CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

Review of PhD Thesis

"*Garcinia kola*: diversity, utilisation and domestication in Cameroon"

by Anna Maňourová, 2023.

This 235-page thesis has 9 chapters with References and Appendices and is based on field work done in Cameroon between 2016-2022, with laboratory analyses done in Prague.

Four of the chapters have already been published as multi-author papers in academic journals spanning different disciplines. The publication of these papers in refereed science journals indicates that their content is acceptable to both the editors and the peer reviewers. Consequently, my comments on these published chapters are purely for the information of the PhD candidate, and no action is expected. Two further chapters (Chapter 6 and 8) have already been submitted for publication but have not yet been accepted. I provide more substantive comments on Chapter 6; and some comments on Chapter 8. I also comment on the four remaining unpublished chapters which provide supporting materials (Introduction, General Methodology, Conclusions and Literature cited). I have not commented on Chapter 11 (Appendices). I hope these comments will assist the candidate to make some revisions.

COMMENTS ON UNPUBLISHED CHAPTERS

Thesis Abstract

The comment "no significant differences in genotype or phenotype were found between wild and cultivated trees, and most variation was found within rather than between populations", which is repeated later in the thesis, is confusing. I assume it means that there were no significant differences in the many individual genotypes and phenotypes making up the wild and cultivated populations studied.

1. Introduction

Page 1. Line 15. You could add "Leakey, R.R.B. 2020. A re-boot of tropical agriculture benefits food production, rural economies, health, social justice and the environment. *Nature Food* 1: 260-265.

Line 27. You could add chew sticks for dental health.

Page 2. 1.1 With regard to priority setting, probably the best citations would be:

"Franzel S, Jaenicke H, Janssen W (1996) Choosing the right trees: setting priorities for multipurpose tree improvement. ISNAR research report 8. International Service for National Agricultural Research, The Hague, 87 p

Franzel S, Akinnifesi FK, Ham C (2008) Setting priorities among indigenous fruit tree species in Africa: examples from southern, eastern and western Africa regions. In: Akinnifesi FK, Leakey RRB, Ajayi OC, Sileshi G, Tchoundjeu Z, Matakala P, Kwesiga FR (eds) Indigenous fruit trees in the tropics: domestication, utilization and commercialization. CABI, Wallingford, pp 1–27

Chapter 6. Phenotypical description and identification of plus trees (unpublished)

Abstract: Line 3. Is it true that most work on G. kola is lab-based?

Line 6. This seems a very small number of fruits per tree. Other studies have used 20-25.

Line 9. Idetify = identify

I**ntroduction**. Line 3. Yes, but research has focussed on quality traits, as productivity (yield) is subject to G X E interactions making selection almost impossible.

Line 14. 'Neglected' seems a bit strong for a rapidly growing area of research.

Line 19. Better citations for ICRAF priority setting are mentioned above.

Page 96. Line 16. The ideotype is also 'market' oriented.

Page 98. Data collection paragraph. As already mentioned, this sample size is not really big enough to capture all the tree-to-tree variation likely to be at one site. Your descriptions are based on morphology, not phytochemistry or physiology. In my view, the measurements you are making (tree height, DBH, crown width, fruit shape) are really much more focussed on developing 'Descriptors' for *G. kola* rather than for 'Ideotypes'. Ideotypes have been based on assessments of tree-to-tree variation with a view to identifying ideal traits for selection to meet a given use or market for fruits and/or nuts – and then capturing elite genotypes using vegetative propagation. The use of vegetative propagation also allows the development of clonal trials to determine whether traits like tree shape/height etc are characteristics of the clone or have been moulded by the environment (especially mutual shading, competition/interference, etc.) i.e. by G x E interactions.

Page 100. Bottom lines. What you are describing here are traits that would probably be important in a tree breeding programme (making improvements over decades/centuries). The use of vegetative propagation offers opportunities to markedly transform plant height/stem and crown development by capturing the mature state, so the flowering and fruiting occurs on small trees within 2-3 years. I therefore suggest that descriptions of tree shape and height are not really very relevant to the clonal approach to domestication.

I got the impression from earlier chapters that you see tree domestication more from a geneticist's/forester's viewpoint (ie., tree breeding) than from a horticultural perspective. This seems then to be confusing when you advocate Participatory (decentralized) Community-

based Domestication, which is based on making rapid gains/benefits by propagating elite individual trees.

Page 101. Leading on from the above, 6.3.1.1, averages of all these traits hide the really important information that you gained by your measurements of individual trees. So, for example, in my view the much more important information is that fruit weight had "a maximum of 515.9 g and a minimum of 50.3 g". A bigger sample size would quite possibly have found trees with even bigger fruits – so an ideotype might include fruit weights of around 600-700g. Likewise, finding that seed weights ranged from 2.1g to 19.9 g indicates that the ideal seed might be 25 - 30g. As you then go on to say, seed number may be a reflection of the condition' of the tree. Yes, or perhaps more often the pollination success (affected by pollinator populations – which of course can be boosted by setting beehives). Of course, seed number may have a genetic component, and as you say elsewhere, this raises the question about whether the ideal *G. kola* for edible seed production has one big seed or many smaller seeds. This may affect the market prices. However, if the nut market is for medicinal uses, the ideal may be different, and will undoubtably also be interested in the variations in phytochemistry, leading perhaps to numerous different ideotypes (Levels 2-3 in the hierarchy diagram in Leakey and Page, 2006).

Figures 6.2 to 6.5. Do fruit shape, fruit colour, nut colour affect the desirability of the products in the domestic household and/or the market. If yes, then this is important for the ideotype, if not, then it isn't. This information is then part of a Descriptor, not an Ideotype/Plus tree. Is nut colour affected by ripeness or dryness? The same questions apply to leaf shape and size.

Figure 6.6. Again, crown shape and branching characteristics are highly likely to be a response to the growing conditions, to management, to damage by birds and animals. In other words, they are subjects of G x E interactions and probably not of great interest to tree domestication unless they have a genetic origin. This could be tested by planting clonal trials. If farmers want short stemmed, broad crowned trees for easy harvesting then this may be achieved by marcotting or grafting techniques using scions from high in the mature crown of trees that have desirable nut traits.

I made comments on page 68 of Chapter 5, as follows. The same thoughts apply here:

Tree architecture breaks down into about 23 named forms of branching patterns (see book by Hallé et al., 1978). Some species related to *G. kola* conform to Attim's Model, which is close to Rauh's Model. Studies of *Triplochiton scleroxylon* have developed a Predictive Test which can be used in seedlings to determine the likely form of mature trees (see Ladipo et al., 1991a/b. 1992).

It is impossible to know if the form of a single tree is a representation of its genotype without doing clonal field trials (aided by Ladipo's predictive test). It is highly likely that forking etc. is a response to damage of some sort.

Page 105. 6.3.2 Many of the above comments relate to this section too.

Page 108. 6.3.3 This section seems again to be missing the point regarding ideotypes for clonal cultivars (versus tree breeding). An ideotype does not have to be site related. Excellent elite trees (plus trees) can exist in the worst population! A more detailed look

at the tree-to-tree variation would be useful. Could any of this site variation be attributed to soils, rainfall, tree age, etc?

Figure 6.8. This clearly shows site variations. However, I am not sure how you combine tree, fruit and seed superiority into a single trait. Or, am I mis- understanding (these techniques have come into use since my time)? It may be useful to look at the dendrogram in Pauku et al. 2010. (Domestication of indigenous fruit and nut trees for agroforestry in the Solomon Islands. Forests, Trees and Livelihoods 19: 269-287) which expresses relatedness in terms of different traits.

Figure 6.9 Again these are techniques that I have never used or been involved in. Does this in effect show the range of tree-to-tree? How are the different traits represented in this Figure? I get the feeling that you could present all this data in ways that would be much more useful to those engaged in practical tree domestication.

Page 111. 6.4.1

Last line. This recent paper perhaps deserves inclusion:

F.B. Yakubu, A.A. Olaniyi, V.I. Alaje, M.O. Nola, O.O. Fadulu and K.K. Adeniyi 2023. Quantitative variations in fruits/seeds of *Garcinia kola* Heckel from lowland rainforest zones of Nigeria: prospects for domestication, Journal of Agriculture and Environment 19(1): 205-212 ISSN: 1595-465X (Print) 2695-236X (Online)

Page 112. First paragraph. You indicate that seed size (related to fruit size and shape) is the only trait of importance in *G. kola*. But what about nutrient content, flavour/astringency and all the phytochemical traits that are of value to local people? Should they not be part of the ideotype. Fig 2 in Leakey and Page (2006) shows three levels in an ideotype hierarchy - 1. basic at the farm level, 2. chemical at the laboratory level and 3, entrepreneur business level. Your comments about D/P. edulis, really only relate to Level 1.

Some species can have ideotypes for different products (e.g. fruits and nuts) which will be very different. *G. kola* is primarily a nut species, so what is the ideal nut? To that ideal you can add a long list of medicinal and nutritional traits (your chapters 7 and 8). But it would also be possible to have fruit ideotypes for some of the medicinal uses, then there are twigs for chewing sticks, another ideotype!

Line 8. I don't know the paper by Mboujda et al., 2022 but it seems to me that maybe they have misunderstood the Ideotype concept.

I've already commented several times about tree-to-tree variation and the ideotype. I sure that there is continuous tree-to-tree variation in all the traits you have identified and so the 'ideal tree' equates to the very best individual for each product (fruit, nut, twig) and its various traits.

As mentioned earlier, I personally would not include 'harvesting factor' in the ideotype as it can be controlled by management.

Page 113. Lines 7-8. I agree that regional variation (provenance) may be important for 'tree breeding' but I am pretty sure you will find elite individuals of the species even in the least good provenances. These would merit domestication as clonal cultivars.

Lines 24-27. You mention 'cultivar' development here, but it is not clear whether you are referring to clonal cultivars or cultivars from tree breeding progenies.

Page 116. Lines 1-2. Yes, plus trees for breeding but also candidates for clonal propagation as horticultural cultivars.

Chapter 8.

I found this chapter very confusing. 1. It is not clear which is Part 1 and which is Part 2. It needs to be clearer in the text of this chapter what is your work, and what is the work of others being reviewed by you. 2. Maybe if I had a better understanding of the chemistry and medicinal benefits of these products, I would have found it easier to relate them to the information in Chapter 7.

Page 191. 8.2.1 Did you keep the seeds of different individual trees separate, or did you bulk up the samples? The Results section implies that the samples were bulked up by village. That's a pity. It would be great to know the tree-to-tree variation in all these traits.

Table 8.1 It is not clear that this is mean data of different samples, or where it came from. The same applies to the other tables below.

Page 200. 8.4 All these "disparities" are, I imagine, the result of people using bulk samples from different locations and different trees. There is clearly a need to unravel all this variation if *G. kola* is to become really useful as a tree for medicinal use and functional nutrition.

I think you could use this paper to much more forcefully point out all the opportunities and the research needs.

Chapter 9.

Page 203. I think the chapter heading should be more distinctive (Final / Overall Conclusions).

9.1 You may wish to revise some of this section in the light of my earlier comments – especially about ideotypes and breeding versus clonal cultivars.

Maybe it would be useful to read: Leakey, R.R.B. and Simons, A.J. (2000). When does vegetative propagation provide a viable alternative to propagation by seed in forestry and agroforestry in the tropics and sub-tropics? In: Problem of Forestry in Tropical and Sub-tropical Countries - The Procurement of Forestry Seed - The Example of Kenya, H.Wolf and J. Arbrecht (Eds), 67-81, Ulmer Verlag, Germany.

9.2 Likewise, some of my comments may lead you to revise some of this section.

Maybe it would be useful to see: Leakey, R.R.B. and Akinnifesi, F.K. 2008. Towards a domestication strategy for indigenous fruit trees in the tropics. In: Indigenous Fruit Trees in the Tropics:

Domestication, Utilization and Commercialization, 28-49, F.K. Akinnifesi, R.R.B. Leakey, O.C. Ajayi, G, Sileshi, Z. Tchoundjeu, P. Matakala, and F. Kwesiga (eds), CAB International, Wallingford, UK.

Maybe it would be useful to see: Leakey, R.R.B. (1991). Clonal forestry: towards a strategy. Some guidelines based on experience with tropical trees. In: Tree Breeding and Improvement, Ed. Jackson, J.E., 27-42. Royal Forestry Society of England, Wales and Northern Ireland, Tring, England.

Chapter 10. References.

This reference list runs to about 5 pages, but the reference list for chapter 7 is many times longer.

So, what is this list, and how does it relate to the earlier ones?

From the readers perspective, it would be preferable to have one list for all the citations in the thesis. This would obviously mean moving the chapter specific lists to here. You say that the published chapters are "adapted" from the published papers. Perhaps this is just another adaptation.

ADDITIONAL MINOR COMMENTS ON PUBLISHED CHAPTERS

Chapter 3.

Page 12. Line 11. What does "Adapted from" mean before the citation of your paper. I see you also say this for other chapters too.

Page 14. Line 12. *G. kola* is also one of the overlooked by science "Cinderella species".

Page 19. Line 5. It is also recognized as a stimulant.

Page 26. Line 19. Are there any clinically approved uses?

Page 31. 3.9. Line 9. You could mention here that there are three basic approaches to tree domestication: tree breeding, which is very slow and only makes small gains; vegetative propagation of putative cultivars based on tree-to-tree variation, which is rapid and makes substantial gains; and biotechnology for gene editing, which is still to be proven useful.

Page 32. Line 22. Personally, I would add "determination of tree-to-tree variation" to this list of missing topics.

Chapter 4

Page 48. Line 17. Is there any evidence of pricing based on nut size, flavour, or other traits?

Line 27. How did you define an 'agroforestry system'? Typically, home gardens are recognized as agroforestry systems, as you recognize, so what are the other practices here?

Page 49. Line 1. While cultivation alone may be recognized as a very basic form of domestication, most people recognize elements of selection in the process. Did you see or hear any evidence of selection?

Page 51. Lines 1-6. What was the reason for this geographic variation? – the state of the natural resource, the livelihoods of the people, etc? How do these usage patterns differ from Nigeria, Benin, etc?

Page 53. Line 7. Consistency. You use *D. edulis* elsewhere – which is currently correct *D. pachylobus* of *D. edulis*?

Line 25. I wonder whether this is really true. I suspect that there may be other explanations.

Page 54. Lines 15-16. The best examples of sustainable bark harvesting are seen in Cinnamon.

Page 56. Line 17. Any evidence of seasonality of production? If so, is it important for market price?

Page 57. Line 8. Why are you suggesting that breeding is needed when there are other quicker and more effective asexual horticultural routes using vegetative propagation to develop clonal cultivars?

Line 14. I very much doubt that planting seedlings from a tree with a particular form will lead to a progeny with the same form. First, there is huge tree-to-tree variation even in a single progeny (see forestry literature - eg. *Triplochiton scleroxylon*) and secondly tree form is strongly influenced by the growing environment (G X E interactions).

Line 19. I would recommend adding a study of tree-to-tree variation for a wide range of traits, as a first step to domestication by the development of clonal cultivars. This can then lead to ideotype identification that can include seed quality traits and phytochemistry for new business/industries.

Chapter 5.

Page 65. Line 13. See my earlier comment (Thesis abstract) on the use of the words 'genotype' and 'phenotype' for populations

Line 15. If you do a study of tree-to-tree variation you can use the data distribution patterns to assess whether farmers have been selecting trees for planting/domestication (see: Leakey, R.R.B., Tchoundjeu, Z, Smith, R.I., Munro, RC., Fondoun, J-M., Kengue, J., Anegbeh, P.O., Atangana, A.R., Waruhiu, A.N., Asaah, E., Usoro, C. and Ukafor, V. 2004. Evidence that subsistence farmers have domesticated indigenous fruits (*Dacryodes edulis* and *Irvingia gabonensis*) in Cameroon and Nigeria. *Agroforestry Systems*, **60**: 101-111).

Page 66. Line 27. Is there any evidence of G x E interactions in *G. kola*?

Line 30. Personally, I think a more crucial next step is to examine the tree-to-tree variation as a quick entry point into tree selection for clonal cultivar development.

Page 67. Line 3. One of the reasons for the decentralized tree domestication approach (= participatory domestication) is that it avoids the loss of genetic diversity. About 85% of the tree-to-tree variation exists at the site level (you have found this in *G. kola* too), so different communities can develop their own cultivars without affecting the overall cross-site variation.

Page 68. 5.2.1.1 Tree architecture breaks down into about 23 named forms of branching patterns (see book by Hallé et al., 1978). Some species related to *G. kola* conform to Attim's Model, which is close to Rauh's Model. Studies of *Triplochiton scleroxylon* have developed a Predictive Test which can be used in seedlings to determine the likely form of mature trees (see Ladipo et al., 1991a/b. 1992).

Lines 9-13. It is impossible to know if the form of a single tree is a representation of its genotype without doing clonal field trials (aided by Ladipo's predictive test). It is highly likely that forking etc. is a response to damage of some sort.

Line 16. If the trees are indigenous to this area, why are some trees older than others unless they have been specifically planted by someone?

Line 20. Fig 5.1. Variation in crown shapes could also be due to G x E interactions relating to rainfall, soils, management, plant spacing, etc. I don't think you can really assess these differences without clonal field trials.

Page 70. 5.2.1.2 Line 11. Did you look at tree-to-tree variation patterns in these sites? I think the results may be interesting. (refers also to Figure 5.5).

Page 71. Bottom lines: Could you identify any reasons for these differences?

Page 72. Figure 5.3. It would be interesting to know the tree-to-tree variation in these seed traits.

Page 73. Lines 5-6. Were there age differences between trees in fields v. wild populations?

Figure 5.4 I find this figure confusing *vis à vis* the text. I guess there was overlapping continuous tree-to-tree variation in fruits and seeds at each site.

5.2.2 Bottom line. This seems to contradict the earlier statements that there was large within population variation (see page 76 = 91%).

Page 79. Line 8. I very much doubt the validity of this statement about the effect of domestication (mostly cultivation of unimproved wild seed). Ditto: Line 13.

Page 80. Line 16-17. Maybe this was age-related?

Lines 25-29. You are referring to provenance variation for a tree breeding programme – so this may be true, but if the domestication was by the development of clonal cultivars using vegetation propagation of elite trees, this might not be true as you can

find very superior individual trees in the worst provenances. A study of tree-to-tree variation might have illustrated this point.

Bottom line. I have commented on this statement twice earlier. I don't think populations can be said to have the same genotype and phenotype.

Page 81. Bottom lines. Yes, but participatory domestication also provides other areas of capacity building. With regard to vegetative propagation, once farmers know and use the skills, they can apply them to any species of their choice.

Page 82. Lines 1-2. The development of clonal cultivars by vegetative propagation is NOT tree breeding! It is an asexual form of propagation.

Because a project is called "participatory domestication" does not necessarily mean that communities have control and capacity to benefit. For example, relatively 'top down' approaches to participatory domestication in southern Africa, Peru and Papua New Guinea basically failed to deliver community benefits.

Page 84. Bottom lines. Your tree sample size was very small compared with most of the other studies on indigenous trees where at least 24 fruits per tree were collected for 60-100 tree per site (based on recommendations of biometricians).

Page 87. 5.5 Conclusions. You repeat here things on which I have commented earlier: 1. Low population genetic diversity despite high within population diversity, 2. Possible misuse of the term 'tree breeding', 3. Phenotype/genotype of populations.

Chapter 7.

Abstract. Page 124. Lines 7-8. Lots of scope here for medicinal ideotypes!

Page 128. 7.2.1 Nutrition is another trait for possible inclusion in an ideotype. Typically, these chemical studies only publish the average concentration of these chemical constituents. This usually conceals substantial tree-to-tree variation in this content, so missing the potential to develop new drugs/functional foods based on selected elite trees and domestication. See: Leakey, R.R.B., Fuller, S., Treloar, T., Stevenson, L., Hunter, D., Nevenimo, T., Binifa, J. and Moxon, J. 2008. Characterization of tree-to-tree variation in morphological, nutritional and chemical properties of Canarium indicum nuts. Agroforestry Systems 73: 77-87.

Anti-nutrient content is also important and can be incorporated in the ideotype.

Page 164. Can you relate any of this information to your potential for ideotypes? I imagine that there needs to be a study of the tree-to-tree in all these phytochemical attributes/activities. See *Canarium indicum* paper mentioned above.