9
Towards a Robusta Variety Resistant to CWD

9.1 Main Findings

- A breeding programme in Uganda has screened thousands of Robusta plants for resistance to coffee wilt disease (CWD).
- The initial screening produced over 1500 lines potentially resistant to the disease.
- Further screening and agronomic trials have reduced this to seven final candidates for release to farmers in 2010.
- In addition to CWD, these clones have been evaluated for a range of agronomic traits including yield, cup quality, bean size and resistance to other coffee diseases.

9.2 Introduction

In the previous chapters, we have seen accounts of some basic steps required to develop a breeding programme that include:

- the search for and characterization of the extant CWD strains;
- the collecting of candidate coffee genotypes for conservation and testing;
- the development of a reliable rapid screening method; and
- the rapid screening to eliminate susceptible varieties.

With these steps undertaken and valuable data created, a breeding programme can begin to develop and test resistant material to the stage that it can be multiplied to the point of release to farmers.

In the case of CWD, Uganda has the most developed breeding programme and hence this chapter outlines mostly the work of the group at the Coffee Research Institute (CORI) led by Drs Hakiza and Musoli, with support from the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) in France.

9.3 Towards a Breeding Strategy for CWD-Resistant *Coffea canephora*

Based on the results outlined above, an outline breeding plan for *C. canephora* is given in Figure 9.1.

Breeding a new coffee variety normally takes 20 years or more. The reason for this is that it takes many years to complete enough crosses and backcrosses with existing genotypes that provide the other required qualities (yield, cup quality, resistance to other diseases, etc.) to produce a final product that has all the characteristics that the farmer is used to from his previous varieties plus the additional factor of being resistant to CWD.

Phiri N. and Baker, P.S. (2009) *Coffee Wilt in Africa Final Technical Report*. CAB International.

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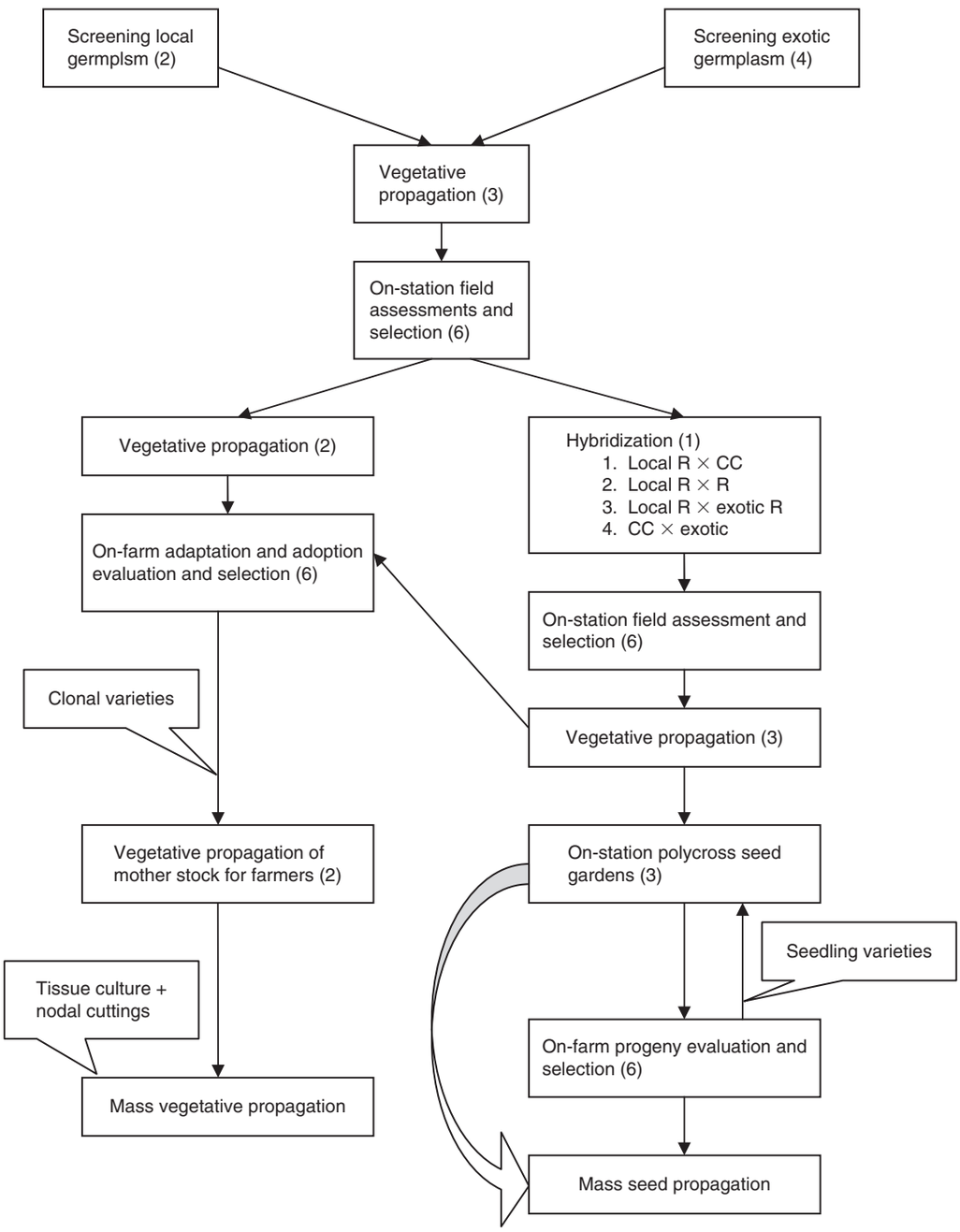
Figure 9.1: Proposed scheme for developing *Coffea canephora* varieties resistant to coffee wilt disease (CWD).
Figures in parentheses are the minimum time in years a particular breeding stage could take. R = resistant and CC = current commercial varieties.



In the present case, the devastation caused by the disease generates a great urgency of finding a resistant variety in the case of Ugandan Robusta, which means that the process needs to be speeded up as much as possible. Fortunately, with the Robusta species considerable genetic variation exists, much more so than Arabica, and more has come to light in the present project with the collection of materials from the Ugandan forest zones (Chapter 6).

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This means that the strategy taken in Uganda has concentrated on the left column of Figure 9.1, where large-scale screening of genotypes from different localities and through artificial inoculation experiments has fairly quickly produced a number of candidate individuals for further testing in the field. If these candidates pass in this field phase, then they can be cloned through vegetative cuttings.

9.3.1 Large-scale germplasm screening

Since a quick and effective way of screening for resistance was developed during this project (Chapter 7), large-scale screening of germplasm was carried out to find resistant strains present in the markedly variable populations of *C. canephora* genotypes available. In Uganda, 1519 candidate CWD-resistant genotypes have so far been identified through mass screening of coffee seedlings and cuttings derived from germplasm available in the conservation plots at the research institutes and from coffee trees surviving in wilt-devastated gardens.

Two main sources of material were used:

1. Conservation plots at Kawanda Agricultural Research Institute (KARI) provided the largest amount of open-pollinated seedlings and rooted cuttings screened.

2. Wilt-devastated gardens provided the rest, where open-pollinated seed and semi-hard wood cuttings were obtained from coffee trees that had survived the disease unscathed.

For both provenances, at age 6 to 8 months, seedlings and cuttings were inoculated with isolates of the pathogen prepared from tissue obtained from the susceptible commercial clone 257/53. Due to the work carried out on strain identification (Chapter 4), breeders were confident that the CWD strain used was the only extant Robusta strain and one that displayed essentially no genetic variability, making it unlikely that resistant strains would break down due to CWD strain natural selection.

The plants were inoculated using the root-dip method with the standardized inoculum of 1.3×10^6 conidia per millilitre. All inoculated plants were incubated at room conditions in the screen house and monitored for CWD symptoms. At the end of each trial, any healthy-looking plants were re-inoculated 6 months after the first inoculation. These re-inoculated plants were again incubated in the screen house and monitored for the disease symptoms. Again after another 6 months, healthy-looking plants were selected for another inoculation. Survivors of these re-inoculations were considered resistant and hence the source of potential clones for future varieties. All the resistant genotypes were then planted in mother gardens and each of them was cloned through cuttings to raise sufficient material for carrying out full-field evaluation trials.

It was found that only a very small proportion (3–15%) of the plants raised from the on-station germplasm plots survived the first inoculation (Figure 9.2). In contrast, the proportion of survivors raised from the genotypes surviving in wilt-devastated gardens was relatively higher (15–20%). It was also found that after re-inoculation, about 50–70% of the re-inoculated plants die. Therefore, the need is for mass screening of very large numbers of progeny in order to find enough material for later selections and agronomic field trials.

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Figure 9.2: *Coffea canephora* seedlings that survived the coffee wilt disease (CWD) infection after the first inoculation (*right*) and seedlings that did not (*left*).

Meanwhile, in Tanzania, over 270 resistant genotypes were identified through mass screening of germplasm. The resistant genotypes have been planted in mother gardens for vegetative multiplication.

9.3.2 Field germplasm screening

Open-pollinated seeds and semi-hard wood cuttings were obtained from coffee trees surviving in wilt-devastated gardens. Seedlings and rooted cuttings, respectively, were raised from these materials for inoculation and selection of resistant genotypes following the procedures described in Chapter 7.

Germplasm screening was also carried out in field observation plots. Because of restrictions in plot availability, time and labour costs, field evaluations inevitably handle fewer plants and the assessment must be prolonged over many years, in order to have a high degree of certainty that resistant genotypes selected are truly resistant under a range of agro-ecological conditions.

At CORI, clones were assessed for CWD resistance in a field trial started in 1999 through to 2006. A range of responses between clones was observed, with one clone (J/1/1) still resistant by November 2007. However, rooted cuttings raised from this resistant clone, when tested for resistance in the screen house, succumbed to the disease although the level of disease among its cuttings was lower than cuttings of the other clones within this trial.

9.4 Multiplication of the CWD-Resistant Varieties

Since *C. canephora* is an outcrossing species, progeny are heterozygous and therefore resistant varieties will not breed true. Consequently, all disease-resistant candidates

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must be propagated by vegetative methods, particularly rooted nodal cuttings or tissue culture. At CORI, cuttings are used for propagating materials for further evaluation, while tissue culture is exclusively dedicated to raising planting materials for farmers.

9.5 Field Evaluation of the Resistant Varieties

Not all the CWD-resistant genotypes have the required qualities of commercial varieties. Hence, the candidate CWD-resistant genotypes must undergo field evaluation for yield, resistance to other diseases (coffee leaf rust (CLR) and red blister disease in Uganda), physical bean qualities and cup quality.

To this end, small trials were set up with a large number of selections, to screen out those with higher potential for further evaluation in different agro-ecological localities. As of November 2007, 1519 CWD-resistant clones, which had been identified through screening in Uganda, were planted in single rows of six trees in an on-station trial at Kituza.

To date, seven superior clones have been selected from this process. The seven clones are being multiplied through nodal cuttings and tissue culture for further evaluation in different agro-ecological areas. Good yielding clones (at least 2 t of green bean per hectare) which are resistant to CLR and red blister disease and have good liquor and physical bean qualities will be recommended for farmers' use, either across the entire country or for particular agro-ecological localities, depending on the performance of individual clones in the multi-location trials.

The CORI team is currently propagating the CWD-resistant clones for distribution to private propagators and farmers. This is being done both through cuttings and tissue culture propagation. Currently (August 2009), the tissue culture is operating under capacity, though Common Fund for Commodities (CFC) funds have been allocated to purchase spare parts for 250 bioreactors which are being used to multiply the clones. To date, 7000 plantlets have been propagated through the tissue culture facility.

Three private propagator companies (who sell directly to farmers) were given five seedlings each for each of the seven resistant clones for developing mother gardens in Bushenyi and Mpigi districts (Box 9.1). The plan is to give 50 seedlings of each resistant clone to nursery operators and this is being funded by the Uganda Coffee Development Authority (UCDA). Clearly, however, the scaling-up programme is underfunded.

However, even if all seven clones perform well and are released to farmers, this may not be enough to ensure both enduring resistance to the disease and good agronomic performance under a wide range of field conditions. Quite apart from the need to retain some heterogeneity to lower the chances of the disease overcoming resistance, a mix of clones is recommended to ensure good yields, since *C. canephora* is a species that outbreeds, and hence good pollination and yield are more likely to come from fertilization between non-identical genetic materials.

Hence, the search for more resistant material should continue as well as the research to develop these to the commercial stage.

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Box 9.1

Scientists plot end of coffee wilt.

(*New Vision*, Kampala; Friday, 14 August 2009; by Macrines Nyapendi.)

SEVEN new varieties of disease-resistant seedlings will be released to farmers next season to beat coffee wilt, Dr Africano Kangire, the head of the Coffee Research Centre in Kituuza, has said.

To speed up multiplication of the planting materials, scientists at Kituuza are using technology which enables them to get thousands of seedlings from a piece of leaf.

The new varieties developed at Kituuza can yield over 3,250 kg per hectare.

'After the mass production and distribution of the wilt resistant varieties, the war on coffee wilt disease will come to an end', Kangire said.

9.6 Hybridization for CWD Resistance

The CWD-resistant clones which do not exhibit high-enough agronomic and commercial qualities can be crossed with other lines which possess these complementary traits. The hybrid progenies can then be evaluated for all the traits in screen houses and through field trials. The clone strategy ensures that only highly resistant genotypes proceed to the field. The hybrid strategy (the right arm of Figure 9.1) is less effective for evaluation of CWD resistance, since it relies on field responses only, but it retains more variation that may include better commercial quality as well as being sufficiently resistant for many situations.

The current commercial clones (1s/2, 1s/3, 1s/6, 223/32, 257/53 and 258/24) used in Uganda have complementary traits, but are all susceptible to CWD. The commercial clones yield 2.5–3 t of green coffee beans per hectare, have good bean qualities (e.g. over 90% retained by screen 18/64) and have good cup qualities. Hybrids have been generated through artificial pollinations to combine the CWD resistance and the complementary traits found in these commercial clones. The progenies generated have been planted in field trials at Kituuza for evaluation, as individual trees. The individual genotypes that are found to be within acceptable limits of the traits will be selected, multiplied as clones and planted in multi-location trials in different agro-ecological localities for reassessment. Clones that perform well in these trials will be selected for release to farmers as clonal varieties either at agro-ecological zone level or for the entire country, depending on the performance of the variety.

Hybridization can also be made between the CWD-resistant clones possessing complementary traits. The hybrid progenies generated in such crosses are also evaluated as individual trees for resistance against CWD and for field performance in the other traits. Good performing individuals can be selected, cloned and planted in multi-location trials for adaptation and adoption tests. These clones will then be selected and released to farmers.

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If there is an entire progeny of a cross between resistant and susceptible parents which perform well (resistant to CWD, CLR and red blister disease, have good qualities and are high yielding), then parents of such progenies can be planted in polycross seed gardens for production of seeds to be given out to farmers. It is anticipated that progenies involving parents from different populations, particularly from distant geographical locations, will benefit from hybrid vigour derived from double heterozygosity of the parents. As already indicated above, given the specificity of the pathogen populations affecting the different commercial coffee species, resistant varieties can be derived through interspecific hybridizations, bearing in mind the complications associated with such hybridizations and the difficulty to derive a variety of desired quality.

As known from many breeding programmes, incorporation of CWD-resistant genes into commercial clones that should also have good quality traits is likely to take a very long time and, at times, it is a gamble. Therefore, molecular techniques can be adopted to facilitate the breeding and selection process. Studies had been initiated at CORI, Uganda to characterize CWD resistance using molecular markers and it is hoped that a follow-up of these studies can continue, which should lead to mapping of the resistance genes. Mapping studies could be initiated using the double haploid CWD-susceptible or CWD-resistant parents and their progenies to identify molecular markers and or quantitative trait loci (QTLs) associated with the CWD resistance to assist in breeding resistant varieties and isolation of resistance genes for creating bacterial artificial chromosome (BAC) libraries. Use of double haploids will minimize the effect of heterozygosity.

9.7 Grafting

Since currently the mechanism of resistance against the CWD pathogen is not known, we are not certain that a resistant rootstock can prevent the pathogen from reaching the scion. However, if it is established that this phenomenon is possible, then grafting scions of varieties with good agronomic characteristics, but which are susceptible to CWD, on to a CWD-resistant rootstock could be a good and probably quicker means of deriving appropriate planting materials for farmers of both *C. canephora* and *C. arabica*. In Uganda, grafting of current commercial clones on to the CWD-resistant clones are being evaluated for: (i) compatibility between the scion and rootstock; and (ii) for the ability of the rootstock to prevent the CWD getting through the vascular system of the rootstock to the scion. If these results are satisfactory, then grafting should be adopted for continuing with a large-scale multiplication of the current commercial varieties.

Rootstock of other *Coffea* species can also be explored for this purpose. Successful interspecific grafting involving *C. canephora* and *C. liberica* has been reported (Couturon, 1993). However, Bertrand *et al*. (2001) reported depressing effects of *C. liberica* rootstock on yield and quality of *C. arabica* scion varieties. Therefore, other agronomic properties of the grafted varieties such as yield and quality should be studied and well understood before recommending grafting for producing planting materials for farmers on a large scale.

9.8 Availability of the Improved Material for Replanting in the Democratic Republic of Congo (DRC)

The current situation in DRC with respect to CWD requires rehabilitation of existing production areas, and the extension of these production areas using planting material

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of desirable characteristics. Currently in DRC, when starting new plantations, farmers seldom use seeds from the research centres that have guaranteed characteristics for good production. Generally, the vegetative material used is from cuttings and often this material performs poorly. However, the elite clones selected at Yangambi, which include seven clones of Robusta (L 36, L 48, L 93, L 147, L 215, L 251, SA 158) and five clones of small Kouillou (LAF 93, LAF 159, S 9, S 19, S 23) are available and those can be used preferentially by the farmers.

One of the priorities for the DRC government, as described in the strategic action plan, is the revival of the coffee production by the systematic replanting of the old plantations. The Government proposes to rehabilitate all the centres of production and the multiplication of the elite clones of Institut National des Etudes et de la Recherche Agricole (INERA). The plan also includes the reorganization of the supply chains of quality coffee material by the creation of multiplication centres and the dissemination of improved material in each of the four principal zones selected above. This requires the involvement of the Ministry of Agriculture (technical and financial support) and the Office National du Café (ONC) as partners already on the ground (Beni/Nord-Kivu and soon in Boma and Isiro).

The ONC and the farmer trade union (SYDIP) have set up seven centres for multiplication of rooted cuttings at Beni, Oïcha, Irango, Bingo, Mutwanga, Vuyinga, Mayi Moya in North Kivu. The INERA Institute has recently acquired an *in vitro* propagation unit to enhance the multiplication.

9.9 Conclusions

Uganda has made great strides towards the development of resistance to CWD in a comparatively short time, since normally it takes 20 or more years to develop a new variety. The speed of progression is an indication of the seriousness of the CWD situation in Uganda. However, the whole programme needs further funding support, both to find more clones as well as to bulk up the many millions of plants that will be needed.

It remains to be seen how well the seven clones – those that will be released shortly into the field – will behave. Seven clones is a small number, but there is hope that the very limited genetic variation of the CWD strain will mean that the material will remain resistant.

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