190 seedlings were selected which accounted for 5% of the total seedlings screened. As 5% is still a high selection rate, these selections have not been transplanted yet but are currently being subjected to further *Phytophthora* pressure for a period of four weeks. This seems to be worthwhile as some of the selections are already showing signs of *Phytophthora* related deterioration.

Clonal propagation

The seedling rootstocks which were grafted to nurse seedlings for etiolation are listed in table 2. The mean number of cuttings which could be taken, after etiolation, from each successful graft on a nurse seedling as well as the percentage of these cuttings which rooted, are presented in the table. These records are kept to obtain information on the clonal propagation ability of the seedlings. Selection 92-1-2/5 was notably more prolific by producing a mean number of 50 cuttings per nurse seedling. The rooting ability of this particular selection, when compared to the other selections and to Duke 7 (25%) was highly satisfactory. The selection 94-7-1/2 (Teague) and two selections from the Paarl had a rooting percentage of more than 70%. Budwood from the different seedlings is still limited as the mother plants have yet to respond to the transplantation to the larger bins. Clones from the mother trees will be planted in orchard J9 at Nelspruit during March. Trees planted in orchard J9 are now two years old and are thus ready to be top worked to available budwood of the selections. This will enhance clonal propagation.

CONCLUSION

A further 3 323 seeds were germinated and screened for resistance. Five per cent of the seedlings were selected. Results of the clonal multiplication of the previous selections (1992 95) show a variability among the selections regarding cutting production as well as rooting ability. These results have an influence on the selection of material to be horticulturally evaluated in the Phase II evaluation programmes.

With regard to future prospects a new dimension has been introduced to the breeding programme with the implementation of the polycross nursery. Exploiting new sources other than the Duke 7 family opens the prospects of introducing different modes of tolerance and even resistance in a locally bred rootstock. This could lead to a range of local rootstocks to choose from. The upgrading of facilities to cope with the large amount of clonal cuttings to be screened in the future will be finalized during 1997. Exciting results are expected from the statistical screening of cuttings.

Table 2
Number of cuttings per nurse seedling and percentage of rooting of the selections in the multiplication process.

Selection	*Mother plant*	*Number of cuttings per nurse seedling*	*% Rooted*
92-1-1	Duke 7	5,5	63,6
92-1-2/1	Duke 7	13,0	20,5
92-1-2/2	Duke 7	11,0	9,1
92-1-2/5	Duke 7	50,0	66,0
92-2-2	Barr Duke	28,5	57,9
92-4-3	Barr Duke	11,0	54,5
92-5-1	Barr Duke	14,0	35,7
93-2-5/3	Barr Duke	0,0	0,0
93-5-2/3	Barr Duke	0,0	0,0
94-1-9	Duke 7	0.0	0,0
94-1-10	Duke 7	0,0	0.0
94-1-12	Duke 7	12,7	55.3
94-2-4	Barr Duke	14,0	39,3
94-7-1/2	Teague	11,0	77,3
94-18-1	D9 (SP)	0.0	0,0
2-62-1	(Pietou, Paarl)	11,0	72,7
2-64-1	(Bruwer, Paarl)	9,7	75,9
Wurtz sel	Wurtz	5,7	35,3
Duke 7	Clonal	4,0	25,0

REFERENCES

BIJZET, Z., SIPPEL, A.D. & KOEKEMOER, P.J.J. 1993. Avocado breeding: a progress report. *South African Avocado Grower's Association Yearbook* 16: 86 - 89.

BIJZET, Z., BREEDT, HJ. KOEKEMOER, P.J.J. & CILLIERS, B. 1996. Avocado rootstock breeding New developments and intricacies. *South African Avocado Growers' Association Yearbook* 19: 14 - 15.

BREEDT, H.J., KOEKEMOER, P.J.J. & BIJZET, Z. 1995. Vorderingsverslag: Seleksies van *Phytophthora* bestände Avokado onderstamme 1992 tot 1994. *Suid-Afrikaanse Avokadokwekersvereniging Jaarboek* 18: 4 - 6.

ERNST, A.A & HOLTZHAUSEN, L.C. 1978. New promising technique for rooting difficult-to-root avocado *(Persea Americana* Mill.) Cuttings. *Citrus Subtrop. Fruit J* 532: 6 - 10

FROLICH, E.F. & PLATT, R.G. 1972. Use of the etiolation technique in rooting avocado cuttings. *Calif. Avocado Soc. Yearbook* 55: 97 - 109.

KOEKEMOER, P.J.J., BREEDT, HJ. MANICOM, B.Q. & BIJZET, Z. 1994. Selection *of Phytophthora* tolerant avocado rootstocks. *South African Avocado Growers' Association Yearbook* 17: 72 - 74.

MOLL, J.N. & WOOD, R. 1980. An efficient method for producing rooted avocado cuttings. *Subtropica* 1(11): 9 - 12.