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PROSPECTS FOR GENETIC IMPROVEMENT OF *ACACIA SENEGAL*: CAN MOLECULAR APPROACHES DELIVER BETTER GUM YIELD AND QUALITY?

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ABSTRACT

In the drylands of Africa, land degradation and soil fertility depletion are major threats to natural resource conservation and food security. A potential solution is to exploit the properties of native N_2-fixing legume trees. One candidate is *Acacia senegal*, the main species producing the internationally traded 'gum arabic', which also provides a multitude of ecosystem services and supports the livelihoods of many impoverished rural populations in the drylands of sub-Saharan Africa. The development of improved planting stock would help meet the demand for reliable, high quality gum and, at the same time, could enhance income generation and promote wide-scale planting giving environmental benefits for the fragile drylands ecosystem. This requires a strategic improvement programme, but traditional breeding methods require significant time. Molecular approaches are frequently touted as providing a means of increasing efficiency in breeding programmes; however it is as yet unclear whether they can deliver this efficiency, particularly in the regions in which *A. senegal* is an important crop. Here we review the status of genetic improvement of *A. senegal* highlighting modern molecular approaches to advance the breeding efforts for gum and other important traits to enhance adaptability and sustainable management of genetic resources in the changing global climate.

Key words: *Acacia senegal,* Africa, drylands, gum arabic, genetic improvement.

1 INTRODUCTION

Acacia senegal (L.) Willd. (*Acacia* sensu lato) is renowned for its exudate, a water-soluble gum (gum arabic) used internationally in processed food and drinks, pharmaceuticals, cosmetics and for many other purposes. The tree also provides local environmental and socio-economic benefits: it can improve soil fertility due to its inherent ability to fix N_2, which also supports food crop production in the gum arabic agroforestry systems;[1,2] it provides shade, fuelwood, livestock fodder, bee forage and honey; and the gum itself is an important source of nutrition in the drylands of sub-Saharan Africa, eaten by local communities. The inclusion of the species in agricultural landscapes also acts to increase complexity, diversity and stability of the drylands ecosystem. *Acacia senegal* is native and adapted to the drylands which cover more than 50 % of the land area of sub-Saharan Africa region (Figure 1). These drylands ecosystems are very fragile with low rainfall and high temperatures, and they support an ever-increasing human population living below the poverty line. In the past five decades there have been several initiatives by governments and non-governmental organizations to plant *A. senegal* in the region to combat desertification and alleviate poverty.

Given the absence of any previous significant breeding programmes on which to build a selection programme, molecular markers should now be central to any future tree improvement programme and several examples of their application exist, although these are mainly for temperate timber species (Table 1).

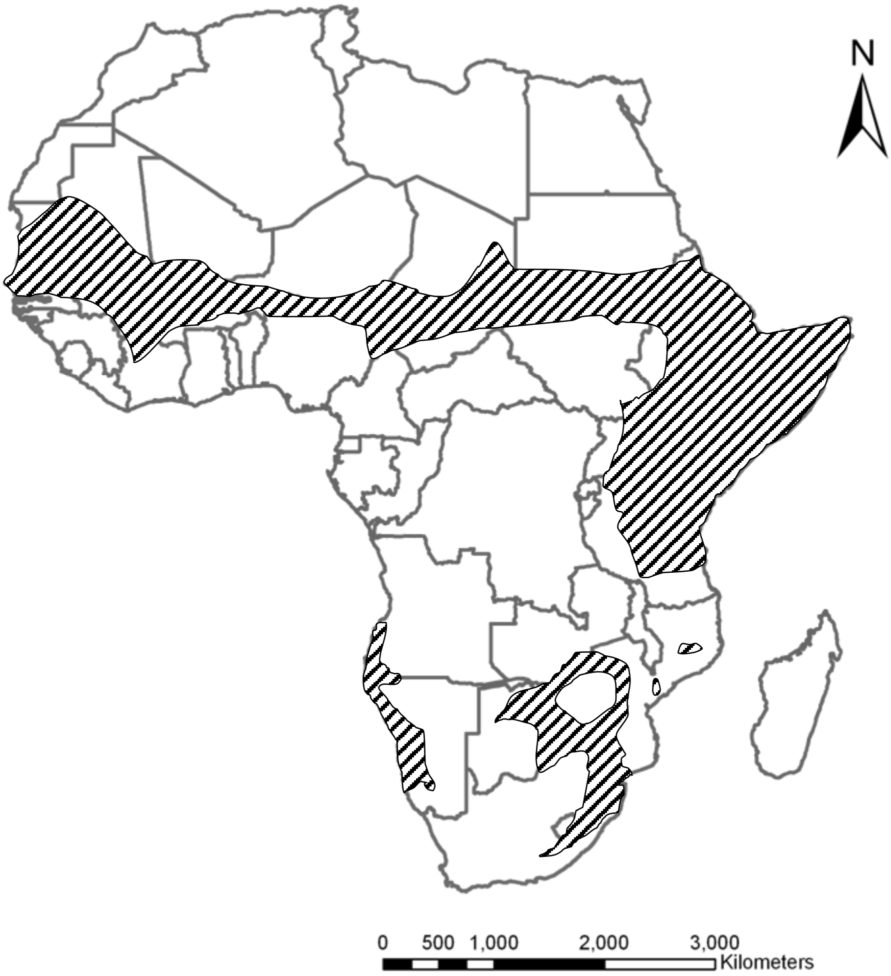

Figure 1 *Approximate distribution of* Acacia senegal *(hatch shading) in Africa (after Fagg and Allison, 2004). This distribution occurs mainly in the drylands.*

The relatively few studies on tropical forestry (agroforestry) species using molecular markers have largely been for the purposes of genotyping and evaluation of genetic resources.[3] There have been even fewer such studies on trees from the drylands of Africa. This perhaps depicts the true picture of the huge gap in global investment for improvement of forest species considered of high value (e.g. timber, energy, pulp and paper) and those which produce valuable products and ecosystem services (e.g. food, fuelwood, pollination, nutrient inputs and cycling, soil stabilization) but are of limited commercial value, such as numerous tropical agroforestry species. However, molecular breeding for *A. senegal* will benefit from the work on commercial species and Australian acacias (that are in a separate genus and tribe from African acacias). Considering its wide ecological range and importance, *A. senegal* could become a model for molecular-assisted breeding of drylands tree species in sub-Saharan Africa. DNA-based molecular data are now beginning to accrue for the species and the opportunity to capitalise on this should be seized.[4,5,6] In this review, we assess the current state of knowledge and discuss possible strategies for improvement of gum arabic production and quality using DNA-based molecular approaches.

2 GUM SOURCE, YIELD AND QUALITY

The name "gum arabic" reflects the early trade history of the product when it was exported to Europe through various Arabian ports. According to the Joint FAO/WHO Expert Committee on Food additives (JECFA), gum arabic is a dried exudate obtained from the stems and branches of *A. senegal* or *A. seyal* Del.[7]. However, *A. senegal* is by far the most important species in terms of both production and quality.

Sudanese gum arabic has been the benchmark for quality largely due to the long history of production coupled with efficient and well established collection and post-handling systems.[8] In particular, Kordofan gum, named after a region within Sudan's gum belt, produces high quality gum fetching the highest premium in the international markets. Considerable variation in gum yield production within and among populations or

Table 1 *Molecular markers used in tree improvement programmes*

Species and regions	**Methods and applications**	**References**
Mainly temperate species	Genotyping and measures of genetic diversity, SNPs[1] and QTL[2] and SNPs, candidate genes, association mapping	González-Martínez *et al.*, 2006; Grattapaglia *et al.*, 2009
Eucalyptus spp., Tropical and sub-tropical	Genotyping and measures of genetic diversity, QTL, MAS[3], association mapping, molecular breeding	Henery *et al.*, 2007; Grattapaglia & Kirst, 2008; Grattapaglia *et al.*, 2009
Elaeis guineensis Jacq. (Oil palm), Tropical	Marker assisted recurrent selection, genomewide selection, molecular breeding and modelling	Wong & Bernardo, 2008
Acacia mangium/*A. auriculiformis*, Tropical	Genotyping and measures of genetic diversity, QTL, Molecular breeding	Butcher *et al.*, 1998; *Acacia* Project[4]
Various tropical (agroforestry) species	Genotyping and measures of genetic diversity	Jamnadass *et al.*, 2009

[1]SNPs, single nucleotide polymorphism; [2]QTL, quantitative trait loci; [3]MAS, marker assisted selection; [4]The *Acacia* Project (http://pkukmweb.ukm.my/~acacia/highlight.html)

provenances of *A. senegal* has been reported in both natural and planted stands.[1,2,9] Apart from environmental factors such as rainfall, temperature and soil type, gum yield can be improved by tapping - deliberate and systematic slashing of the bark from the branches, conducted at the onset of the dry season. Gum arabic is a complex polysaccharide whose quality is defined by biological, physical and chemical parameters, including botanical source, specific optical rotation, intrinsic viscosity, emulsification properties, sugar content, amino acid and mineral content.[10] Both gum yield and quality are complex traits and their variation remains poorly understood, but is likely to involve both genetic and environmental control. Studies to understand these traits, and to use them to improve *A. senegal* are overdue.

3 THE STATUS OF GENETIC IMPROVEMENT OF *ACACIA SENEGAL*

3.1 Early selection and improvement initiatives

Although *Acacia senegal* was one of the earliest domesticated tropical dryland species, the process of improvement has hardly progressed beyond first cycle progeny trials. In Sudan, new gum producing trees and plantations have continually been recruited from high yielding individuals or populations through natural regeneration, coppicing, direct seeding or transplanted seedlings propagated from local material in rotational bush-fallow agroforestry systems, traditionally known as the gum gardens.[8,9] However, there is no readily available information on how these collections have been deployed, although seed from the gum belt region are now widely used in establishment of trials, new gum plantations or afforestation programmes both within and outside Sudan.[2,8]

3.2 Provenance trials and adaptive variation

Over the years, there has been a series of isolated provenance trials across the geographic range of the species. A good example is that of a single site provenance trial evaluation located in a clay plain near Ed Damazin in the Blue Nile State of Sudan.[1] Eight *A. senegal* provenances originating from contrasting conditions (semi-arid with clay soil and arid with sandy soil) of the gum arabic belt in Central Sudan were used to evaluate the genetic variation in shoot growth, water-use efficiency (as assessed by $\delta^{13}C$) and gum production. The results showed that clay provenances were distinctly superior to the sand provenances in all growth traits, but water use efficiency was better with the sand than the clay provenances. Other trials have also been reported; three established in Burkina Faso, West Africa across latitudinal, temperature and rainfall gradients,[11] and one in India,[12] some of which are still being maintained and assessed. Significant differences in survival and growth parameters among the provenances were reported 5-6 years after planting with the locally or regionally sourced material generally showing superior performance.

Recently, results of provenance trials established at Dahra and Bambey (Senegal) with 16 accessions (13 from the Sudano-Sahelian region, two from India and one from Pakistan) showed that Malian provenances were the best performers in terms of growth (height), whereas provenances from Pakistan and India performed poorly with high mortality 7 years after planting.[13] Gum production was significantly different within and among the provenances with individual production ranging from 6 to 500 g/tree/yr. Other provenance studies have also been established to compare growth and gum production potential of *A. senegal* varieties in a range of Zimbabwean sites.[8] To the best of our knowledge, the

Dahra site (Senegal) also hosts the only documented *A. senegal* progeny trial, but this is still in its first breeding cycle.

4 MOLECULAR APPROACHES SUITABLE FOR *ACACIA SENEGAL*

4.1 Characterisation of *A. senegal* genetic resources

Several recent publications have carried extensive reviews on forest genomics and application of molecular markers for tree improvement, most of which have been based on temperate timber species.[14,15,16,17] In this section, we look at how some of these methods can be applied to improvement of *A. senegal* (e.g. see Table 1).

Early genetic characterisation studies of *A. senegal* involved isoenzyme markers which invariably showed high variation within populations and low variation among populations. However, these studies also showed geographic structuring, clearly differentiating populations at the local and regional level. Only very recently have any DNA-based (AFLP, amplified fragment length polymorphism; RAPD, randomly amplified polymorphic DNA; ISSR, inter simple sequence repeat; SSR, simple sequence repeat cpSSR, chloroplast simple sequence repeat) molecular data on *A. senegal* been published.[4,5,6] In a study of *A. senegal* var. *kerensis,* populations in Kenya were analysed using both nuclear and chloroplast microsatellite markers (loci). High levels of genetic diversity were found within all populations and genetic differentiation among populations was low.[6] The high levels of genetic diversity from the nuclear data (mean H_E= 0.67) were similar to those reported for the acacias from which the microsatellites were transferred: *A. brevispica* Harms (H_E= 0.72) and *A. mellifera* (Vahl) Benth. (H_E= 0.67).[18,19] Similar genetic and geographic patterns have been found with other African acacias, e.g. *A. karroo* Hayne (Brain *et al.*, 1997) and *Faidherbia albida* (Del.) A. Chev. (syn. *A. albida* Del.).[20] By comparison, Australian acacias have shown variable data on genetic variation and partitioning within and among populations, indicating differences in breeding systems, seed dispersal mechanism, gene flow, geographic distribution, and even sampling strategies and markers used.[21,22] Genetic diversity in the nuclear genome of *A. mangium* Willd. was estimated with restriction fragment length polymorphism (RFLP) loci for 10 natural populations representing the geographical range of the species.[21] The level of genetic diversity varied significantly among the populations (ranging from H_E = 0.01 - 0.21), and was higher than previously detected with isoezyme markers.[23] More recently, Krauss & He used AFLP to assess genetic diversity and differentiation in two widespread coastal species, *A. rostellifera* Benth. and *A. cochlearis* (Labill.) H.L.Wendl., commonly used in restoration programmes in SW Australia.[24] They found very high levels of genetic differentiation among populations with more than 50 % of the total genetic variance partitioned among populations.

4.2 Candidate genes and genome scanning

Choice of candidate genes should be made on the basis of function and traits of interest, in this case gum yield and quality. A number of genes involved in the biosynthesis of polysaccharides, lignin and cell wall proteins, have been identified and used in association genetics for wood-based properties.[25] The aquaporins (a gene family facilitating the diffusion of water and small neutral solutes across plant cell membranes) are also potential candidate genes since *in situ* gum production is related to water availability driven by seasonal (wet and dry) changes.[26] Expressed sequence tags (ESTs) databases have been important sources of information for the selection of candidate genes for mapping and association studies, and can quickly generate a large array of partial genes for the organism of interest to provide a pool for single nucleotide polymorphism (SNPs) discovery. In order to identify candidate genes involved in gum traits, cDNA libraries can be synthesized *de novo* from RNA isolated from cambial tissue. Single pass sequencing and creation of ESTs libraries, followed by homology searches (nucleotide-nucleotide: blastn; nucleotide-protein: blastx) should identify candidate genes.[27] For example, Namroud and other workers recently analysed a total of 534 SNPs representing 345 expressed genes from six natural populations of white spruce (*Picea glauca* [Moench] Vos), of which 14 % were identified as candidates for local adaptation.[28] Both average heterozygosity and population differentiation estimates (H_E =0.270 and F_{ST} = 0.006) were comparable to those previously obtained with allozymes and ESTs in the same populations.[28,29] These results also demonstrate the utility of SNPs for studies of genetic diversity and population structure, none of which have been applied to African acacias.

4.3 Association mapping

The key difference between association or linkage disequilibrium (LD) mapping and a QTL mapping study is the nature of the mapping population. The basic principle underlying LD mapping is that alleles at loci close to a particular gene or trait of interest will show strong statistical association with that trait, whereas alleles at distant loci will not. This approach has been applied for crops e.g. *Zea mays* L., maize and is increasingly being applied to forest trees, especially those linked to wood properties.[25,30] Recently, Buckler and others applied the nested association mapping (NAM, a variant of association mapping) approach to the outcrossing crop species maize, to study flowering time, a complex trait that controls adaptation to local environment.[30] They concluded that the genetic architecture of flowering time was controlled by small additive QTL with few genetic or environmental interactions (the simple additive model). As *A. senegal* is principally outcrossing,[31] it should be amenable to the association mapping approach.

Successful application of association genetics may depend on the source of genetic information. It is likely that, in most cases, any given phenotypic trait will be controlled by multiple small contributions from different genes. Current methods for accessing selectively important variation offer ‘top down’ approaches - identification of ‘candidate’ genes of known or implied effect from homology with published genomes - and ‘bottom-up’ approaches - screening the target genome at large numbers of anonymous loci and searching for associations with the trait of interest: genome scanning. The former allows searches for variation to be targeted in regions of the genome thought likely to be important in the physiological process being examined and takes advantage of available genomic resources from model organisms, but is limited by the physical capability to select and screen individual genes for variation. However, high-throughput transcriptomic approaches (next-generation sequencing) offer increasingly rapid access to variation in coding regions, so the efficiency of the ‘top-down’ approach should improve. The latter approach makes fewer assumptions about the loci likely to be involved in the trait of interest, samples much more of the genome and offers wider scope to screen for covariation among different loci an approach that is possibly more likely to identify genes of small effect but must necessarily involve a substantial amount of redundancy,[32] as most of the loci studied will not contribute to control of the trait of interest.

4.4 Marker assisted selection (MAS)

Lack of in-depth molecular data has precluded development of a strategic improvement programme for *A. senegal*. Quantitative trait loci (QTL) analysis has been widely used in crop breeding strategies that aim to assemble more desirable combinations of genes in new varieties.[33,34] The approach requires that each population is genotyped and phenotyped: a long and tedious process. For example, in standard techniques for inbreeding cereal crops, selection of desirable plants begins in early generations for traits of high heritability, but for traits of low heritability, selection can go on for several generations until lines become homozygous. They are then subjected to field evaluations and the entire process can take up to 10 years, at considerable expense. Genetic linkage maps have been constructed for several tree species using this approach. As would be expected, emphasis has been placed on wood properties but other traits have also been tackled, e.g. disease resistance, growth, flowering, frost tolerance and plant secondary compounds. For example, it was recently reported the QTL for foliar concentrations of terpenes and formylated phloroglucinol compounds in *Eucalyptus nitens* using pedigree populations developed to third generation progenies.[35] These traits show large within-population variation and are highly heritable, and indicate the feasibility of identifying QTL for other plant secondary compounds, like gum arabic yield and quality. However, the QTL approach has several practical limitations for trees. The long generation times of trees mean that if the required pedigrees do not already exist, it will be decades before they are developed. Other limitations include the need for QTL verification in different seasons, environments and genetic backgrounds. In addition, QTL studies are principally relevant within the pedigrees being evaluated.[25,36] Gum trees can start producing from 2 years,[8] but commercially acceptable quality should target gum produced from 7 years; peak production is normally between 7-15 years and should form the timeline for at least the first breeding cycle for QTL studies. These challenges have made the QTL approach less attractive for the application of MAS in commercial tree breeding programmes.

4.5 Management of breeding populations and plantations

Planting of non-local provenances, improved material or seed stands within the range of natural populations may impact on the local gene pools to varying degrees, requiring adoption of management strategies to reduce the risk of genetic disruption or contamination of the natural populations. Such translocations may pose significant risks of genetic contamination, especially when the non-local type is located near fragmented or small natural populations. Over time, outbreeding depression due to breakdown of co-adapted gene complexes through recombination may reduce the fitness of the local population or taxon, making it vulnerable to extinction, or pollen swamping may result in loss of genetic integrity.[37] Genetic contamination has been demonstrated in a planted stand of the outcrossing Australian *A. saligna* subsp. *saligna* (Labill.) H.L.Wendl. with long distance pollen dispersal (1,500 m) detected from the natural background population of *A. saligna* subsp. *lindleyi*.[38] Similar gene flow and genetic contamination have been reported in eucalypts (e.g. Potts *et al.*, 2003; Sampson & Byrne, 2008) and, in some instances, very long distance pollen dispersal events have been reported (e.g. up to 2.3 km, for the tropical tree *Dipteryx panamensis* in a fragmented Costa Rican landscape).[39] *Acacia senegal* is an outcrossing species, with the potential for long distance pollen-mediated gene dispersal, because of its effective pool of pollinators and seed dispersal.[6,31] It naturally hybridises with other species within the *A. senegal* species complex.[8] Management strategies to limit gene flow into natural populations may involve: controlling the location, size and isolation of non-local plantations; and controlling mating. There is, therefore, a need to undertake studies to quantify the volume and spatial capability of gene dispersal among populations. This will provide information for mitigation of the impacts of germplasm transfer, where it is recommended, and to ensure conservation of existing genetic resources in natural populations.

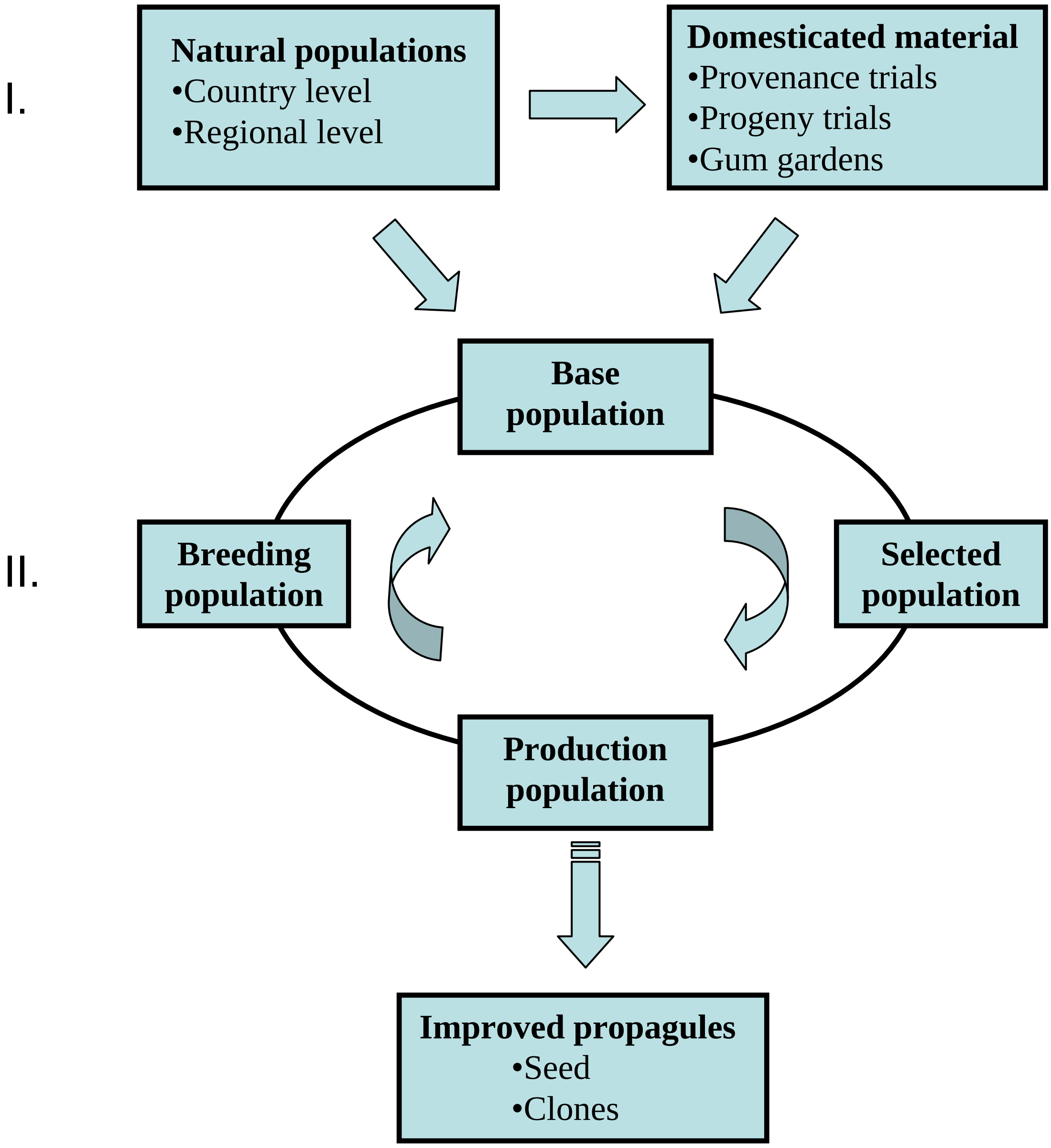

Figure 2 *Strategy for improvement of* A. senegal *for gum and other important traits. Phase I, selection and characterisation; Phase II, breeding and production of improved planting material.*

5 IMPROVEMENT STRATEGIES AND CONCLUDING PERSPECTIVES FOR *A. SENEGAL*

On the basis of what we now know about the status of improvement of *A. senegal*, molecular approaches can help deliver better gum yield and quality. The wide range of methods presented and the data available from other species with similar breeding and ecological attributes (e.g. Australian acacias and eucalypts) will act as models on which a breeding programme to improve gum yield and quality can based. The improvement strategy should include the following key components:

- *Molecular markers*: to establish genetic integrity of selected material (germplasm)
- *Short-cut strategy*: target existing provenance or progeny materials to start and fast-track breeding programmes
- *Long-term strategy*: prospect new material informed by phenotypic traits, genetic structure, geographic source or adaptively divergent environments
- *Application of molecular tools*: from characterisation to the breeding cycle and validation of planting material (Figure 2)
- *Concerted actions, networking and partnerships*: to widen geographic coverage and be cost effective
- *Integrated and interdisciplinary approaches*: e.g., the ACACIAGUM consortium (http://inco-acaciagum.cirad.fr) to ensure sustainability, and provision of socio-economic well-being and ecosystem services

White's classical framework for tree improvement programmes presents four key activities (selection, provenance/progeny testing and seed orchard establishment) from this we have adapted an improvement scheme for *A. senegal* (Figure 2).[40] We recommend a two- tier approach for the improvement of *A. senegal*. The first is to fast-track the breeding process for gum and other desirable traits by targeting the existing base populations (including provenance and progeny trials). Their genetic integrity should be established with appropriate markers to permit the development of pedigrees to feed the breeding cycle and utilise such breeding tools as MAS, NAM or marker assisted recurrent selection. The second tier would constitute a long-term approach. This takes into account the current available molecular data on the population genetic structure and phylogeography of *A. senegal* to inform the establishment of 'fresh' base populations.[6] The assumption here is that previous germplasm collections originated from populations that were either poorly characterized or used less precise markers to identify productive individuals. We recommend identifying geographic regions, defined by eco-climatic factors and physical barriers, knowledge on phenotypic trait variation and the scale of gene flow that would be likely to comprise adaptively divergent environments. When appropriately characterized (genotyped), these eco-geographic or genetic units would then be amenable to the application of the recent advances in forest genomics, such as association genetics, quantitative genetics or the genome-wide approach now attracting the attention of both crop and perennial plant breeders.[17,41] The variety of new high throughput techniques now available means that molecular data can quickly be generated by approaches that either survey multiple candidate genes or screen the whole genome using SNP discovery methods. The approach that is adopted will depend on the available resources and the questions being asked. For instance, although our review has focussed on gum yield and quality, the spectrum of genes being studied should encompass other equally important traits such as drought tolerance. Indeed, given increasing global temperatures and the impacts of climate change in the drylands, drought tolerance may prove to be more important in maintaining the genetic resource base of the species.

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