

Review

Application of biotechnology for the domestication of *Dacryodes edulis* (G. Don) H. J. Lam in Cameroon: A review

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Cultivation of *Dacryodes edulis* (G. Don) H. J. Lam commonly known as safou to improve the livelihood of the local population has been growing rapidly in the range of occurrence of safou and recent emergence of the market chain introduced by the World Agroforestry Center (ICRAF) experts has stimulated this further. Domestication through conventional genetic improvement (breeding) of safou has relied on phenotype selection and quantitative genetics through field trials breeding. Technologies to increase productivity, increase sustainable resource use, respond to climate change and enhance efficiency are required to meet demand. Biotechnology applications give a scope for rapid improvement and also facilitate the breeding program. Advantages of biotechnology application using molecular markers in breeding programs includes: study of genetic diversity, DNA fingerprinting of individuals, easy identification of specific traits or genes of interest, rapid propagation of improved genotypes and integration of gene(s) of interest into the species. It also provides genetic basis for selecting individuals and particular regions of the genome in a breeding program, reduce breeding population, can lead to early selection of traits and the development of a new variety with combination of characteristics. The complementary role of these techniques will be necessary for a successful genetic improvement program in the species. This review examines the achievements obtained using classical techniques, emphasizes missing gaps for the application of molecular techniques and discusses the complementary role of biotechnology techniques for a sustainable genetic improvement program in the species.

Keywords: *Dacryodes edulis*, domestication, population, genetic diversity, multiplication, biotechnology and genetic improvement

INTRODUCTION

Dacryodes edulis (G. Don) H. J. Lam belongs to the family *Burseraceae* and is known in Cameroon as African plum, bush butter or safou. It is a dioecious, small to

medium-sized tree up to 20–25 m tall. It is an important fruit tree at the national and international levels, has high nutritional values and plays an important role in the

economy of the rural communities (Tchiegang et al., 1998). The species originates from Central Africa and the Gulf of Guinea and is presently cultivated from Sierra Leone to Angola along the Atlantic and further inland as far as Uganda and northern Zimbabwe. Safou is an important fruit tree nationally and internationally, whose production has improved the living standard of many rural communities through the domestication program by the World Agroforestry Center (ICRAF).

Cultivation of safou to improve local livelihoods has been growing rapidly in the range of occurrence of the species and recent emergence of the market chain introduced by ICRAF experts has further stimulated this (Tabuna, 2002). Technologies to increase productivity and increase sustainable resource use, respond to climate change and enhance efficiency are required to meet demand. Domestication through genetic improvement is the alteration of the genetic composition of species through the selection of desirable traits from the original (wild) population to the planted population. The achievements obtained using classical technique includes: selection of plus trees from natural stands, farmlands and homestead gardens and phenotypic diversity studies. This was followed by testing their progenies with quantitative genetic studies in field trials (forward selection) for control crosses or interspecies hybridization. The best parents were selected (Backward selection) for the establishment of clonal trials. The main purpose of the tree improvement programs is geared at enhancing the genetic value of the population while maintaining genetic diversity (Namkoong et al., 1998). Each generation of selection changes the genetics structure of the population by changing allele frequencies and the association of alleles on chromosomes. Early generation of selection maintain high levels of genetic diversity within the large population size (Williams et al., 1995).

However, after a few generations of selection there will be a trade-off between increase genetic gain and maintaining genetic diversity. In addition, domestication by phenotypic selection methods could take many decades to increase the productivity of forest species (Bradshaw and Strauss, 2001). The rapid developments in plant biotechnology in the last decade have witnessed an explosion of efforts to utilize molecular tools in identifying genes and understanding their functions and the application in safou cannot be an exception. The field of biotechnology tools in the study of the entire genome has significantly impacted agricultural production and many reviews have been cited for forest trees (Riemenschneider et al., 1988; Jain and Priyadarshan,

2009; Schnell and Priyadarshan, 2012). Biotechnology techniques (genetic markers and recombinant DNA technology/genetic engineering) can be used to facilitate the breeding program. The advantages of genetic markers in breeding programs includes: the study of genetic diversity, DNA fingerprinting, easy identification of specific traits or genes of interest, rapid propagation of improved genotypes and integration of gene(s) of interest into species. It provides the genetic basis for selecting individuals and particular regions of the genome in a breeding program, reduces the breeding population, can lead to early selection of traits and the development of a new variety with combination of characteristics. The complementary role of these techniques will be necessary for a successful genetic improvement program in the species. This review examines the achievements obtained using classical techniques, emphasizes the missing gaps for the application of molecular techniques and discusses the complementary role of these techniques for a sustainable genetic improvement program in the species.

DOMESTICATION INITIATION PROGRAM

Prior to domestication program, studies on safou focused on the reproductive biology (Kengue, 1990), economic importance, uses and management (Ayuk et al., 1999) which are all factors which affect breeding operation. Management activities in Safou can be ascertained since many farmers have kept on working on what can be done for their trees to produce more fruits with the available manure, fertilizers and land with little emphasis on the genetic potential of the planting stock being used. Domestication through genetic improvement is the alteration of the genetic composition of species due to selection of trees with desirable traits from the original population to the planted population. International organizations such as the World Agroforestry Centre (ICRAF), Center for International Forestry Research (CIFOR) and some national agricultural research centers like the Institute of Agricultural Research for Development (IRAD), have been involved in the domestication of high value indigenous forest trees of West and Central Africa for the last ten years. Five species (*Irvingia gabonensis*, *Dacryodes edulis*, *Ricinodendron heudelotii*, *Garcinia kola* and *Pausinystalia johimbe*) were selected during the initial process of domestication based on farmer's preference and the market potentialities of the species. *D. edulis* (G. Don) H. J. Lam was ranked second amongst trees that farmers selected for the domestication program (Franzel et al., 1996).

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Germplasm collection and conservation

Several literatures have indicated that safou is cultivated and marketed in the Humid Lowland Zone (HLZ) of Cameroon notably in the South West, West, Littoral, Center, South and East regions (Tabuna, 2002). The collection of plus-trees was concentrated on populations located in these regions. Collected germplasms (plus-trees) were from homestead gardens, cultivated farmlands and secondary forests. The distance between populations was 200 km and between plus-trees was 100m. Phenotypic mass selection and progeny test have been the most common practice for the quantitative evaluation and selection of superior trees (Tchoundjeu et al., 2002; Tchatchoua et al., 2012). The efficiency of selections when trees are growing in natural forests or in an unimproved plantation depends on the traits, species and the experience of the investigator. Only by careful study of the open-pollinated progeny, controlled-pollinated progeny and replicated clonal trials, can we determine if a given tree is really genetically superior (Zobel and Talbert, 1984). The selected plus-trees supplied seeds for the provenance-progeny trial and seedlings for cuttings while marcots were obtained for the vegetative propagation stands in Mbalmayo and Minkoameyos field trials. Unfortunately, many of the identified and labeled plus-trees on farm lands were subsequently destroyed or the farmers may not be available, so future genetic improvement program will need to be centered on the available genebank stocks.

Population variation

ICRAF started with the collection of germplasm in 1998 with desired characteristics in the range of the distribution of this species (Tchoundjeu et al., 2002). Safou has a great deal of variability in fruit traits. Selected traits for on farm establishment included: fruit characteristics such as size, shape, taste, colour and nutritional qualities. Many farmers and through market survey records indicated that fruits with particular traits are preferred by the population. In such cases, selecting superior individuals with potential traits for planting can increase production but such an approach needs the testing programs, that is, testing of the progenies or clones.

A genetic improvement strategy in its broadest sense includes the whole process of selection, breeding and testing from species level, through the population and family, down to the clonal level, and conservation of variation within them. Understanding the genetic structure among populations for the desired traits is essential for developing tree improvement programs by selecting population for conservation and use. Research straggling in safou and suggest the presence of population variability in fruit traits. Leakey and Ladipo (1996) reported continuous variation in fruit characteristics from different

regions in Cameroon. Youmbi et al. (1989) distinguished two morphological types based on their chemical compositions. Waruhiu (1999) identified tree to tree variation in fruit traits from three regions in Humid Forest Zone of Cameroon. Additional work by Kengue and Singa (1998) indicated existing population variations on growth traits from safou collections grown in Barombi-kang, Cameroon. Population selection indicates a method designed to improve the phenotypic performance of an inter-mating population by increasing the frequency of favorable alleles controlling traits of interest in which case the improved population can be directly used as a cultivar.

Genetic variation

The genetic characterization of plant is usually the initial stage after species selection whereby the germplasms are collected in the distribution range of the species to find and create enough genetic diversity in the population. Selecting trees from natural or planted tree populations must have a certain degree of genetic diversity. Genetic diversity is of fundamental importance to the long term survival and evolution of the species and it gives an opportunity to tree breeders to carry out selection from their breeding populations and thus conservation and management of the germplasm (Poltri et al., 2003). Improving all the traits of interest simultaneously due to pleiotropy effect of genes was identified by Leakey and Ladipo (1996) and Tchatchoua et al. (2012). It was shown that selection for yield favoured seed traits and unfavourable for nut production. This is scientifically attractive because one could promote significant gain for yield, even with a small breeding program. However, it is important to keep in mind that unfavorable genetic correlations can offset the advantage brought by the traits making the ideotype. Several methods for the study of genetic diversity includes: Phenotypic and molecular techniques. Phenotypic characterisations are influenced by environmental conditions and can lead to overestimation of the diversity of important traits. Also, major traits for which improvement is desired are the results from complex interaction of genes at multiple loci as such improvement of these traits can be possible using biotechnology tools. Work has been initiated on the study of genetic diversity using microsatellite markers in some Cameroonian populations (Benoit et al., 2011; Donfagsiteli et al., 2016).

MULTIPLICATION OF GERmplasm

Propagation by seeds

Propagation through seed has been the common method used by farmers for decades until the introduction of

grafting by ICRAF. Propagation through seed provides opportunity for variation and genetic improvement in the species. Propagation by seeds has been a very successful method for safou production in Cameroon. Many seedling management experiments have been conducted in the nursery of ICRAF leading to a provenance-progeny trial established in Minkoameyos from four populations representing two agro-ecological zones in Cameroon. A seedling stand from four populations and a comparison stand of seedling and marcots from five families were established in Mbalmayo. The establishment of the trial consisted of field visits to identify candidate trees in terms of desired fruit traits which was conducted by ICRAF technical staff in 2001 with the help of farmers (Tchoundjeu et al., 2002). Fifty seeds were collected from each tree and sown and nursery bred in polythene bags at ICRAF's research nursery in Yaounde-Cameroon. After six months of growth, 30 seedlings per population were planted in manure-filled holes of 40 x 40 x 60 cm in the experimental field trial at Minkoameyos near Yaounde. Distance between trees was 5 by 5 m. The field trial was intercropped with maize for weed control and manual weeding was done three times a year. The experimental design consists of four populations planted in separate plots of 15 replicates with 5 families per population and two-tree plot per family per replication. Border trees from unknown provenances were planted around the separate population plots.

Vegetative propagation

Vegetative propagation method is an effective means of mass propagating and deploying genetically improved materials. The use of clones in tree improvement programs cannot be overemphasized as clones not only fruit earlier but they accumulate high genetic gain through clonal trials. Clonal trees whether grafted or cuttings can be used to measure and understand environmental effects and the importance of genotype by environment interaction can be determined by genetically comparable studies on multiple sites. Clonal trials established though with a few clones and many unknown populations indicated that using clones in plantations and on farms can be feasible in safou.

Multiple approaches for vegetative propagation of safou including grafting, cuttings and marcotting have been reported. There has been a transition in vegetative propagation by cutting, from a very difficult to root species in the 50ths (Philippe, 1957) to about 100% success in rooting of young seedlings with the development of new techniques of propagation. The published report by Mialoundama et al. (2002) recorded 80% rooted cuttings from juvenile leafy stem cuttings in sawdust or sand/sawdust medium. Clonal propagation to

capture all the genetic effects (dominance, additive and epistatic) of selected individuals or plus trees have been employed in clonal trials established in Minkoameyos in June 2001 and some selected farmer's fields in West and Centre regions (ICRAF personal comm.). Use of mycorrhiza to improve rooting ability was investigated by Mbeuyo et al. (2013) and many investigations still need to be done on the effect of clones on rooting, use of mycorrhiza to improve rooting ability, source of rooting materials from the tree, etc. In addition, nut grafting process involving the removal of the hypocotyls and root from a germinated nut followed by inserting of the scion into a slit cut into the nut as reported in some forest trees (Jaynes and Messner, 1967) is yet to be investigated. Vegetative propagation by marcotting has yielded very excellent results from experiments using different branch types, branch diameter, substrate and hormone concentrations (Mialoundama et al., 2002). Many marcots from ICRAF plus-trees have been planted on farm and in comparison, a stand of seedlings and marcots from five families in Mbalmayo. A clonal orchard was established in Mbalmayo with five families. Experimental design is complete randomized block with single tree-plot of five replicates. More research needs to be done on using silvicultural methods to improve marcots and cutting growth. Clonal variation is an important issue to be investigated in the genebanks to help in future breeding opportunities. Knowledge of the methods by which each tree can be propagated will be useful in our future research endeavours. Limitations in propagation through seed range from biological factors unusable variation that may occur in the production population while vegetative propagation may be limited by genotype specificity which can be overcome by *in-vitro* or micropropagation techniques.

Hybrids through control-cross pollination

Cross-pollination programs have started very timidly. Controlled cross pollination are necessary to efficiently combine traits into single genotype and to study the genetic influence of both simple and complex traits. Controlled crosses that produce full-sibling families are expected to be almost twice efficient as open-pollinated half-sib families for meeting improvement goals (Kung et al., 1974). Controlled crossing of elite individual trees is an important method to understand both the specific genetic combining ability of trees and to develop superior individuals for seed orchards or clonal propagation. The problem in using controlled crosses is that the cost of producing a large number of progeny is high, and if only a relatively small number of progeny are produced, the rate at which genes are fixed is much greater. In addition, the large size of trees of reproductive age makes controlled breeding difficult and the length of time from seed

germination to flowering can slow down breeding programs. Interspecific crossing based on DNA fingerprints will provide variation means for improving genetic variation in population while selection for important traits may be further increased by the use of DNA markers.

BIOTECHNOLOGY APPLICATION

The continuous application of traditional breeding methods in a given species could lead to the narrowing of the gene pool from which cultivars are drawn, rendering crops vulnerable to biotic and abiotic stresses and hampering future progress. This technology deals with the complementarity between parents characteristics without identifying the gene responsible for the control of the trait or traits of interest. The use of biotechnology tools in the study of the entire genome has significantly impacted agriculture. Several different markers have been developed for trees (Neale et al., 1992; Haines, 1994) and specifically for tropical forest trees (Muchugi et al., 2008) and new marker types are developed every year. These markers range from biochemical markers (monoterpenes and allozymes) to the most complex molecular markers: single allelic dominant markers (AFLPs, RAPD) and the multiallelic codominant markers (RFLPs, SSRs, CAPs, EST and SNPs). Molecular markers are classified into two groups based on DNA–DNA hybridization or on polymerized chain reaction (PCR) with properties of a good molecular marker as follows:

1. Highly robust and repeatable across different tissue types and different laboratories, that is, it can be reproducible in any laboratory experiment or between different laboratories performing identical experiments.
2. Unaffected by environmental and developmental variation
3. Inexpensive to develop and apply
4. Polymorphic, this is the variability among individuals and reveals high levels of allelic variability.
5. Reveal markers that are codominant. Depending on the type of application, the selected technology must be able to detect the marker's different forms, distinguishing between homozygotes and heterozygotes (codominant inheritance). A heterozygous individual shows simultaneously the combined genotype of the two homozygous parents.
6. Evenly distributed throughout the genome. Polymorphism is better assessed when the marker is more distributed and densely covers the genome.
7. Discriminating, that is, can be able to detect differences between closely related individuals.
8. Neutral. The allele present at the marker locus is independent of, and has no effect on, the selection

pressure exerted on the individual.

The advantages these genetic markers offer in breeding programs include:

a) Rapid and easy method to study genetic diversity. Genetic diversity is the diversity that occurs in genes of individuals and species. Genetic diversity can be found among species, populations, within populations and within individuals. If we consider the long-term genetic improvement of yield for the major crops, plant breeding has worked whenever there is genetic variation within the germplasm pools accessible to plant breeders and selection has focussed on the right traits measured in the right environments. Several markers have been identified in the study of genetic diversity which include biochemical markers used to determine the differences in chemical composition to molecular markers (restricted fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSRs), single nucleotide polymorphism (SNPs), etc) which can detect variation ranging from mutation at single nucleotide to variation due to changes in an entire chromosome.

b) Genetic fingerprinting. DNA fingerprinting is the method by which an organism is genotyped across a range of genetic loci (markers) to develop their individual genetics fingerprint. It was originally developed in the 1980s (Jeffrey et al., 1985a). Microsatellite loci are used most frequently in DNA fingerprinting, however single nucleotide Polymorphisms (SNPs) are becoming more frequently used. Plant genotyping is required for a variety of end uses including marker assistance selection MAS, associating phenotype with polymorphism, DNA barcoding, genetic diversity studies, conservation genetics and improving genome assemblies. Methods of genotyping depend on the facilities available and questions to be answered.

c) Easy identification of specific traits or genes of interest for marker assistance selection (MAS). A marker can either be located within the gene of interest or be linked to a gene determining a trait of interest. As such MAS can be executed as a selection for a trait based on the genotype using associated markers rather than the phenotype of the trait. MAS program for a given trait involves: 1) Characterizing germplasm for useful traits; 2) Selection of diversified parents; 3) Developing mapping population; 4) Selection of suitable combination of molecular markers and genotyping of parents and mapping population; 5) Construction of genetic or linkage map; 6) Phenotyping of mapping population for the selected traits; 7) QTL, analysis by combining the data of linkage and phenotyping; 8) Identifying mapping and validation of QTLs and 9) Executing MAS for the target traits with benefits as follows: early selection, decreasing breeding cycle, increase selection intensity and relative efficiency of selection on low heritability traits (Meuwissen

et al., 2001). The development of whole genome sequencing using next generation sequencing technology has made SSPs and SNP the markers of choice for genetics and plant breeding. Its application in this species can speed up or revolutionise the present research. This new DNA marker technology can be used to facilitate the breeding program.

d) GMO for rapid introduction of trait of interest. Biotechnology involves *in vitro* propagation approaches including micropropagation (axillary shoot multiplication), organogenesis (adventitious shoot production) and somatic embryogenesis are techniques that can be used in the production of plus trees and elite individuals for establishment on farms and plantation. These techniques can also be useful in gene transfer for the genetic engineering process (GMO). Transgenic technology offers the possibility of transferring single traits, without the problems encountered in traditional breeding that arise from the introduction of often additional undesirable genetic material.

CONCLUSION

Globally, *Dacryodes edulis* (safou) is an important fruit tree whose production has improved the living standard of the rural communities through the domestication program by the World Agroforestry Center (ICRAF). During the past decades, ICRAF has undertaken efforts for the conservation of safou genetic resources and major collections have been established. Collection of data in different sites and location permit selection of germplasm on a geographical scale. There is the need for future germplasm characterisation that combines both the classical and genetic characterisation using biotechnology tools, to ensure a more complete and informative characterisation that reveals the true genetic diversity of the population.

The reliability of selection based on field trials may be further increased by the use of DNA markers whereas interspecific crossing based on parent genotypes will provide means for improving the introduction of genetic variation. Genetic engineering will provide the means for introducing traits of interest from other species that are not available in the target plant gene pool or its wild relative. Phenotypic selection and quantitative genetics are very important for germplasm conservation, management and improvement. However, knowledge of genetic diversity and relationship among elite germplasm using biotechnology tools will improve breeding efficiency in the domestication efforts.

Conflict of Interests

The authors have not declared any conflict of interests.

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