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Title: Domestication potential of Marula (*Sclerocarya birrea* subsp. *caffra*) in South Africa and Namibia: 3. Multiple trait selection

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Domestication potential of Marula (*Sclerocarya birrea* subsp. *caffra*) in South Africa and Namibia: 3. Multiple trait selection.

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ABSTRACT

Twenty four ripe fruits were collected from each of 55 marula (*Sclerocarya birrea*) trees in Bushbuckridge, South Africa and from 63 trees from the North Central Region of Namibia. The South African trees were in farmers's fields (20), communal land (29) and natural woodland (14), at three sites: Acornhoek road, Allandale/Green Valley and Andover/Wits Rural Facility. The Namibian trees were all from farmers' fields in three areas: North east (20), North west (10) and West (25). The fruits were partitioned into in skin, flesh/juice, nut, shell and kernel. Each component of each fruit was weighed fresh separately and its individual identity maintained throughout the series of assessments. Detailed analysis of this data provides a good understanding of the extent of the variation found and of the dry matter partitioning among the fruit components of different trees. **ADD**

These results are discussed within the context of similar studies in other indigenous fruits elsewhere in Africa.

INTRODUCTION

Sclerocarya birrea (A. Rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro is one of the traditionally important indigenous fruits of southern Africa, which in recent years has also become commercially important as its fruits and other products have entered local, regional and international trade (Shackleton *et al.*, 2002; Wynberg *et al.*, in press). With this growing commercial importance have emerged several domestication initiatives. The first was by Holtzhausen *et al.* (1990) of Pretoria University who started to develop cultivars from 'plus-trees' taken from the wild using grafting techniques. Applications for Plant Breeders Rights on three out of six cultivars have been lodged by Professor Holtzhausen. The second initiative was by Veld Products Research in Botswana (Taylor *et al.*, 1995; 1996), again using grafting and establishing these and some of Holtzhausen's clones in field trials. The third was an external domestication programme, initiated by Ben Gurion University in Israel, using material obtained in southern Africa and planted in the Negev Desert (Nerd and Mizrahi 1993; Mizrahi and Nerd, 1996). The fourth programme was launched in 1995 by the International Centre for Research in Agroforestry (ICRAF), now the World Agroforestry Centre, with a participatory mandate, in which subsistence farmers are the planned beneficiaries of the domestication activities (Maghembe, *et al.*, 1995;

1998). Rangewide germplasm collections of *S. birrea* and *Uapaca kirkiana* have been made as the first step in a domestication strategy (Leakey and Simons, 1998). These collections provide material for conservation and future utilisation.

In many countries, non-timber forest products (NTFPs) are an under-utilised resource, and it is only in recent years that domestication projects for agroforestry trees have been initiated (Leakey and Newton, 1994; Leakey *et al* 1996). However, without parallel efforts to promote the commercialisation of these products, domestication is unlikely to yield the expected benefits in terms of poverty alleviation, and there is a need for agroforesters to work closely with the private sector (Leakey, 1999). The present study, as part of the Resource Inventory, quantifies the phenotypic variation in fruit, nut and kernel traits in marula (*S. birrea* ssp. *caffra*), within the framework of a broader project examining the benefits and opportunities for domesticating and commercialising the fruits and kernel oil of marula in South Africa and Namibia. Recent collections of marula fruits and nuts from individual trees in Makueni district of Kenya have been analysed for a wide range of nutritional compounds and minerals. The skin and flesh was found to vary considerably in the vitamin C content (85-319mg/100g), while the kernels were rich (56-64%) in oils (Thiong'o *et al.*, 2002).

METHODS AND MATERIALS

Marula fruits fall from the tree just before they ripen. Ripe, unblemished fruits were collected from beneath the crown of marula trees in villages in Limpopo Province, South Africa (Bushbuckridge) and Namibia (North Central Region), between 21st January and 5th February 2002 (Table 1). In South Africa, marula trees were found in farmers' fields, in communal grazing land, and in natural woodland, while in Namibia, fruiting trees were only found in farmers' fields. In general, fruits were usually plentiful and so were collected at random sampling from four quadrants (6 fruits per quadrant), according to the procedures developed by Leakey *et al.* (2000). Fruits from each tree were separately bagged and labelled. As soon as possible (usually 2-3 days later), the fresh fruits were weighed using a 0.1g electronic balance. Keeping the fruits in the same order, the skins were then peeled off and weighed, while the nuts, still in the same order, were soaked and scrubbed to remove the flesh before being set in the sun to dry for about 10 hours. When dry, the nuts were weighed and numbered so that their identity was maintained for subsequent cracking and kernel removal. Flesh mass were derived by difference (Fruit - skin - nut = flesh).

Table 1. Origins of trees sampled in South Africa and Namibia, by village and land use.

	Natural	Communal land	Farmers fields	Total
South Africa				
<i>Bushbuckridge</i>				
Acornhoek road		29 trees	6 trees	35 trees
Allandale			10 trees	10 trees
Green Valley			4 trees	4 trees
Andover	11 trees			11 trees
Wits Rural Facility	3 trees			3 trees

TOTAL	14 trees	29 trees	20 trees	63 trees
Namibia				
<i>North west</i>				
Omanjoshi			7 trees	7 trees
Omarkango			9 trees	9 trees
Okamukwa			4 trees	4 trees
<i>North east</i>				
Onangwe			8 trees	8 trees
Oilyateko			1 tree	1 tree
Elope			1 tree	1 tree
<i>West</i>				
Tsandi: - Otjito			4 trees	4 trees
Tsandi: - Omukoko			6 trees	6 trees
Onesi: – Otjeviya			4 trees	4 trees
Onesi: - Otjihawu			2 trees	2 trees
Eunda			2 trees	2 trees
Eunda: – Oukwanangaya			2 trees	2 trees
Ongosi			5 trees	5 trees
TOTAL	0	0	55 trees	55 trees

In addition, one fruit sample was collected from the Mhala Development Centre (MDC), a project of the Mineworker's Development Agency in Bushbuckridge that is processing fruits for fruit juice and for kernel oil. A further sample from a superior tree ('Namibian Wonder') was analysed in the same way. The origin of this tree is not disclosed in order to protect the villagers' rights to this germplasm.

Analysis of Variance, Duncan's Multiple Range tests, and tests for skewness and kurtosis have been using SPSS 10.0 for Windows.

RESULTS

Relationships between desirable traits

In South Africa, there were no relationships between fruit mass and tree yield (number of fruits), or between fruit mass and stem basal area. Equivalent data was not collected in Namibia.

In South Africa, the relationships between fruit mass and nut mass were weakest in natural woodland ($r^2 = 0.43$) and strongest in communal land ($r^2 = 0.69$) and farmers' fields ($r^2 = 0.82$), although the slope of the lines were very similar (Figure 12). By contrast, in Namibia, the relationships between fruit mass and nut mass were strong in the West ($r^2 = 0.73$) and North west ($r^2 = 0.58$) and weak in the North-east ($r^2 = 0.20$).

Generally, the relationships between fruit mass and kernel mass were weaker than between fruit mass and nut mass, indicating the difficulty of predicting kernel size from fruit size. In South Africa, the relationship between fruit mass and kernel mass was very weak in natural woodland ($r^2 = 0.13$) and weak in communal land and farmers' fields ($r^2 = 0.42$ to 0.49), while in Namibia, it was weak in the West and North-east ($r^2 = 0.41$ to 0.46) and very weak in the North west ($r^2 = 0.03$).

Relationships between kernel mass and shell mass were always weak (South Africa: $r^2 = 0.14$ to 0.34 ; Namibia: $r^2 = 0.08$ to 0.31), indicating that it should be possible to find a large kernel in a relatively light (= brittle) shell. However, the large mean kernel mass of 'Namibia Wonder' was associated with a large mean shell mass.

At the population level, the relationship between mean kernel mass and mean number of kernels per nut was not linear (Figures 13a and b) because the individual kernel weight of nuts averaging 1-2 kernels was greater than in nuts averaging 2-3 kernels. In individual trees, however, there were fruits with up to four kernels per nut and the relationships between kernel mass and mean number of kernels per nut was linear (Figure 14a and b: AR23, $r^2 = 0.74$; AN31, $r^2 = 0.89$; AN32, $r^2 = 0.93$; AL3, $r^2 = 0.86$; AL4, $r^2 = 0.74$; AL5, $r^2 = 0.89$; GV3, $r^2 = 0.71$), although in different trees these relationships could be both strong and weak. Interestingly, however, the slope of the lines for different trees were different, suggesting considerable genetic variation in the potential mass of individual kernels from different trees, even under conditions where the competition for assimilates between developing kernels would be constant. This suggests that trees differ in their capacity to partition dry matter to developing kernels, a trait that would be desirable in cultivars being developed for kernel production. Earlier it was suggested that the occurrence of individual fruits with few kernels per nut, could result from poor pollination success. If this is the case, the linear relationship between kernel mass and mean number of kernels per nut suggests that increases in pollination success could have considerable impact on kernel production (Figure 15), especially in those trees with a genetic propensity to partition assimilates to kernels. The combination, therefore, of genetic selection and better pollination management could have important practical outcomes for the production of kernel oil.

Development of ideotypes

Domestic use of marula products by local people is primarily for the flesh and juice for beer making, and for the edible kernels. The commercial uses of marula fruits are focussed on the use of the pulp with and without the skin, and on kernel oil. Women of Mhala Development Centre (MDC) extract the flesh and juice, discarding the skin, for sale to fruit juice companies. Cape Distell Pty, the makers of 'Amarula Cream' liqueur, on the other hand use the flesh, juice and skin of the fruits. MDC and CRIAA-SA-DC in Namibia extract the kernel oil for export to the cosmetics industry in Europe. Consequently, any cultivars developed should be selected with the intention of maximising the yield and quality of these products. Thus an ideotype for products based on the fresh fruit should include:- fruit mass, pulp mass, skin mass and flesh/juice mass, while that based on kernels, should include:- kernel mass, oil content and shell mass (shell brittleness). At a later date, it would be possible to add nutritional quality and taste/fragrance to the fruit ideotype, and oil quality to the kernel ideotype.

Examination of the data from this study illustrates that trees producing large fruits, often have high values for flesh/juice mass and pulp mass (Figure 16a, b and 17a, b) and some of these also have high kernel mass and oil content. Examination of the inter-relationships between the traits indicates some changes in the relative ranking of trees, particularly between kernel mass and oil content, but also to a lesser degree between the components of pulp (skin and flesh /juice). These differences in ranking

and the poor relationships between fruit traits and kernel traits, suggest that there is some benefit in identifying the multiple trait combinations (ideotypes) towards which selection should be directed, that would be important for different marketable products - eg. fruits and kernels. However, examination of how well the superior trees for each particular trait fit the ideotype reveals that few trees conform well (Figure 18a and b), although the data from 'Namibian Wonder' illustrates that rare individuals exist in the wild population which do conform fairly well to these ideotypes (Figure 19). There is, thus, opportunity for future improvement, and consequently, the search for these desirable individuals, which have potential to be developed as cultivars needs to continue. In particular, it is clear that that selection for thinner and lighter nutshells would be advantageous. The weak relationship reported above between kernel mass and shell mass, suggests that individuals with large, easily extracted kernels in thin shells may exist in the wild population.

Frequency distribution

The data for fruit, nut and kernel mass of each tree in each site displayed considerable variance about its mean, and the means for the trees of each population showed some variation (eg. Figure 20 for fruit mass at Allandale, South Africa). The frequency of these individual data sets, and of the overall site mean were close to a normal distribution (Appendix 2). When the data for South African trees were aggregated by land use, the frequency distributions for natural woodland was also close to normality (skewness = -0.03 to 0.83), but that for communal lands and farmers fields (especially for fruit mass) had a greater tendency towards being positively skewed (Figure 21), the latter also having some bi-modality (especially kernel mass: Figure 21c). In a similar way, the distribution pattern of the same traits in Namibia, was close to normal (Figure 22), but with some degree of bimodality in fruit mass from the populations from the North east and North west (Figure 22a). Kernel mass in populations from the West had a small number of large kernels (Figure 22c).

DISCUSSION

The very considerable tree-to-tree variation in fruit and kernel characteristics in marula are consistent with results from other indigenous fruit trees, such as *Irvingia gabonensis* (Atangana *et al.*, 2001; 2002; Anegbah *et al.*, in prep a) and *Dacryodes edulis* (Leakey and Ladipo, 1996; Waruhiu, 1999; Anegbah *et al.*, in prep b) in west and central Africa. As in the case of these moist forest species of west and central Africa, this variation, coupled with the considerable market opportunities, indicates that there are domestication opportunities for marula, as has already been recognised (Holtzhausen *et al.*, 1990; Taylor *et al.*, 1996; Mizrahi and Nerd, 1996; Maghembe, *et al.*, 1998). However, this study quantifies the variation in dry matter partitioning between constituent parts of the fruits for the first time, and provides good fundamental knowledge about the range of variation in several important traits across geographically separated, as well as environmentally and culturally different sites. This allows an analysis of the relationships between different commercially traits of importance to the development of cultivars that can be used to determine 'ideotypes' that maximise the Harvest Index across several different fruit and nut traits, and hence to identify potential cultivars for cultivation that could meet the needs of farmers with proximity to different markets.

It is clear from this study of the phenotypic (tree-to-tree) variation in populations in South Africa and Namibia (Figures 16 and 17), and their relationship to fruit and kernel ideotypes, based on the data from 'Namibian Wonder' (Figures 18 and 19), that there is very considerable opportunity to identify individual trees with fruit and kernel characteristics well above the average of the species. While this is evident from the range of continuous variation identified in each of the populations studied, populations of only 14-63 trees, the potential is really highlighted by the discovery of 'Namibian Wonder', a much more rare member of the population. Such extreme examples of phenotypic variation are only found at far greater selection intensities. Unfortunately, the incidence of trees of this sort are not known, but it is worth noting that this tree has the biggest fruits ever reported in marula, and Prof Holtzhausen of Pretoria University has previously implemented an intensive search for such fruits in South Africa and Namibia. In the present study, the Namibian populations seem to have greater promise of superior trees than that of South Africa, but the geographical range surveyed in South Africa was considerably narrower than in Namibia, although neither survey was comprehensive. Clearly, if the domestication of marula is to be pursued, more comprehensive surveys will be required, and these should extend into the neighbouring countries of Botswana, Zimbabwe, etc. From the studies so far, it is not possible to predict where the most promising trees might be found, and of course, it is possible that trees with desirable fruit traits, may be found in different locations from those with desirable kernel or other traits.

The extent to which all the trees characterised to date are allocating large amounts of dry matter to shell production, is also clear from the ideotypes. The nut:kernel ratios and weak relationship between, kernel mass and shell mass, indicate that it may be possible to find trees that partition more dry matter to marketable products. This is also indicated by the different relationships between kernel mass and the number of kernels found in different trees. In this case, it seems that there are opportunities for improvements in kernel mass from both genetic selection and from better management. However, the lack of any evidence that kernel mass is any greater in nuts with a single kernel suggests that a strategy to select for a single large kernel is not appropriate. The management opportunity stems from the finding that not all fruits contain the possible four kernels. It seems likely that the reason is inadequate pollination. If this is shown to be true, and simple studies could determine this, then it may be possible to improve kernel yields by judicious use of beehives. In this connection, it must be remembered that *S. birrea* is a dioecious species, with male and female trees and that male trees should not be removed from the population for other products (eg. wood carving, firewood, etc.).

The ideotypes described here are based on the characteristics measured in this study. Recent data from Kenya (Thiong'o *et al.*, 2002), illustrates that in nutritional terms, traits for nutritional values (protein, carbohydrate, vitamins and minerals) and for taste/sweetness (carbohydrates, acidity) could be added to the ideotypes. The phenotypic evaluation of organoleptic traits is being added to those of morphological traits in the similar studies with the indigenous fruit, *Dacryodes edulis*, in West Africa (Kengni *et al.*, 2001; Leakey *et al.*, 2002).

Studies of the frequency distribution of fruit and kernel trait data in *Dacryodes edulis* and *Irvingia gabonensis* in West Africa, similar to that reported here, have indicated that farmers, by their own procedures of genetic selection (truncated selection), have

made a 40-65% gain in fruit mass (Leakey *et al.*, in press a). A similar analysis here, is not conclusive, but there are certainly some results that suggest that a similar process of farmer domestication is underway in marula in southern Africa. For example, several data sets (eg. fruit mass from trees in farmers' fields – Figure 21a) are positively skewed (with a tendency to bimodality), with a tail of unusually large fruits. This contrasts with more normally distributed data from trees in natural woodlands. Interestingly, other studies within this project (Shackleton, C. pers com) have found large differences in marula tree fruit yield between trees in farmers' fields and in communal land and natural woodland. Some of these trees were the same as in this study and in these trees the mean yield/tree from 13 trees in crop fields was 33,187 fruits, while that from 12 trees in natural woodlands was only 6135 (Shackleton, C. pers com). It is not clear to what extent these differences in fruit yield represent genetic selection or cultivation and reduced competition in farmers' fields from other plants.

How could the opportunities for domestication, described above, be captured and developed to enhance the livelihoods of local people in South Africa and Namibia? This question will be addressed in much greater detail at a workshop in Cape Town in November 2002, when the results of the present study will be discussed with regard to the interesting results that have been obtained by other components of this project examining: (i) the current commercial activities with marula in these two countries, and (ii) the important traditional and social values of the species. However, as examples of what could be done, the women supplying fruit to Mhala Development Centre (MDC) at Thulamahashe, could select superior trees and develop cultivars from them. These selected trees would produce more fruit juice and have greater kernel oil yields, for marketing to fruit juice and cosmetics companies respectively, MDC is funded by a DFID bilateral programme, so future funding aimed at poverty alleviation (Poulton and Poole, 2001) could set up a participatory tree domestication programme, based on the experience of ICRAF in Cameroon and Nigeria (Leakey *et al.*, in press b). A participatory domestication programme would train and assist local villagers to set up simple, low-technology propagators, appropriate for rural development programmes (Leakey *et al.*, 1990), that do not require running water or electricity, to root stem cuttings of selected trees, and so to develop cultivars. With this training, the community could make genetic copies of their best trees, either for fruits, or for kernels, using the ideotypes developed above for guidance. Because, this participatory domestication process, leaves the germplasm and indigenous knowledge with the people, it empowers the community and allows them to determine their own commercial opportunities (Leakey *et al.*, in press b). In this study, a sample of the fruits passing through the MDC juicing process were sampled and characterised. It was found (Figure 3a) that the women were processing fruits representative of the best 84% of the trees in the area (= they had discarded the worst 16%). Through domestication, they could improve their trees and produce fruits equivalent to the best 5% in the area. This would reduce the labour involved in juicing the fruits, increase the proportion of the pulp extracted (nb. this project has identified that the women are not recovering a high proportion of the juice – Mander, pers. comm.), and with appropriate selection could probably improve the flavour of the product. Interestingly, the 'Amarula' factory of Distell Corporation use both the skin and the pulp in their process, so maximising the product they obtain from the fruits. An opportunity for the future would be to add an assessment of the genetic and non-genetic (consequences of storage and processing) organoleptic (flavour and fragrance) properties of different

fruit samples as these are important commercially in the juice and liqueur industry. It would be important to interact with commercial companies to determine which properties are desirable and which are undesirable.

Improvements in kernel production and oil extraction could be made by following similar domestication strategies, although as mentioned above to maximise kernel production it would also be necessary to examine ways to improve kernel number by improving the pollination success, perhaps by siting bee hives in appropriate positions between male and female trees. There may also be a requirement for education and awareness raising of the need to maintain male trees to maximise pollination and kernel production, as people tend to see them as redundant and this perception may need to be changed.

In Namibia, village communities could undertake similar participatory domestication programmes. For the village where ‘Namibian Wonder’ has been found, the opportunity for a very rapid increase in the quality of their marula trees could be made by developing this exceptional tree as a cultivar, although it would be wise to also develop other cultivars as well, in order not to severely narrow the genetic base of the population. The commercial opportunities for this village could be greater than just producing better fruits and kernels locally, as there would also be great opportunity to market plants of this and other cultivars. However, it would first be essential to register this cultivar (take out ‘plant breeders rights’) in the communities name to prevent other entrepreneurs from multiplying the cultivars for their own commercial gain. The important need for intellectual property rights (IPR) protection for ‘Namibian Wonder’ cannot be over-emphasised if the community is to be the beneficiary of the domestication outputs. Information about the origin of this tree should be maintained confidential until this protection is achieved. This could be achieved through a participatory domestication project similar to the one suggested for MDC. In this instance an NGO like CRIAA-SA-DC could be the implementing organization.

The impacts of domestication and wider commercialisation of Marula could also be positive benefits on the environment. For example, many of the trees on farms are mature and there is little regeneration in progress. So a planting programme would help to restore the natural resource. The need for people to plant and nurture marula trees could be part of an educational package, poster pamphlet. New plantings of marula would also provide future ecological niches for a wide range of wildlife, above and below ground. In addition, the move towards agroforestry may encourage farmers to plant other useful trees, so diversifying the farming system with likely benefits on sustainability, through the creation of an agroecological succession culminating in a mature or climax phase. Hopefully, such developments would also help to sustain some of the traditional values of marula in the culture, for example the ‘first fruits’ ceremony and the traditional role of marula beer in society.

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