# Characterization of Robusta Diversity

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There is a great urgency for intensifying studies of the still existent wild coffee populations, especially in those geographic areas where such populations are most threatened by extinction. The preservation of this coffee germplasm should be secured, either by conservation of the natural forest habitats or by establishing living collections.<sup>1</sup>

## 6.1 Main Points

 Robusta material was collected from Ugandan forest sites of Kibale and Itwara, and genetic diversity analysed using simple sequence repeats (SSR) marker technology.

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- The material was compared with cultivated genotypes from Kalangala Islands on Lake Victoria as well as Nganda and Erecta phenotypes.
- The genetic analysis revealed the significant differences between the groups collected. The Ugandan genotypes were found to be sufficiently different from the Guinean and Congolese types to represent new, genetically distinct diversity groups within the *Coffea canephora* genome.
- The variability inherent in the Ugandan Robusta genome therefore represents a very valuable resource for future breeding programmes.
- More collections from Uganda and other countries should be undertaken to maximize available genotypes for future breeding purposes.
- In Uganda, this valuable genetic resource is under threat from deforestation and coffee wilt disease (CWD) itself – it is urgent that steps are taken to collect and conserve this material in safe sites.

## 6.2 *C. canephora* Diversity

Berthaud (1986) first identified two C. canephora groups:

- A Congolese group, which comprised genotypes from the Central African Republic (CAR), Cameroon and the Democratic Republic of Congo (DRC) basin;
- A Guinean group, which consisted of genotypes of wild origin from Côte d'Ivoire.

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

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<sup>&</sup>lt;sup>1</sup> Charrier and Berthaud (1985).

Montagnon *et al.* (1992) proposed a substructure in the Congolese group with two subdivisions, SG1 and SG2. Dussert *et al.* (2003) added two extra groups, B and C, to the Congolese group. Subsequent studies using SSR markers on *C. canephora* among other coffee species (Poncet *et al.*, 2004) and on *C. canephora* alone, have confirmed the structure described by Dussert *et al.* (2003). Most recently, Gomez *et al.* (2009) separated five groups within the Guinea–Congo zone, with Guinean *C. canephora* as diversity group D, geographically separated by the Dahomey Gap from diversity groups A, B, C and E in the geographical area of DRC, Cameroon and CAR.

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# 6.3 Seeking Sources of Resistance

As part of the research programme, a major search was undertaken to find sources of genetic diversity within *C. canephora* that might display resistance to CWD. The subject of this chapter is largely based on the work of Musoli (CORI) and CIRAD collaborators.

### 6.3.1 Collections

Various centres were contacted for contributions including:

- The collections of Uganda (Coffee Research Institute CORI) and DRC (University of Kinshasa – UNIKIN);
- The Côte d'Ivoire national collection, which accepted and exchanged planting material and carried out evaluation;
- The French Guiana collection (the Centre de Coopération Internationale en Recherche Agronomique pour le Développement – CIRAD); and
- The collections of Costa Rica (Centro Agronómico Tropical de Investigación y Enseñanza – CATIE).

#### 6.3.2 Surveys

Surveys were made by Musoli *et al.* (2006) of coffee trees in the following places:

- 1. The primary forests of Uganda, to enhance knowledge of the genetic structure of this species. Collections were made and disease occurrence recorded from Itwara forest (four sites) and Kibale (three sites).
- 2. The Kalangala Islands on Lake Victoria (five sites), which is an area that was previously cultivated but now wild.
- 3. The cultivated trees of Erecta (10 progenies) and Nganda (16 progenies) cultivars were also collected;
- 4. The remnant unaffected commercial coffee trees in Uganda and DRC in zones where infection rates were very high.

The material collected is tabulated in Table 6.1.

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	Source	Type of material	Sampled sites	Studied individuals
Ugandan sources	Itwara	Wild	5	55
	Kibale	Wild	4	54
	Kalangala	Semi-wild/ cultivated	5	35
	Nganda	Cultivated	1	31
	Erecta	Cultivated	1	21
Core sources	Guinean	Wild	3	106
	SG1	Cultivated	4	25
	SG2	Wild	1	9
	Congolese B	Wild	1	39
	Congolese C	Wild	1	10

Table 6.1: Collected Coffea canephora (Robusta) material.

## 6.4 Genetic Analysis of Collected C. canephora Material

Samples from a total of 196 Ugandan coffee trees from the above sites were analysed using 18 microsatellite markers (using SSRs; Musoli *et al.*, 2006) and compared to individuals from other *C. canephora* diversity groups (Cubry *et al.*, 2005).

Ugandan *C. canephora* was found to be highly diverse with a genetic substructure consisting of wild, feral and cultivated populations (Table 6.2). Ugandan *C. canephora* was found to be different from previously known diversity groups, implying that they form new groups within the *C. canephora* species genome (Figure 6.1).

Substantial differences in heterozygosity and gene diversity were observed within and between all sources. Interestingly, wild Kibale and Itwara accessions had lower observed heterozygosity and gene diversity than cultivated populations, including Kalangala, suggesting that cultivated genotypes were the result of outcrossings rather than clonal selection.

The mean number of alleles is high in Ugandan sources, compared to other sources. Among the Ugandan sources as well as the reference Guinean material, the wild sources of Itwara and Kalangala were found to have the highest number of private alleles (i.e. gene variants that are unique to one specific locality). Within these sources, however, there was strong genetic similarity.

Considering separately the three Ugandan sites, Kibale had a highly significant fixation index ( $F_{sT}$  coefficient), which gives the estimation of genetic differentiation between groups, meaning that sites within this source are highly differentiated into subpopulations. On the other hand,  $F_{sT}$  coefficients were not significant within Itwara and Kalangala, meaning that the populations in each site are very similar.

In the case of Kalangala, there were clear similarities to cultivated regions, suggesting that isolation for some generations has allowed some specific alleles to dominate.

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Source	Observed heterozygosity and <i>p</i> value	Gene diversity and <i>p</i> value	Mean number of alleles	Private alleles (Ugandan sources)	Private alleles (all sources)
Itwara	0.396 (0.044)	0.585 (0.041)	193 (8.04)	37	14
Kibale	0.288 (0.043)	0.531 (0.051)	177 (7.38)	19	4
Kalangala	0.405 (0.045)	0.628 (0.049)	206 (8.58)	34	17
Nganda	0.407 (0.043)	0.623 (0.048)	194 (8.08)	14	7
Erecta	0.397 (0.045)	0.625 (0.048)	172 (7.17)	12	6
Guinean	0.35	0.50	179 (5.29)		24
SG2	0.41	0.69	229 (6.74)		12
SG1	0.27	0.37	242 (7.12)		11
Congolese B	0.37	0.50	173 (5.09)		7
Congolese C	0.37	0.45	101 (2.97)		14

Table 6.2: Diversity statistics for Ugandan Coffea canephora collected, comparedto the Congolese and the Guinean accessions. (Gene diversity for Guineanand Congolese regions was adopted from Cubry et al., 2005.)

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Number of private alleles for Ugandan regions was detected with 24 markers among 196 individuals. Number of private alleles for all regions was detected with 18 markers on 232 individuals.

Figure 6.1: Phylogenetic tree of 232 individuals using weighted neighbour joining method among 18 microsatellite loci. G (black) indicates Guinean accessions; SG1, SG2, B, C (brown) indicate Congolese individuals. All the rest are Ugandan genotypes: I (dark green) indicate Itwara forest, Ki (red) indicates Kibale forest, N (red) indicates Nganda, E (green) is Erecta and Ka (blue) is Kalangala Islands. (From Musoli *et al.*, 2006.)



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Hence, the results indicate that Ugandan materials are outside the previously known *C. canephora* genetic diversity and are potentially a valuable source of material for future breeding programmes.

Nganda and Erecta varieties appeared as a mixed group on the phylogenetic tree, meaning that their phenotypic differences were not clearly distinguishable by the genetic markers.

Pairwise  $F_{st}$  values among Ugandan cultivated regions are very low, indicating a low genetic differentiation.

## 6.5 Conclusions

Results of diversity analyses and population structure in this study established that Ugandan *C. canephora* is different from known diversity groups, displaying high genetic variation. This diversity of Ugandan Robusta, for both wild and cultivated populations, indicates the importance of this country as a source of *C. canephora* diversity, especially given the general global lack of coffee genetic material.

The phylogenetic trees that were constructed confirmed Ugandan genotypes as a new diversity group within the species.  $F_{\rm ST}$  values observed between sites and sources confirmed a high genetic differentiation within the species. A common genetic ancestor exists between the Congolese SG2 group and cultivated Ugandan genotypes, since their  $F_{\rm ST}$  values are much lower than values for Ugandan Robusta relating to other regions.

The analyses also pointed out the specificity of Kalangala, which has a high mean number of alleles and private alleles. Itwara sites, however, show much lower differentiation, and they can be considered as a single population. The short distances between sites in Itwara could partly account for this observation. The correlations between geographical and genetic distances allowed researchers to establish that wild Ugandan genotypes have a genetic isolation related to geographical distance.

Deforestation and CWD are both depleting the gene pools of *C. canephora* in Uganda. It is therefore very important to protect these genetic resources, and, as a matter of urgency, further collections should be made and conservation plots set up at secure sites (Box 6.1).

Genetic variation among *C. canephora* populations is high, so samples of a few genotypes should be obtained from many populations so as to capture as much of the

#### Box 6.1

#### Uganda: environmentalists point to worrying pace of deforestation

(http://www.irinnews.org/Report.aspx?ReportId = 84972).

KAMPALA, 24 June 2009 (IRIN) – Uganda has lost nearly a third of its forests in the last 2 decades and could lose most of its tree cover in about 40 years unless measures are quickly taken to reverse the situation, environmentalists have said.

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'Climate change does not happen in isolation....It interacts with existing problems and challenges – notably deforestation, soil degradation, declining food security, declining fish stocks – and makes them worse', said Frank Mulamuzi, environmental advocate and executive director of the National Association of Professional Environmentalists (NAPE).

Uganda had more than 5 million hectares of forest in 1990, but only 3.5 million hectares remained by 2005. 'If deforestation continues at the present rate, Uganda will have lost all its forested land by 2050', the National Environment Management Authority (NEMA) warned in its State of the Environment for Uganda 2008 report published in mid-June.

It attributed the rapid rate of deforestation to expanding farmland, rapid population growth and increased urbanization.

'Water resources will disappear, water catchments areas will dwindle, agricultural productivity will suffer and livelihoods will be affected tremendously', Annet Nakyeyune, an environmentalist at Makerere University, told IRIN.

The effects, she said, would be felt across many social sectors including health 'because diseases are going to increase'. There would not be enough energy, habitats for some animals would disappear, while some species would either migrate or become extinct.

natural genetic variability as possible, for conservation in different localities and subsequent use in future breeding programmes.

In Uganda, the *C. canephora* samples can be collected from different sites in their natural habitats such as Itwara, Kibale forests and other relict forests where wild *Coffea* exists. Samples should also be collected from isolated coffee localities such as Kalangala Islands in Lake Victoria. Due to the threat from CWD and other unforeseen natural disasters on coffee genetic resources, new locations should be identified for local *ex situ* conservation. In addition, representative samples of the genetic diversity of these species should be conserved in multiple international germplasm collections located in different countries through international collaboration.

Coffee genetic resources are threatened further by the likely adoption, on a large scale, of the newly released CWD-resistant varieties, which will have a reduced genetic base. There is a risk that even the few coffee plants which have withstood CWD in farmers' fields may be abandoned and most likely destroyed as the fields are planted with the new CWD-resistant varieties.

Hence, implementation of *in situ* conservation and local germplasm utilization programmes for the remnant diversity found in devastated gardens would minimize depletion of gene pools. Such programmes could sensitize local communities to ensure that farmers multiply, conserve and beneficially utilize the plants surviving in their fields. Through such programmes, diversity at regional levels within countries could be conserved. As Musoli *et al.* (2006) conclude:

Other coffee-producing countries such as the DRC, Gabon and Angola should also be surveyed for more information about the genetic diversity and precise relationships between the regions. A systematic survey of all forests considered to be natural homes of wild coffee, sampling coffee trees at every 10km distance, would be sufficient for understanding this discrimination.

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