

HARNESSING BIOTECHNOLOGY FOR CONSERVATION AND INCREASED UTILIZATION OF ORPHAN CROPS

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Abstract

Mankind depend on a wide range of species for food, fibre, nutrition and other needs, however, there are a limited number of crops that meet the needs of staple diets and few major non-food crops that meet the associated needs. Out of 7000 species known that are partly or fully domesticated, only about 30 of them provide 95% of the world's food energy. This large array of plant species that are neglected or underutilized, are recognized as important minor or orphan crops. With global food security and economic growth dependent on such a limited number of crop species, the future supply of food and rural income is at risk. There is an immediate need to conserve and use the world's plant genetic resources for the development and welfare of future generations.

Ethnobotanic surveys have shown that hundreds of such orphan crops are still to be conserved and used, representing an enormous wealth of agro-biodiversity that has the potential to contribute to improved incomes, food security and nutrition mostly in rural areas of developing countries. The rapidly developing field of biotechnology aimed at improving major crops could play an important role in promoting these orphan crops to address the current and emerging challenges in agriculture. This article reviews the application of different methods of biotechnology that could be used to improve the conservation and utilization of orphan crops to improve food security, mainly for rural poor in developing countries.

Keywords: orphan crops, underutilized crops, neglected crops, minor crops, genetic resources, genetic conservation, genetic utilization, biotechnology

Introduction

Agricultural biodiversity is the primary source of food, feed, medicines, shelter and many other products. Mankind have so far used about 40,000 to 100,000 plant species for food, fiber, forage, fuel, crafts, industrial, cultural and medicinal purposes, of which about 7000 species are still cultivated or domesticated worldwide. However, over the past years, about 30 crop species have become the basis of most of the world's agriculture. In addition, the focus of research and crop improvement so far has been concentrated only on a few crop species that although helped meeting the food needs of ever increasing human population, however, it has also narrowed down the number of species on which the global food security and agricultural development depends [1]. For example, only three crop species such as

rice, wheat and maize contribute to more than half of food demands. This nutritional paradox [2], also known as agricultural "simplification", is a process by which some of the crops just got favored over the years over others mainly because of their comparative advantages in terms of simple cultivation requirements, easy processing and storability, nutritional quality, taste, etc. This simplification process although reduced agricultural biodiversity through narrowing of inter- and intra-specific diversity of crop species, but it increased the chances of successful harvests and survival through narrow but abundant sustenance [3].

The impact of this narrow species based global food security or hidden hunger is mostly experienced by the rural poor of developing countries, particularly in marginal areas where people are faced with a restricted set of livelihood options. In recent times, a change in attitude or awareness has been observed among the policy makers and researchers with regard to the need to rescue and improve the use of all those crops which were left behind for research, development, technology, including conservation efforts. In 21st century, these crops, also known as "orphan crops" (referred also by other terms such as minor-, underutilized-, underexploited-, neglected-, local-, traditional-, lost-, new-, alternative-, promising-, or niche-crops) [4, 5], have raised interest among decision makers and many leading organizations including Consultative Group on International Agricultural Research (CGIAR) have started working on these species. This global "opening" towards orphan crops has resulted in a change of attitude towards biodiversity and plant genetic resources of many countries, which is evident in the 1992 Convention on Biological Diversity (CBD) and the FAO IV International Technical Conference on Plant Genetic Resources for Food and Agriculture (PGRFA) held in Germany in 1996 (Activity 12: "Promoting development and commercialization of underutilized crops and species") [6, 7].

The Global Forum on Agricultural Research (GFAR) in 1999 also emphasized the role of underutilized species in raising income of the rural poor [8]. The purpose of this paper therefore is to review the research activities undertaken so far in improving the conservation as well as utilization of neglected and orphan crops, and also to report the possibilities and limitations of applying advanced molecular science to them. Recent achievements made through advances in genetics and genomics in major or model crops such as *Arabidopsis* could now provide new opportunities to understand orphan crops better.

Orphan Crops: What are they?

It is very difficult to define an “orphan crop” and many terms such as underutilized, underexploited, etc. are used interchangeably. However, the terms do not reflect any information in terms of geography (underutilized where?), social (underutilized by whom?) or economic (underutilized to what degree?) implications. With regard to geographical distribution, a crop species could be underutilized in one region and not in others. Similarly, for social implications, many crop species contribute to daily diet of millions of people (for example in sub-Saharan Africa) but their poor marketability makes them underutilized in economic terms. There are also many neglected crops that are grown only in their centers of origin by traditional farmers but are very important for subsistence of local communities. Some species may be globally distributed but they occupy certain niches in local ecology and also in the production-consumption systems. Although some confusion still remains with the definition of orphan or minor crops, however, what is important is to understand the causes of low level of use and/or neglect of certain crops to design ways of their improvement. The Global Facilitation Unit for Underutilized Species (GFU) identified 11 criteria that define orphan or neglected species (**Box 1**). However, for this paper, the orphan crops are defined as those crop species which have been under-exploited for their contribution towards food security, health (nutritional/medicinal), income generation and environmental effects.

Orphan crops: Development Context

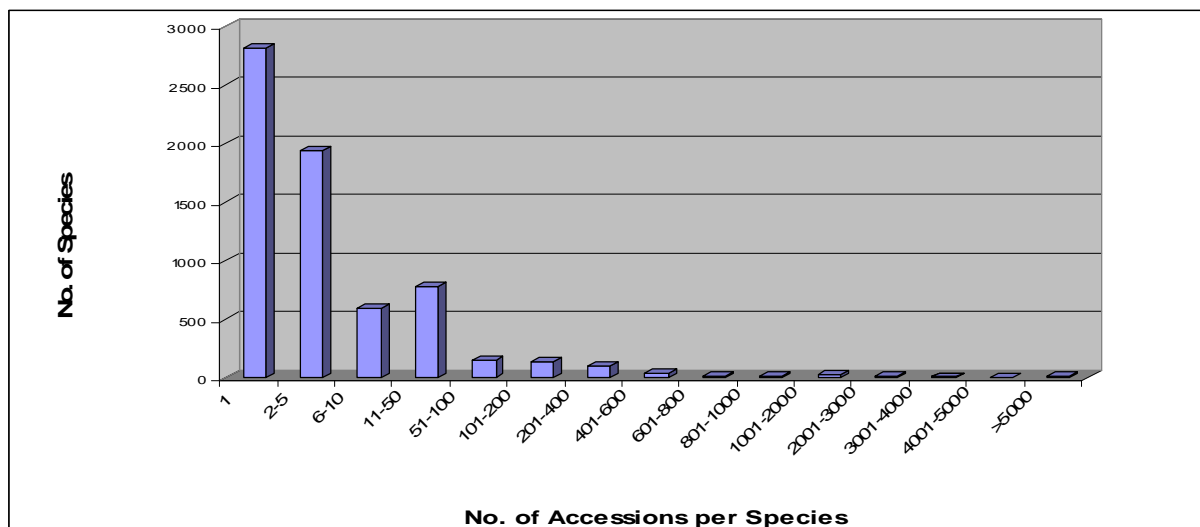
A huge difference exists on the way orphan or underutilized crops received financial investments for research and development and their contribution towards food security and livelihoods, in comparison to other major food crops. One of the major reasons for such low investments could be due to relatively lower returns from these orphan crops, when measured in terms of economic and welfare impacts. However, the role of agriculture based food security goes beyond crop yields and total production. Enhancing food security in poor-

est regions requires investments in research and development for wise management of resources and sustained growth. The rural areas of developing countries are the poorest regions where population growth is on rise, high climatic variability, specific soil and water regimes with phytopathological threats and unreliable market situation [9]. Diversification in agricultural systems is the key for improving the livelihoods of such vulnerable social groups. And this is possible through securing of the resource base of ‘minor or orphan’ crops to address the food needs in a sustainable way [10]. These minor crops have comparative advantage in marginal lands and contribute to the sustainable agricultural production by withstanding the stress conditions with no or low-cost inputs [11]. There is, therefore, research needs to test the role of these minor or orphan crops through traditional and available knowledge in improving the livelihoods of rural communities. There is also an immediate need to enhance better production and marketing strategies of these crops, and promote conservation of their genetic resources through sustainable use. However, for any development work on orphan crops, researchers and other agencies face a major problem of choosing the right crop species. Different criteria have been set out for the selection of underutilized tree species developed by von Maydell [12] (**Box 2**), which could be used as a guide but it is unlikely to meet all the criteria in a single species. The selection process becomes more challenging due to limited knowledge available on these species. Therefore, the selection process should mainly depend on the objective and their contribution towards improvement of food security, enhancing nutritional qualities, sustainability and income generation, although different considerations could also be applied such as cultural and aesthetic values of these species.

Conserving the genetic resources of orphan crops

The rapid globalization of agricultural trade where only a few agricultural commodities are entertained in the market chain is significantly contributing to the narrowing down of genetic base of crop resources. However, this is also opening up new opportunities for producers in developing countries to promote orphan or minor

Figure 1. Representation of minor crops in *ex situ* gene banks [13].



crops and improve livelihoods of rural communities, who use them as subsistence crop in difficult and low production environments. Therefore, conservation of genetic resources of these crops through a complimentary *ex situ* and *in situ* approach will ensure food security for future in these rural areas. The success of crop improvement for major crops, which resulted in green revolution, was mainly dependent on the well collected and conserved genetic diversity. Similar recognition for safeguarding the valuable genetic diversity of orphan or minor crops is needed to ensure the fullest use of these crops.

The Global Plan Action of FAO [7] has already advocated the importance of germplasm collections for underutilized species and several international organizations such as Bioversity International and International Center for Underutilized Crops (ICUC) have already started the promotion process of conserving germplasm collection of these species. However, the *ex situ* conservation of many of these species are still inadequate. It is estimated that more than 6 million accessions are conserved in about 1300 germplasm collections worldwide. The figures in these *ex situ* collections are quite impressive, however, about 80% belong to major crops and their close wild relatives and remaining 20% belong to other neglected and underutilized species, many of which are very poorly represented with less than 8 accessions per species [13] (Fig. 1).

Wheat, rice, maize and soybeans each occupy more than 70 million ha globally per year, covering about 580 million ha area and generating about USD 300 billion in gross value annually within developing countries. These four crops approximately supply on an average of about 1360 kcal of energy and 33 g of protein to individuals in poor countries. Whereas, 27 orphan crops within developing countries occupy areas of between 0.5 and 38 million

Box 1. Criteria for characterizing orphan or neglected or underutilized species [40]

- ⇒ Require only limited external inputs for production
- ⇒ Suitable for organic production
- ⇒ Suitable for cultivation on marginal land (poor soil fertility, etc.)
- ⇒ Suitable for stabilization of fragile ecosystems
- ⇒ Fit into small-scale farming systems
- ⇒ Possess traditional, local and/or regional importance
- ⇒ Easy to store and process by resource-poor communities
- ⇒ Market opportunities available
- ⇒ Possess high nutritional and/or medicinal value
- ⇒ Offer multiple uses
- ⇒ Traditional knowledge

ha with total area of about 250 million ha contributing about USD 100 billion in gross value. These figures nowhere near to that of wheat, rice, or maize but substantial enough to make an impact on food security of developing and poor countries. There are many such thousands of species that make up the world's plant genetic resources for food and agriculture which still needs to be collected, characterized, evaluated and conserved for utilization. It is evident that there will never be enough funding or resources for large scale formal collection of these species. Therefore, the most sustainable way of conserving genetic resources of orphan crops is through close linkage between conservation and utilization i.e. 'conservation through use' is most important. This also means conserving maximum genetic diversity of each species to meet the demand for these species in different production systems, under different environments, user needs and uses. There is also a need for integrated conservation strategy where efforts need to be made for *ex situ* collection as well as proper documentation of traditional knowledge on these crops. Therefore, it is not only important to secure species in production but also to generate information on material that is already in production, which is an important strategy of conserving the diversity of underutilized species. However, there are some issues for consideration while conservation and utilization of underutilized species, which are as follows [14]:

1. Conservation

- How many accessions should be conserved (*in situ* and *ex situ*) to safeguard the representative genetic diversity of these species?
- How broad should be the conservation of the gene pool of these species, considering that there is gene transfer or gene flow in nature? Should tertiary gene pools be also conserved?
- What should be the minimum level of knowledge on the eco-geographical distribution of the species and its status on genetic erosion?

How much local knowledge should be safeguarded?

2. Utilization

- What would be the level at which an under-utilized species will no longer be considered as minor crop?
- What would be the minimum information needed on nutritional aspects, processing aspects etc. required for successful industrial applications?
- What should be the basic information required for understanding the production, multiplication, and regeneration capacity of these species?

What should be the minimum level of information required on pests, diseases and other cultivation-related constraints?

Box 2. List of criteria for the selection of food producing trees and shrubs in semiarid regions [12].

- ⇒ They should solve problems
- ⇒ They should meet demands
- ⇒ They should be accepted by people
- ⇒ There should be no legal restriction
- ⇒ They should have low risk
- ⇒ They should be free from negative properties of effects
- ⇒ They should be adapted to site conditions
- ⇒ They should be easy and safe to establish, with low inputs
- ⇒ They should have fast growth
- ⇒ They should produce high yields
- ⇒ The quality of products should be good
- ⇒ The crops should be compatible with other land use

3. Policy-Legal issues

- What should be the basic policy framework to enable proper deployment of the species among farmers?

What should be the minimum level of policy reforms required to ensure a sustainable use of these resources (particularly for wild species)?

Research issues and opportunities

There is a universal agreement that orphan or minor species are essential to the livelihoods of millions of poor people throughout the world and are part of the threatened commodities, which needs immediate attentions from the perspective of rapid genetic erosion occurring in nature. The strategic way forward will therefore need to address the issue of combining conservation as well as its use to secure the resource base of such crops. The approaches may differ, depending on whether the crop is seed propagated or clonally propagated, annual or perennial, out-breeding or self-pollinated. However the basic questions of achieving this will remain the same: What is the smallest size of *ex situ* collection that can cover substantial amounts of diversity and how can it be most economically maintained? How much diversity will remain in production systems and how can this be monitored? How can resources be secured through linkages and collaborations, involving producers, consumers, the formal and informal sectors, to ensure that both conservation through use and conservation for use can be sustained? (Table 1) [14]. Modern technologies such as molecular genetics, GIS tools etc. will certainly play a major role in developing strategies of conserving minor crops and their further use.

Application of biotechnology: scope

Advances in molecular genetics (genomics as well as transgenics) have resulted in better understanding of the biology of the entire plant kingdom, providing an opportunity for efficient transfer of techniques from model species and major crops to other underutilized or orphan crops [15]. There are four main functionalities of molecular genetics which are routinely used to backstop conventional breeding, such as tissue culture and micropropagation; DNA fingerprinting for genetic diversity assessment; marker-assisted selection and related genomics, proteomics, transcriptomics, metabolomics; and production of genetically modified organisms (GMOs) or transgenic. The application of biotechniques, however, requires huge investment in terms of infrastructure, consumables, staff salaries and training and these needs to be considered when the total funding available for the promotion of a particular crop species is limited. It is because of these costs that biotechnology investment has been limited only to major crops, however, the knowledge and experience generated in these crops could be used for underutilized or orphan crops. It also reflects that spillovers from investments in facilities, human capital, and supplies for major crops could be quite large for orphan crops. Once researchers are well trained in the techniques and have the necessary equipment, they could apply their skills to a wider range of crops, or they could help to train others to adopt these technologies for orphan crops.

1. Tissue culture and micropropagation

Tissue culture is a process of overcoming reproductive barriers between distantly related crop relatives and micropropagation is an *in vitro* process by which vegetative multiplication is carried out through rooting of micro-cuttings, somatic embryogenesis or organogenesis. It can be used to clone large numbers of plants from genotypes of particularly desirable characteristics. Micropropagation is also used to eliminate diseases from germplasm and as a convenient method for the *in vitro* transfer of breeding material.

2. DNA fingerprinting and genetic diversity

Genotypic characterization using molecular markers to assess genetic diversity is an important process to devise effective sampling strategies such as selection of diverse parents for pre-breeding programmes. It also plays an important role in developing conservation strategies such as identification of duplicates or mismatches in rationalising *ex situ* germplasm collections. Molecular markers can also be used to certify varieties, determine the presence or absence of diseases and assess the reproductive biology of species, among other applications. There is a wide range of markers available, each of which has different characteristics. Several DNA based marker systems such as restriction fragment length polymorphism (RFLP), random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and microsatellites or simple sequence repeats (SSRs) [16] are routinely used

for assessment of genetic diversity at DNA level within a germplasm collection of any crop species. More recently, additional molecular markers such as EST-SSRs (SSRs from expressed sequence tagged sites, derived from transcribed DNA), DArTs (Diversity Array Technology) and SNPs (single nucleotide polymorphisms) are available for such studies.

3. Genetic maps, marker-assisted selection (MAS) and other 'omics

There has been a significant amount of knowledge generated in developing linkage maps and identification of QTLs (quantitative trait loci) to understand the association between markers and genes that control a proportion of the variation of a trait. By establishing an association, markers can be used to understand complex traits and assist in selection called MAS, making the process much faster when compared with conventional breeding methods. Genomics, a 'second-generation' biotechnology tool, is used to identify genes and their functions in an organism. By revealing gene sequence similarities and common arrangements of genes (synteny), genomics raises the prospect of information gathered on one species benefiting work on other less researched taxa. Genomics involves a wide range of activities, including the production of expressed sequence tags (ESTs), genome sequencing, gene function determination, comparative analysis (exploring synteny, cross-identification of candidate genes, etc.), physical mapping, through use of another discipline called bioinformatics. The information gathered is then incorporated into selection and breeding programmes. Proteomics (the study of proteins) and metabolomics (the study of metabolites) can be combined together with genomics into a biotechnology meta-analysis, to resolve many issues that cannot be addressed through conventional breeding approaches.

4. Production of GM or transgenic organisms

GM is the use of recombinant DNA and asexual gene transfer methods to alter the structure or expression of specific genes and traits in an organism. The product of GM, a transgenic, is one that has been transformed by the insertion of one or more genes, called 'transgenes', from another, often unrelated, organism. Transferred genes may theoretically contribute to a range of properties, such as resistance/tolerance to biotic and abiotic factors, improved nutritional status, and better management options (such as reduced tillage).

Molecular tools for germplasm improvement

Germplasm improvement of orphan crops needs a holistic approach wherein the developments and knowledge generated in set of crops with similar genetic structures and production constraints could be brought together to address issues. The orphan crops could benefit from the available knowledge from model species or major crops in different forms such as: analyzing crop biodiversity and identification of potentially useful variants; marker-assisted selection of (MAS) of

desired allele (s) and allele combination (s); and cloning and direct transfer of alleles across taxa. Evidences from genetic research have shown that all plant genomes have a vast array of common gene content, biological pathways, and chromosome organizations [17]. Functions such as gene regulation, general metabolism, nutrient acquisition, disease resistance, general defense, flowering time and flower development are largely conserved across taxa. Different plant taxa have different versions of the same genes at a given position or locus in a genome, but the order of loci is conserved to varying degrees across even distantly related crops, a phenomenon known as "synteny". For example, the flowering plants can be divided into two main groups: monocots and dicots. The former includes the grasses, which in turn include the major cereal crops. The latter include the legumes, many roots and tubers, and vegetable crops. The genome of the model dicot species *Arabidopsis thaliana* has been fully sequenced [18], and a great deal of basic research has been done on this species. More recently, the draft genomic sequence of rice (*Oryza sativa*) genome was made available, which is useful both for rice and its monocot relatives. Similarly, the whole genome sequencing of several crops is underway which could be used to generate information for other related or unrelated species through comparative genomics. Goff et al. [19] reported that 98% of the proteins are conserved in maize, wheat, barley and rice. Similarly, it has been found that although the progenitors of rice and *Arabidopsis* diverged 150 to 200 million years ago, more than 80% of the genes documented in *Arabidopsis* have also been found to have related genes in rice [20]. Functions such as gene regulation, general metabolism, nutrient acquisition, disease resistance, general defense, flowering time, and flower development are generally conserved across taxa [21]. Comparative mapping studies among grasses have revealed that the gene order is conserved for chromosomal segments [22, 23, 24]. In few cases, chromosomal colinearity could also be detected between monocots and dicots [19, 25, 26]. Given the similarities among crop genomes, particularly among plant species within a family, it seems possible that research on major crops or model species would benefit a substantial number of related crop species in the same families. The potential spillover benefits are likely to differ, however, across taxa and region depending on the particular biology of the species, the constraints faced by farmers, and seed systems.

Examples of application of biotechnologies to orphan crops

The review on application of different biotechnological tools has been low, exception being in the area of micropropagation. In the future, somatic embryogenesis was seen as likely to become important for conifer propagation. The area of genetic diversity, genetic maps, MAS and genomics were more done on trees. For example, the entire *Populus* genome has been sequenced, and public and private EST libraries for conifers have more than one million entries. Gene discovery and association genetic studies are likely to become important in near future. For GM, most of the work has been done so far

Table 1. List of constraints for research and development work in orphan or minor species [14].

Constraints	Outputs Required	Activities
Lack of genetic material	Improved availability of seed and other planting materials Crop improvement programmes Improved planting materials derived from traditional varieties	Set up local germplasm supply systems among rural communities Initiate participatory and other improvement programmes to obtain clean planting materials and improved varieties
Loss of germplasm and traditional knowledge	Resource base of selected species secured through ex situ and on farm conservation Appropriate traditional knowledge documented and shared among stakeholders	Assess distribution of species and genetic erosion threats Sample germplasm for ex situ maintenance and use Implement on farm conservation through community-based actions Identify and collate traditional knowledge using participatory procedures based on informed consent (including e.g. recipes on uses)
Lack of knowledge on uses, constraints and opportunities	Enhanced information on production levels, use constraints and opportunities Knowledge of gender and other socially significant factors identified	Participatory surveys on uses, constraints and opportunities with communities Analysis of survey data for gender and other socially significant factors
Limited income generation	Strategies for adding value and increasing rural incomes using target crops Enhanced competitiveness of selected crops	Development of value adding strategies (through processing, marketing, commercialization etc.) Investigate and identify improved agronomic and production procedures
Market, commercialization and demand limitations	Enhanced working alliances among stakeholders Improved processing and marketing opportunities identified Improved capacities of marketing associations and producer groups	Strengthen operational links between stakeholders of seed supply system, processing and distribution Develop improved low-cost processing techniques Analyze and identify market opportunities
Lack of research and development activities, and weak national capacities	Enhanced national capacities to work with neglected and underutilized crops Enhanced information and knowledge on the selected neglected and underutilized crops Methods to improve nutritional values developed and documented	Short-term training courses for researchers Develop and undertake community based participatory courses Characterize crops for agronomic, nutritional and market related traits Investigate methods of maintaining and enhancing nutritional value Investigate new areas of crop growth
Inappropriate (inadequate) policy and legal frameworks	Raised awareness among policy-makers of issues and options for improved policy and legal frameworks Links to existing rural and economic development projects enhanced	Identify inappropriate policy/legal elements Undertake public awareness actions among policy-makers Establish close partnerships with extension workers and others involved in agricultural development

on development of protocols rather than on direct deployment activities. It is envisaged that GM will become a regular activity for fruit trees, mainly for tackling disease problems, to enhance rooting and to control fruit ripening. Based on records from NCBI citations (<http://www.ncbi.nlm.nih.gov/sites/gquery>), and reviews by [39, 41], few examples of biotechnology research work on underutilized crops are listed below:

1. Tissue culture and micropropagation

A substantial number of activities including *in vitro* propagation (microcuttings or somatic embryogenesis) have been undertaken on a number of species, such as *Abelmoschus manihot* (bele), *Aegle marmelos* (bael), *Coriandrum sativum* (coriander), *Ipomoea batatas* (sweet potato); *in vitro* samples used for the dispersal of

germplasm; important in the production of transgenics), *Lablab purpureus* (hyacinth bean), *Plectranthus esculentus* (Livingstone potato), *Ricinodendron heudelotii* and *Sesamum indicum* (sesame). There have been reports on exchange of knowledge between institutions to produce disease-free germplasm. For example, ARC-Roodeplaat have used tissue culture techniques to produce virus-free planting material of *Ipomoea batatas* and to rapidly reintroduce *Plectranthus esculentus* to small groups of farmers in areas of South Africa from which the species had been lost. In China's Shandong Province, a micropropagation project distributed virus-free *Ipomoea batatas* that led to a 30% increase in yield and adoption across 500,000 ha area.

2. Genetic diversity

A large number of underutilized species have been characterized using different types of molecular markers to assess genetic diversity. Molecular diversity has been assessed within and among populations such as *Adansonia digitata* (baobab; using AFLPs combined with morphological data), *Bactris gasipaes* (peach palm; using AFLP and isozymes as well as SSR development), *Vitellaria paradoxa* (shea nut; using isozymes, RAPDs, SSRs combined with morphological data); among accessions such as *Artocarpus heterophyllus* (jackfruit; using isozymes), *Sesamum indicum* (Sesame; using AFLPs as well as SSR development); among accessions of different related species such as *Eleusine coracana* (finger millet; using a wide variety of markers, including isozymes, ISSRs, RAPDs and RFLPs along with development of EST-SSRs and SNPs), *Eragrostis tef* (tef; using AFLPs and ISSRs combined with morphological data; nuclear and organellar sequencing, and development of EST-SSRs and SNPs), *Ipomoea batatas* (sweet potato, using RAPDs combined with morphological data and SSR development along with nuclear sequencing), *Iringia gabonensis* (dika nut; using RAPDs), *Leucaena leucocephala* (leucaena; a wide range of studies using a range of techniques, among populations and related species), *Metroxylon sagu* (sago palm; using AFLPs combined with morphological data), *Cajanus cajan* (pigeonpea; using DArTs) and SSR development in *Diospyros kaki* (persimmon). Molecular fingerprinting has also been used to rationalize germplasm collections such as *Ipomoea batatas* collection at CIP using RAPD markers. The molecular profiles of groups of accessions that appeared morphologically identical were compared to identify duplicates in the collection. The germplasm collection was therefore reduced approximately to two-third of its original size, thus decreasing the maintenance cost in gene bank management. Similar approaches are also being used to rationalise the genebanks of other underutilised crops such as cassava and yam; *Eleusine coracana* and *Ceratonia siliqua* (locust bean gum).

3. Genetic maps, MAS and genomics

Genetic/linkage maps have been developed in species such as *Chenopodium quinoa* (quinoa) based on AFLP, RAPD, and SSR markers; and *Lablab purpureus*, based on RFLP and other markers. There has been recent identification of EST sequences in few species such as *Cajanus cajan*, *Ceratonia siliqua*, *Chenopodium quinoa*, *Diospyros kaki*, *Eleusine coracana*, *Eragrostis tef*, *Ipomoea batatas*, *Sesamum indicum* and *Setaria italica* to form the basis for MAS program in these species. For example, in *Manihot esculenta*, *Cajanus cajan* and *Setaria italica*, the identification of markers for drought stress has been targeted, while in *Eleusine coracana* both salt tolerance and drought stress have been considered, with a view to enhance production in marginal and degraded environments. Yu [27] reported genomic SSRs for 18 underutilized crop species (including 11 dicot and 7 monocot species), which could be used for further genetic analyses of these species. Comparative genomics (synteny) have been exploited for blast resis-

tance in *Eleusine coracana*, with another grass species, rice, where mechanisms of blast resistance are better understood and for which the genome has been sequenced. Synteny has also been compared between rice and *Setaria italica*. Expression profiles were compared between developing *Sesamum indicum* seed and *Arabidopsis* seed in order to identify EST candidate genes that may be involved in the biosynthesis of sesame lignans (which have antioxidant and health protecting properties). Related work has been undertaken to create diverse fatty acid compositions in sesame oil, in order to make the oil more competitive in world markets.

4. Genetic engineering/modification

GM activities in underutilized or orphan crops have been more apparent than expected although there has not been any practical field deployment so far. Genes involved in fatty acid synthesis in *Coriandrum sativum* and *Garcinia mangostana* (mangosteen) have been used to transform *Arabidopsis thaliana* and oil seed rape (canola), respectively, in order to understand metabolic pathways of seed oil production. Salt tolerance related to sorbitol accumulation has been studied in *Diospyros kaki* by transformation with a *Malus domestica* (apple) gene. Transgenic *Eleusine coracana* has been produced by various approaches, and an introduced gene from *Porteresia coarctata*, encoding a serinerich-protein, has been shown to increase salt tolerance. *Leucaena leucocephala* was transformed with a gene from aspen that down-regulated lignin biosynthesis and may have a future role in the use of the species for pulp and paper manufacture, as well as in fodder production. All the GM activities although appear to be far from deployment, however, the work on transgenic *Ipomoea batatas* is an exception wherein viral sequences to promote feathery mottle virus resistance have been introduced into sweet potato and field trials have been undertaken. The development of GM sweet potato showing resistance to weevil attack is also under active consideration. Studies involving tolerance to attack by fungal pathogens such as *Sclerotium rolfsii* in *Colocasia esculenta* and resistance to pod-borers in *Cajanus cajan* is underway using transgenic approaches.

Limitations and risks of applying biotechnology to orphan crops

Biotechnology offers a great deal of advantageous for the improvement of orphan crops, however, the most obvious challenge for its application to these species is related to the investments being presently made on these crops. There are also other concerns such as interventions being decentralized, participatory, multidisciplinary with open access to germplasm and information. In addition, if interventions need to be sustainable, then farmer's existing practices along with genetic and species diversity should be given importance rather than imposing completely new management methods [28]. Presently, biotechnology-based interventions do not always follow these guiding principles, more importantly when promotional activities for a par-

ticular orphan crop focus mainly on farmer's practices. In such cases, biotechnology can be applied only through centralized facilities. In fact, research is often undertaken in countries (e.g., in Europe and North America) other than where the underutilised crop is actually grown (e.g., in Africa, Asia or Latin America). The result of this geographic separation between researchers and users is that there is a danger that promotion activities become disconnected from actual practical needs and challenges [29]. For example, the application of biotechnology for an orphan crop may be related to international markets, while local markets may be the better option for improving the livelihoods of smallholder farmers [28].

Further, through centralization farmers may lose the rights over the genetic resources of underutilized plants that they once held [30]. If the biotechnological tool is used with commercial interests, then intellectual property rights protection to biotechnology processes and modifications is likely and may impede benefits to poor farmers [31, 32]. Unlike major crops that are distributed through formal supplier-to-farmer delivery systems, growers normally obtain underutilized species by informal farmer-to-farmer exchange of germplasm. Delivering new, centrally produced, varieties to a diffuse group of producers may therefore be more difficult for underutilized species than for major crops [33]. In addition, biotechnology approaches (mainly in case of industrial use), frequently involve the passage of germplasm through narrow genetic bottlenecks, and often time is associated with the intensification of farming systems, often resulting in losses in diversity, both at genetic and species level [34]. Since underutilised crops are often highly genetically variable and frequently occur in species rich environments, applying biotechnology approaches to their promotion could have particularly negative consequences for biodiversity, reducing the sustainability of farm ecosystems [35]. However, use of molecular markers to characterize the extent and degree of genetic variation present in traditionally cultivated crops in farmers' fields is more feasible, with a view to enhance the management and conservation of this diversity for current and future use.

Recommendations for better application of biotechnology to orphan crops

There are two ways in which biotechnological tools can be better applied to orphan species for the benefit of small-scale farmers in the future: (i) improving the partnerships between the stakeholders involved; and (ii) monitoring the utility of applications. Naylor *et al.* [15] indicated the importance of a wide range of partnerships during the promotion of underutilized crops. These include between: (a) high- and low-income countries, (b) institutions working on major and less-used crops, (c) the public and private sectors, (d) researchers and policy makers within and between low-income countries; and (e) the different stakeholders involved in germplasm and product value chains, from initial production of varieties through to consumer delivery of crop products. Better partnerships between high- and

low-income countries could help address the lack of capacity for underutilized species in molecular and conventional breeding programmes. Similarly, improved partnerships between institutions working on major and less-used crops could expose themselves to the needs of orphan crops and suggest applications based on approaches that have already been applied to major crops. Developing links between public and private sectors is particularly important to facilitate access at reduced cost to potentially useful proprietary biotechnologies and products [15, 32, 36]. NEPAD [31] indicated that good partnerships between researchers and policy makers in low-income countries is a must so that the benefits and risks of biotechnology are properly incorporated into national development strategies along with sharing of capacity and experiences. Most importantly, proper partnerships among different stakeholders in value chains should facilitate the equitable sharing of benefits, and ensure practical deployment of biotechnology products keeping the wider context of farming systems [29, 31]. For example, CGIAR's initiatives such as 'HarvestPlus' Challenge Program, which involves both major and minor crops, may offer particular opportunities. In recent years, a number of crops including minor crops are being sequenced and networks are being developed to share information and other resources on biotechnology use among countries, institutions and sectors, such as the African Agricultural Technology Foundation (<http://www.aatf-africa.org/>) and the Asia-Pacific Consortium on Agricultural Biotechnology (<http://www.apcoab.org/>). However, the emphasis on commercial application of GM is still under debate and biosafety regulations in many countries still needs to be defined. The perceived bias to GM causes mistrust, and hinders public acceptance and policy development on biotechnology deployment more widely [32, 37].

Conclusion

The application of biotechnology to orphan crops will become more feasible if significant practical benefits can be shown and this requires substantial monitoring of interventions and consider the magnitude of impacts. There are no good examples available for orphan crops applying comprehensive monitoring, although *ex ante* analysis of benefits to consumers through biofortification in the HarvestPlus challenge program has been carried out [38]. Other interesting examples for monitoring of minor crops include quinoa, finger millet, tef and sweet potato. To apply biotechnology approaches for the promotion of particular orphan crops, Dawson *et al.* [39] proposed the following points that could be considered:

- ⇒ Develop an analysis that explains how biotechnology interventions integrate with wider promotion activities.
- ⇒ Undertake an analysis of alternative scenarios to address constraints. If alternative approaches are efficient and cost effective, they may be considered over biotechnology approaches.
- ⇒ Undertake an analysis to describe the pathway from biotechnology outputs to the delivery of practical deployment.

- ⇒ Carry out an assessment of how the benefits of biotechnology interventions can be maximised for the poor, compared with other stakeholders, in value chains.
- ⇒ Define how farmers' intellectual property rights can be protected during the development of any variety or process.
- ⇒ Develop a set of indicators and a system for monitoring biotechnology interventions, by which the costs and benefits of activities can be fully assessed, especially costs and benefits for smallholder producers.

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TAILORING BIOINFORMATICS FOR THE GENETIC IMPROVEMENT OF ORPHAN CROPS

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Abstract

The advent and rapid advance of genomics technologies can enable plant breeders to design cost-effective and efficient breeding strategies by exploiting the ability of molecular plant breeding to increase favorable gene action and efficiency of selection. The unparalleled scientific progress in the fields of genomics and bioinformatics can successfully be harnessed to address the challenges of small holding farmers in developing countries. The power of molecular breeding extends to orphan crops with little DNA sequence information through comparative genomics methods. This growing abundance of genomic resources necessitates that plant scientists be equipped with fundamental genomic analysis tools for genomics assisted crop improvement. The role of bioinformatics as a pivotal tool for molecular breeding is growing steadily, particularly in identification of nucleotide variants associated with key traits. Basic bioinformatics skills to utilize selected public databases and integrated resources are outlined. Online resources for self-paced tutorials and other skill building opportunities were suggested. Particular emphasis was made to comparative genomics techniques to develop genomic resources for molecular breeding. Research institutions in developing nations should invest in bioinformatics capacity building in terms of human resources and infrastructure development in addition to forging strong partnerships with advanced research institutes.

Key words: molecular breeding, comparative genomics, database, bioinformatics, orphan crops, markers

Introduction

Agriculture, the main stay of Africa's economy and livelihood, is beset by a web of interacting and interrelated factors, exacerbated by climate change, posing a threat to food security which calls for innovative and effective breeding strategy. A number of recent reviews have provided detailed account of how the advent of genomics and its derived 'omics' technologies can enable plant breeders to design cost-effective and efficient breeding strategies by exploiting the ability of molecular plant breeding to increase favorable gene action and efficiency of selection among other things [15, 29]. The rapid accumulation of genomic data and the ensuing development of functional genomics techniques, tools, and databases ushered the era of molecular breeding as a new paradigm [51]. Numerous powerful molecular tools have been and are being developed to understand fundamental processes underlying key physiological traits desired for germplasm

enhancement [15]. A wide variety of markers have been developed and progressively improved for cost-effectiveness, efficiency, and increased throughput. Nucleotide variation in the forms of SNP and SSR have been broadly utilized to study genetic diversity and to genetically map traits of economic importance across a wide range of crops [1, 2, 16]. The unparalleled scientific progress in the fields of genomics and bioinformatics can successfully be harnessed to address the challenges of small holding farmers in developing countries where orphan crops are grown as staple food or cash crops. Given the meager agricultural input in developing countries genetic improvement is the most plausible option to raise crop productivity for the resource-poor farmers. The advent of new technologies in molecular biology and the parallel evolution of bio-computational tools offer broader opportunities for devising an efficient and effective breeding strategy. In order to extend the power of molecular breeding to orphan crops with little DNA sequence information, plant scientists should be equipped with fundamental genomic analysis tools including comparative genomics. This paper reviews selected bioinformatics tools, databases, and services suitable for plant biologists engaged in improvement of under-researched crops. An attempt has been made to provide a flavor of potential application of bioinformatics databases and tools for a novice molecular breeder in the developing country, taking into account the limited resources and infrastructure in most national agricultural research institutions. While focus is on orphan crops, breeders working on non-orphan crops such as maize, soybean, and rice are also urged to start applying these techniques in their breeding scheme with earnest.

Plant Genome Projects

The completion of genome sequences of the model plant *Arabidopsis* and the first crop plant, rice, heralded the dawn of the genomics era. Following these landmark achievement, the research community is aggressively taking on the challenges of integrating molecular breeding into the existing breeding programs [13]. Knowledge of the genome sequence of plants is of paramount importance in understanding the physiological processes underlying plant traits which can be manipulated to create desirable cultivar. The technology of genome sequencing has dramatically improved as evidenced by the steadily growing amount of genomic information and the completion of vast number of organisms [6, 7, 26]. In fact, with the current trend of rapid development of sequencing technology, it will not be too long before the genome sequence of all agricultural plants will be determined.

Current views on opportunities for tackling the challenge of food security vis-à-vis the increasing world population