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African Orphan Crops: Their Significance and Prospects for Improvement

In Africa and other developing regions, the vast bulk of food is produced and consumed locally.

HIGHLIGHTS:

Enhancing food security in Africa through science, technology and innovation

Role of orphan crops in enhancing and diversifying food production in Africa

The comparative genomics of orphan crops

Harnessing biotechnology for conservation and utilization of genetic diversity in orphan crops

Tailoring bioinformatics for the genetic improvement of orphan crops

The improvement of African orphan crops through TILLING

Importance of tissue culture for orphan crops

Significance of genome sequencing for African orphan crops: the case of tef

Progress in prevention of toxico-nutritional neuro-degenerations

African yam bean: a crop with security potentials for Africa

Who is who in African orphan crops research and development

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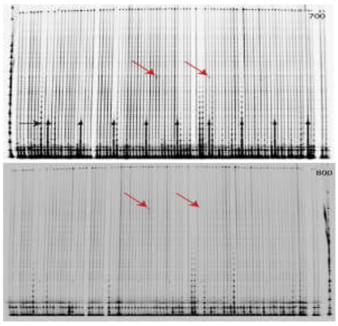

EDITORIAL

This special issue of the ATDF Journal is dedicated to the Orphan Crops of Africa. Although orphan crops are also known by different names (e.g. underutilized-, lost- or disadvantaged-crops), they all refer to a group of crops that are vital to the economy of developing countries due to their suitability to the agro-ecology and socio-economic conditions, but remain largely unimproved due to less attention has been given by the world scientific community. In order to boost food production in Africa emphasis should be given, not only to the major crops but also, to the orphan crops.

The topics covered in this issue range from germplam collection and conservation to the application of modern techniques (Bhattacharjee; Dominique & Daniel). Under '*Comparative Genomics*' Patterson and colleagues showed the relationships of different orphan crops at a molecular level. The same group led the international group of scientists to complete the sequence of sorghum genome (*Nature 457:551-556; 2009*). Sorghum is also another important crop of Africa that was until recently considered as an orphan crop. But due to concerted efforts made by the national and international institutes, significant number of cultivars with desirable agronomic traits reached the farming community. Gedil presents how bioinformatic tools can be applied to the orphan crops. Recently, some orphan crops researchers got an opportunity to implement modern improvement techniques that have proved to be efficient in the improvement of major crops of the world. Among these, tissue culture (Dubois) and TILLING (Esfeld & Tadele) are discussed in detail. In addition, genome sequencing has been recently completed for orphan crops such as cassava and in progress for tef (Plaza *et al.*). Lambein and colleagues report their efforts to remove toxic substances from the two orphan crops that are extremely tolerant to drought but cause neuro-degenerations when consumed without proper processing.

'Who is who in African orphan crops' presents the names and addresses of institutes directly or indirectly involved in orphan crops research and development (Tadele). Some key organizations might be missed from the list but the list provides useful information, at least, for orphan crops researchers who need to establish research partnerships and are seeking for grants.

On behalf of orphan crops researchers, I take this opportunity to thank the editors of ATDF Journal for giving the chance to the African Orphan Crops. In addition, I would like to thank all organizations who provide financial and technical supports for orphan crops research and development. Finally, my personal thanks to all contributors who managed to write the articles despite their other commitments.

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ENHANCING FOOD SECURITY IN AFRICA THROUGH SCIENCE, TECHNOLOGY AND INNOVATION

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Summary of UNCTAD Technology and Innovation Report 2010

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Abstract:

The report focuses on the challenges of improving agricultural performance in Africa and the role of technology and innovation in raising agricultural production and incomes of all farmers, including smallholder farms. The report argues that the main challenge is to strengthen the innovation capabilities of African agricultural systems as a means of addressing poverty, improving food security and achieving broader economic growth and development.

keywords: Technology, Innovation, Smallholder farmers, food security

Sub-Saharan Africa is the developing region most likely to miss the first Millennium Development Goal (MDG1), aiming to reduce by half extreme hunger and poverty by 2015. Per capita food production in least developed countries (among which African countries are over-represented) has declined continuously since the early 1970s, so that in 2003-2005 it was one-fifth lower than in 1970–1972. While Asia and Latin America have seen significant increases in their agricultural productivity over the last three decades, Africa's agricultural productivity has stagnated. This has created serious problems of food insecurity and has presented a major development challenge given that the agriculture sector forms the basis of many African economies and provides the largest source of employment and livelihoods for the vast majority of the continent's population. The core challenge that confronts Africa is one of promoting steady growth in agriculture in the dynamic context of economic transformation of countries. Agriculture will remain important for food security but at the same time, building productive capacities in agriculture and identifying multi-sectoral linkages between agriculture and other sectors will be important to support sustainable economic development of Africa. The onus therefore lies in the identification and support of processes and linkages that promote technological change, productivity increases and innovation.

Technological innovation is not all composed of radical discoveries, and much of what is relevant to African agriculture relates to the ways in which incremental improvements in processes, products, inputs, or equipments are needed to adapt existing technologies to the local environment in ways that enhance productivity and lower costs. The ability to adapt, therefore, is a significant step in technological empowerment, which over a period of time, can lead to the creation of knowledge generation capabilities amongst actors that are demand-driven rather than simply those that aim to replicate the successes of other regions of the world.

Declining agricultural productivity in many developing countries can be reversed through building what are called agricultural innovation systems that provide the enabling framework not only for the adoption of existing technologies and the development of new ones that are suited for African needs. Agricultural innovation systems denote the network of economic and non-economic actors, and the linkages amongst these actors enable technological, organizational and social learning of the kind needed to devise context-specific solutions. The dissemination of already existing technologies from outside could help this endeavour, but a major challenge relates to the ways and means in which innovation that is relevant to African agriculture could be promoted.

However, the ability of the agricultural innovation system to be able to access, use and diffuse knowledge embedded in agricultural technologies depends on the presence of an enabling framework that supports the emergence of technological capabilities by strengthening existing linkages, promoting new linkages and fostering inter-organisational learning that leads to capital accumulation and technical change. Such an enabling environment, by definition, is one that strengthens the absorptive capacity of local actors while protecting their interests through a policy framework that recognises their legal rights and privileges, linkages, socio-cultural norms and historical context. This report defines an enabling environment for technology and innovation in agriculture as one that provides the actors, skills, institutions and organizations required to promote the use, dissemination, diffusion and creation of knowledge into useful processes, products and services.

Creating an enabling environment for technology and innovation is an essential requirement to enable African countries to address the following constraints that impede their agricultural development:

Declining investment: most developing countries already had investment deficits in agriculture well before the onset of the current financial crisis in mid-2008. The long decades of neglect of the agricultural sector in the Africa region are partly a consequence of the policy of strict fiscal austerity imposed on African countries, which has severely curtailed state support of agriculture. The perception that investment has merely to do with the provision of agriculture research has exacerbated the situation further. This has resulted in poor rural infrastructure, low coverage of extension services, reduced provision of subsidies for inputs and finance for farmers, and reduced investment in research and development in the agricultural sector. As a result, farmers in Africa are now poorly equipped to deal with the new challenges that they face, which include climate change, desertification, competition from cheap imports, and highly concentrated global value chains dominating the world's commodity markets. Investing in activities that promote new forms of partnerships, use of local knowledge (including traditional agricultural knowledge), practices and preferences, as well as policy-driven demand-based approaches have been missing to promote the African response to its agricultural challenges.

Land tenure and credit access: access to credit is another fundamental institutional constraint that circumscribes the ability of African farmers to cope with the rising prices of land, seeds and other agricultural inputs. However, this clearly needs to be accompanied by an enabling framework that guarantees better physical and scientific infrastructure of relevance to African agriculture, and improved market access and demand forecasts. Guaranteed land tenure could be vital to accessing credit and investing in the medium and long-term productivity of the land.

A focus on small farmers: focussing on smallholder farmers has proven an effective means to contribute to a country's economic growth and food security. Smallholders make up over half the population in most developing countries and their farms are often efficiently run and enjoy significant growth potential. However, smallholder farms are diverse in terms of the challenges and limitations they face in the light of which adaptation of technologies and reconfiguration of supply chain roles and responsibilities will be critical to enable small-scale farmers to frame the issues of appropriate agricultural outputs and activities on their own terms. Their isolation makes them susceptible to both external and internal shocks, and also hinders resilient responses. A focus on smallholder farms is required to ensure that they are well networked into all available technical and institutional support mechanisms that is so critical for them to consolidate their activities.

Adapting to climate change: climate change is a global challenge with critical development implications. The negative impacts are especially severe in marginal lands. Some 300 million farmers in Africa live and work on marginal lands at increased risk of soil degradation, droughts, floods, storms, pests and erratic rainfall. Climate change technologies and innovations for mitigation and adaptation strategies are needed to accelerate the development, deployment, adoption, diffusion and transfer of environmentally sound technologies from developed to developing countries.

Bioenergy: energy is at the centre of the development challenge in many developing countries, with inadequate supply hindering capacities to expand production and improve human wellbeing. If properly managed, the high technical potential of bioenergy in regions such as sub-Saharan Africa could make a significant contribution to fighting poverty while also addressing climate change and expanding trade opportunities in sustainable energy products.

Structural policy reforms: the thirty-year legacy of structural adjustment and trade liberalization has turned Africa from a net food-exporting continent to one that predominantly imports. The food insecurity situation in Africa is better framed in terms of missed opportunities as a result of serious failings of development strategies. Africa's agricultural sector implemented programmes designed to eliminate price controls, privatize state farms and state-owned enterprises, abate taxes on agricultural exports, remove subsidies on fertilizer and other inputs and encourage competition in agricultural markets. The anticipation that these measures would encourage the private sector to move in and provide these services was not matched by reality. Long-standing policy failures must be reversed. Experience from the most recent crisis also shows that countries that specifically aim to achieve food security can cushion the blows from a cyclical world market. Future trade agreements must ensure that the space to apply such policies is preserved and, indeed, strengthened.

Building locally relevant research and innovation priorities: African agricultural research has not been weak, but it has lacked the right impetus to bridge ongoing research with product development initiatives. There has been a tendency to focus on applying international models of agricultural development without questioning their applicability to local circumstances. An accompanying attitude that looked down on regional research, as against international research (where the latter was considered to be far more superior), has been entrenched since colonial times. In reality, patterns of knowledge change are related to the increasing convergence in the different areas of science and technology, and indigenous capabilities of countries matter. The benefits attending to convergence include new organizational production structures, advances in communication apart from global trade. This calls for policies that help re-orient actors towards local sources of technology and learning, and address the negative perception towards local research.

Amongst options available, *international cooperation* can potentially be a strong factor in helping relevant new technologies be adopted, adapted and diffused throughout host economies. In particular, a handful of South-South cooperation models have already proven their worth as mechanisms for ensuring the right technological tools are made available to African farmers. So-called triangular cooperation, where a Northern neighbour signs on as a sponsor to South-South technology sharing efforts, has also shown promise as a model for the international diffusion of technologies.

It is important to realize that there are no quick fixes. This can be seen in the case of other developing countries which are now benefiting from public and private investments that were made into the development of agricultural technologies and innovation capacity since decades. Brazil, for example, has achieved its current leading position in tropical agriculture technology and increased agricultural productivity as a result of more than three decades of public and private investment in the development of technological packages tailored to its own soil and local agro-ecological conditions.

Amongst options available, international cooperation can potentially be a strong factor in helping relevant new technologies be adopted, adapted and diffused throughout host economies. In particular, a handful of South-South cooperation models have already proven their worth as mechanisms for ensuring the right technological tools are made available to African farmers. So-called triangular cooperation, where a Northern neighbour signs on as a sponsor to South-South technology sharing efforts, has also shown promise as a model for the international diffusion of technologies.

On this basis, when the new African Agriculture Revolution is eventually implemented, it is likely to be built on Africa's own indigenous technology and knowledge requirements, and the nutrition and food security needs of its people. Building capabilities for science, technology and innovation of relevance to local agriculture however, is the only path to achieve this.

Building innovation capabilities in African agriculture

Regardless of the theoretical viewpoints on the kind of approach best-suited to promoting agricultural development, the fundamental issue for a policy maker is how such an approach can be used to devise an agriculture development strategy. The innovation systems framework can be useful to help identify areas of weakness that could ideally be addressed through national policy action. A key issue is how to promote capabilities among African farmers and develop more effective innovation systems for agriculture at the national and sub-national levels.

An agricultural innovation systems approach is recommended as a policy instrument for African agriculture. Agriculture development strategies that incorporate the main components of the AIS approach can be transformed into a workable concept at the country level. The AIS approach depends on identifying the key actors and linkages that together strengthen knowledge flows and enable interactive learning that is important to build capabilities for agriculture. The linkages between these actors derive from two major sources: the policies and institutions (including the constitution, laws, rules, regulations and by-laws) are a very important to guide individual behaviour. Laws, rules and regulations are important offer specific incentives to individuals or groups to collaborate and engage in mutual learning. A second form of linkages derives from socio-political-historical attitudes and practices (that could stem from cultural norms) that dictate how or why individuals interact and what benefits they perceive from such interactions. The chapter has identified short-term and mid-term policy actions that are meant to guide policy makers to enact the AIS approach within their national contexts.

The characteristics of these systems may vary significantly between (and even within) countries, which makes country-specific analysis necessary, but there remain some common issues that affect many African countries to a greater or lesser extent. Common challenges include poor linkages between farmers and others in food value chains and the research and education systems, weak bridging institutions between the two (extension services, for example), inadequacies in infrastructure and financing mechanisms, and policy frameworks that do not provide adequate support for smallholder famers or may even create disincentives to technological development or innovation. Policy-makers need to ensure that national agricultural research systems involve farmers fully as partners, and gear research to solving the pressing production problems that they face. There is also a need for institutional innovations and different ways of organizing smallholders, linking them better to knowledge flows and to potential markets for their produce, and policy options to enable these have also been identified in the chapter. The specific policies needed at the national level will depend on existing capabilities and human and financial resources, the political, social and institutional contexts and agro-ecological conditions.

Adequate policy space is necessary to implement appropriate policies. Promoting the sharing of experiences and relevant knowledge flows internationally and at the national level provide a starting point. Designing effective public policies, however, requires adequately prepared policy-makers and may entail some degree of experimentation to find what works best in a specific situation.

There are other gains that flow from agriculture to non-agriculture based systems that will be important in the African context, and can accrue from building agriculture innovation systems. These include the flow of rural human capital as a result of increased rural spending on education (accruing from agricultural surpluses), the release of rural labour for industrial employment and enhanced foreign exchange earnings and increase in domestic savings, all of which are needed to enable the structural transformation of African economies.

Agriculture and national food security

Achieving food security means much more than simply producing more food. Without policies to improve poor consumers' access to food, policies that increase the availability of food only will not ensure national food security.

In Africa, improving the availability of and access to food demands strengthening local productivity and production. This is because, in Africa and other developing country regions, the vast bulk of food is produced and consumed locally. A lesson from the recent food crisis is that as food import prices rise and global stocks drop, the need to improve local production becomes more acute.

There are four main ways to improve local food production:

1. expanding arable land;
2. achieving higher levels of cropping intensity;
3. increasing yields; and
4. implementing agricultural policy reforms.

Increases in cropping intensity are, however, strongly dependent on increasing the availability of irrigation.

Yet the gains made through such strategies remain vulnerable to a variety of new challenges, particularly soil degradation due, among other factors, to climate change. Desertification is estimated to put the food security of one billion poor people at risk, particularly in dryland areas of Asia and sub-Saharan Africa.

Other important risks include the hollowing out of potentially productive rural communities caused by mass migration to the cities. While the explosive transfer of population from rural to urban settings can weaken the productive capacity of vulnerable agricultural sectors, experience suggests such trends can be slowed and even reversed by well designed policy interventions to improve the attractiveness of agriculture and rural livelihoods.

The failure of developed countries to recognize the negative impacts of their policy actions on food production and demand in the developing countries has in some instances resulted in suboptimal policy choices. To preserve policy coherence, renewed attention to agriculture must go hand-in-hand with the removal of farm and export subsidies for key commodities (cereals) in developed countries, to avoid depressing the prices that small farmers receive in developing nations.

Competition for the use of land from biofuels poses yet another new risk for food security, as acreage that might have been devoted to producing food for human consumption is diverted to the production of biofuel feedstocks. Evidence is mounting that competition over the use of land from biofuels has contributed strongly to the current food crisis. Competition for land to raise livestock and produce animal feed similarly tends to decrease the amount of food available locally for food consumers.

Challenges and opportunities to achieve food security

Financial resources are badly stretched in most African countries. Policy-makers need to invest in the most productive manner possible, putting resources into areas that are most likely to have a large impact on increasing smallholder productivity and improving national food security. Nevertheless, there is also a need for African countries to increase their investment in agricultural development. A combination of smart targeting of investment and greater overall levels of public support for agriculture is needed. Today, only a handful of African countries devote the agreed target of 10 per cent of GDP in public expenditure in agriculture.

Some research from India points to investment in rural roads, research and extension and education as the most effective investments to combat rural poverty, but other evidence suggests that sub-Saharan African countries should also pay close attention to irrigation and rural electrification.

Unfortunately, the international community has shown decreasing interest in support for African agriculture over the past 30 years. Support for agriculture as a proportion of total international development assistance to African countries has fallen by as much as two-thirds from its peak in the early 1980s, as a result of the shift towards structural adjustment lending with an emphasis on liberalization. This has left many African countries badly positioned to face the challenges posed by volatile prices for agricultural inputs and food products and the impacts of climate change.

There are strong structural impediments to replicating an Asian-style Green Revolution in Africa. The heterogeneity of staple crops, farming systems and the paucity of rural infrastructure make it clear that no mechanistic replication of the Green Revolution technology package is possible, or even desirable, in Africa. Specific African challenges and conditions, and the need to pursue sustainable agricultural development, mean that a truly African Green Revolution should be very different from the Asian Green Revolution of the 1960s and 1970s. The package of measures needed must also go beyond appropriate technology mixes and address other constraints to smallholder farmers building stronger technological and innovation capabilities.

For too long, African agriculture has lagged farther and farther behind other developing regions in nearly every measure of agricultural productivity and production. African production growth has tended to rely on unsustainable increases in the area under cultivation, while yields stagnate. A new agro-ecological approach, sometimes dubbed the Rainbow Revolution, is needed to reverse these trends. The interventions needed range from the tried-and-true (increases in land under irrigation) to the use of appropriate innovations to radically increase productivity in African agriculture.

What has become clear, however, is that no intervention can hope to succeed unless African smallholder farmers are brought into the process. Their ability to participate, however, depends on much more than what happens on the farm. Which brings us to the next set of concerns: the need to understand and radically overhaul the enabling environment surrounding food production and extending outwards into financing agricultural investment, agricultural research, transport and education systems, distribution and storage: the panoply of food-related activities that take place outside the farm itself that connect consumers with the food they need.

Transfer and diffusion of agricultural technology

Acquiring and adapting technologies new to the local agro-ecological system, either from abroad or from local sources (research institutes or universities, for example) is a key part of any serious strategy for achieving food security. Selection of technologies appropriate to the conditions within the host food system is crucial . State-of-the-art, high-tech solutions may not always be the most appropriate for the needs of smallholder farmers. Adopting a pragmatic mix of technologies (low-, medium- and high-tech) that best meets their needs is the ideal.

A balanced technology acquisition approach must balance the contrasting challenges of technology selection, adaptation and diffusion. It is not enough for a technique to be technically sound, it must also be adapted to suit the specific conditions found on the ground, and be made affordable and attractive enough to smallholder farmers to achieve wide diffusion. Models of public-private partnership that make not only public institutes but also for-profit enterprises into stakeholders for the diffusion model can be valuable in building a self-sustaining momentum behind dissemination efforts. Such a model stands the best chance of being demand-driven – succeeding because farmers demand its continuation, rather than due to a top-down bureaucratic decision.

International cooperation can also be a strong factor in helping relevant new technologies be adopted, adapted and diffused throughout host economies. In particular, a handful of South-South cooperation models have already proven their worth as mechanisms for ensuring the right technological tools are made available to African farmers. So-called triangular cooperation, where a Northern neighbour signs on as a sponsor to South-South technology-sharing efforts, has also shown promise as a model for the international diffusion of technologies.

Successful adoption and mastery of new technologies by smallholders requires adequate absorptive capacity on their part. Successful technology transfer is not necessarily easy to achieve and entails some cost on the part of the farmer to learn the technology. Still, the returns from successful technology transfer can be very large.

Technology mixes for small-scale farming

A wide range of production and post-harvest technologies are currently available for a wider range of crops than during those which were the subject of the Green Revolution. There is also a better (but still incomplete) understanding of the context and factors that determine technology adoption, diffusion and impact. As research is moving towards ‘precision agriculture’, efforts to build an enabling environment need to incorporate an understanding of contextual factors, and should incorporate efforts to utilize farmers' innovative capacity.

Local agro-ecological conditions play a vital role in shaping the overall technology acquisition strategy, which in turn requires close relationships with local grassroots initiatives coordinated by extension services, NGOs and private enterprise at the district level. Issues of sustainability (particularly in terms of water and energy use) are a major consideration in the choice of technologies, and must take centre-stage when discussing all aspects of technology adoption.

The urgent need for improvement underlines the importance of adopting and diffusing existing technologies as a matter of priority, and ensuring that public and private partnerships work toward the development, dissemination and adoption of technologies. Opportunities to reduce crop losses can be realized when farmers, processors and traders have access to reliable information and appropriate techniques and technologies to improve quality, throughput, labour and time efficiencies.

Technology needs assessments should identify gaps in technology, infrastructure or information in order to better facilitate the deployment and use of technologies that reduce post-harvest losses of smallholder rural produce.

In the future, climate change will render the need for new agricultural technologies more important for strongly affected parts of Africa. There is a need for: (a) increased agricultural R&D that is relevant to African agro-ecological conditions; (b) much stronger innovation capabilities among African institutions and smallholder farmers; (c) promotion of new techniques and technologies; and (d) training end-users in their application and improvement.

To download the report, go to http://www.unctad.org/Templates/webflyer.asp?docid=13234&intItemID=2068&lang=1

ROLE OF ORPHAN CROPS IN ENHANCING AND DIVERSIFYING FOOD PRODUCTION IN AFRICA

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Abstract

Orphan- or understudied-crops are considered as the major staple food crops in many developing countries because of their particular role in food security, nutrition, and income generation to resource-poor farmers and consumers. Like other crops, orphan crops are also categorized under cereals, legumes, root crops, and fruit crops. Orphan crops are in general more adapted to the extreme soil and climatic conditions prevalent in Africa than the major crops of the world. However, due to lack of genetic improvement, orphan crops produce inferior yield in terms of both quality and quantity. The major bottlenecks affecting the productivity of orphan crops are low yield [for example, in finger millet (*Eleusine coracana*) and tef (*Eragrostis tef*)], poor in nutrition [cassava (*Manihot esculenta*), and enset (*Ensete ventricosum*)], and production of toxic substances [cassava and grass pea (*Lathyrus sativus*)]. Environmental factors such as drought, soil acidity and salinity, pests, diseases and weeds also contribute to large losses in yield. Hence, an agricultural revolution is required to increase food production for under-researched crops in order to feed the ever increasing population in Africa. Hence, modern crop breeding techniques developed for major crops of the world also need to be applied to orphan crops. The application of these techniques to the understudied crops is vital in order to boost productivity and feed the largely underfed and malnourished population of Africa.

Key words: orphan crops, understudied crops, Africa, agricultural revolution

Description of orphan crops

Different names are used interchangeably to describe the range of orphan crops. Some of these names are, underutilized crops [1], lost crops of Africa [2, 3, 4], minor crops [5], neglected crops [6], and crops for the future [7].

According to Wikipedia three criteria must meet in order for the plant to be considered underutilized or orphan crop, i) proven food or energy value, ii) the plant has been widely cultivated in the past, or the plant is currently cultivated, in a limited geographical area, and iii) currently cultivated less than other comparable plants [8]. According to Naylor *et al.* [9] twenty-seven orphan crops within developing countries are annually grown on about 250 million ha of land. **Table 1** shows the list of some orphan crops and their useful agronomic traits.

Although orphan crops are many in number, brief description is given below for the most important ones interms of the area they are grown and/or population they feed. These include cereals (e.g. millet, tef, fonio), legumes (cowpea, bambara groundnut, grass pea), and root crops (cassava, yam, enset).

Finger millet (*Eleusine coracana*) is the most important small millet in the tropics and is cultivated in more than 25 countries in Africa and Asia predominantly as a staple food grain [25]. The plant is tolerant to drought. The seed of finger millet contains valuable amino acid called methionine [2], which is lacking in the diets of hundreds of millions of the poor who live on starchy staples such as cassava. Finger millet is also a popular food among diabetic patients because of its slow digestion.

Tef (*Eragrostis tef)* is grown annually on over 2.5 million hectares of land mainly in Ethiopia. The plant is tolerant to abiotic stresses especially to poorly drained soils where other crops such as maize and wheat could not withstand. In addition, the seeds of tef produce healthy food because they do not contain gluten for which large portion of the population are allergic [24]. Unlike other cereals, the seeds of tef can be stored easily without losing viability under local storage conditions, since it is not attacked by storage pests [23].

Fonio (Acha, *Digitaria exilis* and *Digitaria iburua*) is an indigenous West African crop. It is grown mainly on small farms for home consumption. Fonio is not only tolerant to drought but also a very fast maturing crop. It is also nutritious because it is rich in methionine and cystine, the two amino acids vital to human health and deficient in major cereals such as wheat, rice and maize [26].

Cowpea (*Vigna unguiculata*) is a leguminous crop annually grown on about 10 million hectares of land mainly in Africa. The crop is tolerant to drought and heat. It also performs better than many other crops on sandy soils with low level of organic matter and phosphorus [27]. Since cowpea has quick growth and rapid ground cover, it is a useful crop in controlling erosion [28].

Bambara groundnut (*Vigna subterranea*) is an annual legume crop grown for human consumption. The seeds of bambara groundnut are known as a complete food because they contain sufficient quantities of protein, carbohydrate and fat. The average composition of the seed is 63 percent carbohydrate, 19 percent protein, and 6.5 percent oil [3].

Grass pea (*Lathyrus sativus*) is another leguminous plant commonly grown for human consumption in Asia and Africa. The plant is extremely tolerant to drought and is considered as an insurance crop since it produces reliable yields when all other crops fail. Like other grain legumes grass pea is a source of protein particularly for resource poor farmers and consumers. However, the seeds of grass pea contain a neuron-toxic substance called ODAP [β-N-Oxalyl-L-α, β-diaminopropanoic acid [29].

Cassava (manioc; *Manihot esculenta)* is staple food for about a billion people [30]. The plant is tolerant to drought and also performs better than other crops on soils with poor nutrients. The major problems related to cassava are its very low protein content and the roots contain poisonous compounds called cyanogenic glycosides (CG) which liberate cyanide [31]. Konzo is a paralytic disease associated with consumption of insufficiently processed cassava.

Yam (*Dioscorea sp*) represents different species under genera Dioscorea. It is grown on about 5 million hectares of land world-wide [32] and staple food in west Africa. The roots are the edible part and looks like sweet potato (*Ipomoea batatas*) although they are not taxonomically related.

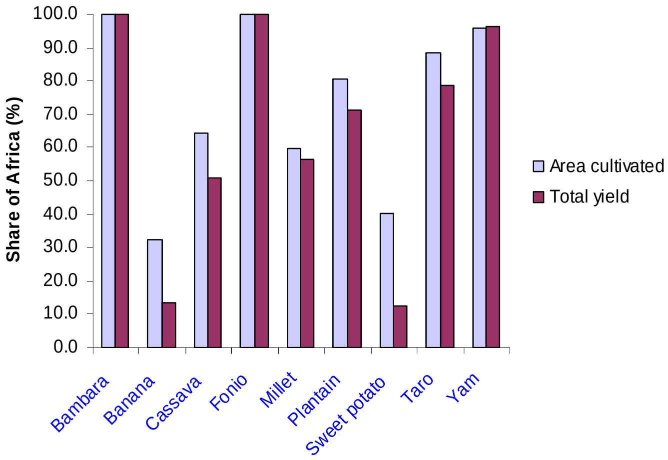
Enset (*Ensete ventricosum*) is commonly known as 'false banana' for its close resemblance to the domesticated banana plant. Unlike banana where the fruit is consumed, in enset the pseudostem and the underground corm are the edible parts. Enset is the major food for over 10 million people in densely populated regions of Ethiopia. The plant is considered as an extremely drought tolerant and adapts to different soil types [33]. Since enset flour is rich in starch but not in other essential nutrients enset-based diets need heavy supplementation.

Role of orphan crops in African economy and socio-economic conditions

Orphan crops play particular role in food security, nutrition, and income generation to resource-poor farmers and consumers in developing countries. These crops perform better than major crops of the world under extreme soil and climatic conditions prevalent in developing world particularly in Africa. Most of African orphan crops including finger millet and bambara groundnut are extremely drought tolerant while some others withstand water-logging for longer period than the major crops of the world (**Table 1**).

In general, orphan crops are extensively grown in Africa. The total global production of three orphan crops, namely bambara groundnut, fonio and yam comes from Africa (**Fig 1**). Africa also devotes large area of land for cassava, millet, plantain and taro cultivation. However, the total acreage and total production are not comparable for last four crops in Africa. For example, regarding cassava, Africa contributes for about 65 percent of the global area but produces only 50 percent of the total world production. This might be due to the use of unimproved planting materials and poor management practices.

Figure 1. Share of Africa in the world acreage and production for selected orphan crops in 2008.



Source: Adapted from FAOSAT [34].

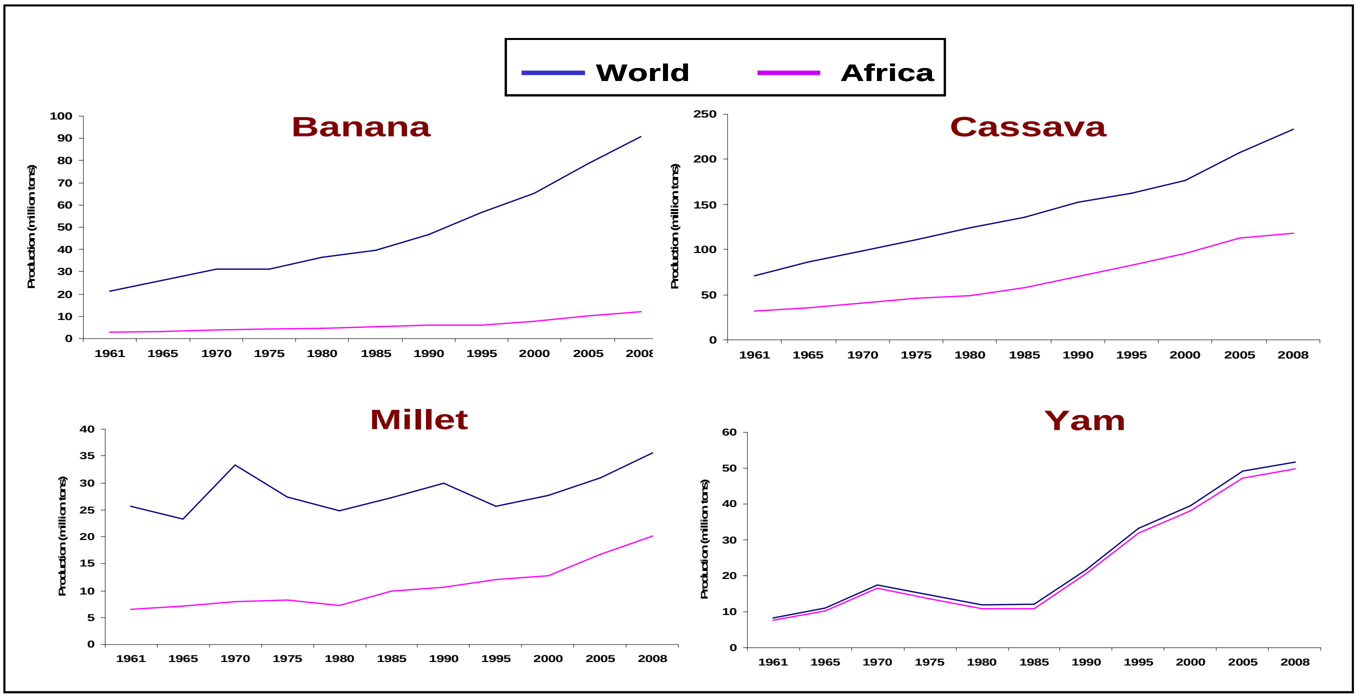
Fig 2 shows the trend of total production for four orphan crops in Africa as compared to the world production. Except for banana, the production of three other crops (cassava, millet and yam) has steadily increased over time in Africa. Yam is exclusively grown in Africa and the production of this crop has tripled in the last 25 years (**Fig 2**).

Orphan crops are also compatible to the agro-ecology and socio-economic conditions of the continent. However, when these crops are replaced by other newer crops for the locality, some problems were reported. The best example is from the study made in the Northwestern Ethiopia where the incidence of malaria has been elevated in the years when the cultivation of exotic crops specifically maize was increased at the expense of indigenous or orphan crops [36, 37, 38]. Malaria is the major health problem in the world particularly in Africa. In the year 2006, there were an estimated 247 million malaria cases causing nearly a million deaths, mostly of children under 5 years [39]. The study by McCann and colleagues [36, 37, 38] indicated that the pollen from maize facilitates optimum conditions for mosquito breeding. Mosquitoes carry *Plasmodium* parasites, the causal agent for malaria. Larvae of the mosquito had a survival rate of 93 percent when it fed on maize pollen, as opposed to a survival rate of about 13 percent when it fed on other possible food sources. As a result, the cumulative incidence of malaria in high maize cultivation areas was 9.5 times higher than in areas with less maize [36].

Limitations or negative characteristics of orphan crops

Although orphan crops perform better than major crop under extreme environmental conditions and fit to the

Figure 2 The trend of production for four orphan crops from 1961 to 2008.



Source: Adapted from FAOSAT [35].

socio-economic conditions of the developing countries especially in Africa, they have also a number of limitations. The major bottleneck is related to the little genetic investigation made on these crops. Almost all orphan crops are studied by poorly funded researchers based in the developing nations where resources for conducting research are limiting. The majority of these researchers have little chance to establish partnerships with the scientific community especially with those in the developed countries. Some of the outstanding bottlenecks related to orphan crops are indicated below:

- **Poor grain yield:** most orphan crops particularly cereals such as tef, millet and fonio produce extremely low seed yield.
- **Poor in nutrient content:** although root and tuber crops such as cassava and enset produce high yield, the products are largely starchy materials that are deficient in other essential nutrients particularly in protein. Although these crops are staple food crops for large number of Africans, supplementation with other nutrients is required.
- **Unfavorable agronomic characters**: Some of the negative features associated with the African rice (*Oryza glaberrima)* unlike the Asian rice (O. *sativa) are rapid shattering of the* seeds, difficulty of milling the grain, and lower seed yield (Linares 2002).
- **Abiotic stresses:** Since most fertile lands are used to grow other crops than the orphan crops, the productivity of orphan crops under the less fertile and moisture deficit soils is extremely low.
- **Hazardous or toxic products:** The following orphan crops produce a variety of toxic substances that affect the health of human.

⇒ **Cassava.** The roots of cassava contain poisonous compounds called cyanogenic glycosides (CG) which liberate cyanide [31]. Konzo is a paralytic disease associated with consumption of insufficiently processed cassava.

⇒ **Hyacinth bean** *(Lablab purpureus).* The pods and seed of hyacinth bean can be poisonous due to high concentrations of cyanogenic glycosides and can only be eaten after prolonged boiling [1].

⇒ **African yam bean** *(Sphenostylis stenocarpa).* The seeds of African yam bean contain anti-nutritional factors such as cyanogenic glycosides and trypsin inhibitors. Cooking is required to reduce toxins to safe levels, though this also decreases the level of nutrients in seed [1].

⇒ **Grass pea.** the seeds of grass pea contain a neuron-toxic substance called ODAP [β-N-Oxalyl-L-α, β-diaminopropanoic acid [29]. ODAP is the cause of the disease known as neurolathyrism, a neurodegenerative disease that causes paralysis of the lower body. Serious neurolathyrism epidemics have been reported during famines when grass pea is the only food source [40].

Genetic improvement of orphan crops: lessons from major crops

Crop production could be increased by either expanding the arable area or through intensification, i.e., using improved seed, fertilizer, fungicides, herbicides, irrigation, etc. According to Food and Agriculture Organization, agricultural intensification represents about 80 percent of future increases in crop production in developing countries [41]. Based on this goal, crop breeders are focusing towards achieving improved cultivars that produce higher yields and at the same time tolerate to the sub-optimal soil and climatic conditions.

Among plant characters or traits that contributed for higher productivity in the last century, those which alter the architecture of the plant rank first. Architectural changes include alteration in branching pattern and reduction in plant height. The major achievement of Green Revolution in 1960's was due to the introduction of semi-dwarf crop varieties of wheat and rice along with proper crop production packages. These broadly adapted semi-dwarf cultivars were responding to fertilizer application; which led to tremendous increase in productivity. Currently, a number of genes affecting plant height are identified from major cereal crops including wheat, rice and maize [42]. According to the International Food Policy Research Institute, Green Revolution represented the successful adaptation and transfer of scientific revolution in agriculture [43]. However, since this agricultural revolution did not occur in Africa, crop productivity remains very low.

Modern improvement techniques are not yet employed in orphan crops. Breeders of orphan crops are mostly dependent on the conventional techniques such as selection and hybridization. Only limited numbers of breeders implement modern techniques such as marker-assisted breeding and transgenics. Genomic information such as whole-genome sequencing are not yet available for orphan crops. In order to feed the ever-increasing population of Africa, agricultural revolution is needed to boost productivity of orphan crops through the implementation of modern technologies proved to be effective for major crops of the world.

Application of modern improvement techniques to the orphan crops

A number of molecular markers are implemented in modern plant breeding. These include Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNAs (RAPDs), Amplified Fragment Length Polymorphisms (AFLPs) and microsatellites (Simple Sequence Repeats, SSR). Marker assisted selection (MAS) is the identification of DNA sequences located near genes that can be tracked to breed for traits that are difficult to observe. According to Collard and Mackill [44] the following factors should be considered before selecting what type of DNA marker to be used in MAS: reliability; quantity and quality of DNA required; technical procedure for marker assay; level of polymorphism; and cost. Comparative mapping studies have revealed that the genomes of plant species within families are conserved for chromosomal regions [45]. Hence, orthologous genes from orphan crops could be identified and isolated based on information from major crops.

Conventional breeding technologies including selection, hybridization and mutation breeding are all considered as non-transgenic methods. From modern techniques, marker-assisted breeding and TILLING are also non-transgenic. TILLING (Targeting Induced Local Lesion IN Genomes) is a high-throughput and low cost method for the discovery of induced mutations. Transgenic and a modified form known as cisgenesis are widely applied to major crops such as rice and maize. Only few orphan crops have so far benefited from the techniques. Transgenic is considered as other advancement towards boosting crop yields and improving nutritional quality of crops. Due to high adoption rate, the global area under transgenic crops is tremendously increased from just 1.7 million ha in 1996 to about 134 million ha in 2009 [46].

Future perspectives and recommendations

In order to boost productivity and diversify the food system in Africa orphan crops should be given due attention. The following points need to be considered in order to promote orphan crops research:

- Apply modern improvement techniques to orphan crops research. These include MAS, TILLING, tissue culture and transgenic techniques. The goals and techniques to be employed might vary for different orphan crops. However, the majority of research on orphan crops focuses on four areas, i) improving productivity per unit area, ii) breeding for tolerance against biotic and abiotic stresses, iii) enhancing nutritional quality through biofortification, and iv) removing toxic substances from some plant species.

- Financial and technical supports for researchers and institutions involved in orphan crops research since research on these crops are mostly underfunded and dependent on locally available meagre resources. These supports could be invested in training African scientists and developing infrastructure for African research institutes.

- Establish partnerships with public and private institutions. The partnerships could be made within and between research institutes, universities and private organizations.

- Create a network of orphan crops researchers at different levels: national, regional and international. The network will be an effective information exchange mechanism either among orphan crops researchers or with those working on major crops. This can be facilitated, for example, by forming internet discussion forum.

- Organize conferences, workshops or trainings related to orphan crops research and development.

The First International Conference on African Orphan Crops was held in September 2007, in Bern, Switzerland in order, i) to address the major crop productivity problems related to orphan crops in Africa; ii) to propose the strategy of implementing modern techniques to orphan crops; and iii) to discuss the prospects and feasibility of modern crop biotechnology in African agriculture in general and orphan crops in particular through round-table discussions involving prominent scientists. About 80 researchers from four continents participated in the conference. The Proceedings of the conference has been recently published [47]. The second conference on African orphan crops is expected to take place in Africa in near future.

Conclusion

Orphan- or Understudied-crops provide food for resource poor farmers and consumers in Africa. They also grow under extreme environmental conditions, many of them poorly suited to major crops of the world. Since Green Revolution did not occur in Africa, the continent did not benefit from the positive effects of this agricultural revolution that boosted the productivity of food crops in other parts of the world. The next Green Revolution for Africa needs to also include these locally adapted crops that are mostly known as orphan- or understudied-crops. Although these crops are largely unimproved, the implementation of modern improvement techniques on these crops has many advantages.

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Table 1. Major orphan crops of Africa and their important traits

Common Name	Botanical name	Other names	Type of crop	Country or region of importance in	Important trait	Reference
African eggplant	*Solanum aethiopicum*	Mock Tomato, Ethiopian eggplant	Leafy vegetable	All regions	High yielding	[3]
African rice	*Oryza glaberrima*		cereal	Western	resistant to diseases and pests	[10]
African yam bean	*Sphenostylis stenocarpa*		Root crop	All regions	High protein content	[3]
Amaranth	*Amaranthus spp.*		Leafy vegetable	All regions	Fast growing	[3]
Bambara groundnut	*Vigna subterranea*		legume	All regions	Rich in protein, drought tolerant	[3]
Banana	Musa spp.		fruit	All regions	High yield	[11]
Baobab	*Adansonia digitata*		Leafy vegetable/fruit	All regions	Drought tolerant	[3]
Barbados cherry	*Malpighia glabra*		fruit	All regions	Rich in vitamin	[1]
Cassava	*Manihot esculentum*	manioc	Root crop	All regions	Drought tolerant	[13]

Common Name	Botanical name	Other names	Type of crop	Country or region of importance in Africa	Important trait	Reference
Celosia	*Celosia argentea*		Leafy vegetable	Western	High productivity	[3,12]
Chickpea	*Cicer arietinum*		legume	Southern & Eastern	Protein source	[14]
Cowpea	*Vigna unguiculata*		legume	All regions	Drought tolerant	[3]
Dika	*Irvingia gabonensis, I. wombolu*		fruit	western	oil-rich	[3]
Enset	*Ensete ventricosum*		Trunk/root	Ethiopia	Drought tolerant	[15]
Ethiopian Mustard	*Brassica carinata*	Ethiopian kale, gomen zer	Leafy vegetable & oil crop	All egions	Resistant to blackleg pathogen	[16]
Finger millet	*Eleusine coracana*	African millet, Ragi	cereal	All regions	Rich in iron, protein; low in glycaemic index	[1, 12]
Fonio	*Digitaria exilis*	Acha	cereal	Western	Fast maturing	[2,12]
Foxtail millet	*Setaria italica*	*Italian millet*	cereal		Drought tolerant	[17]
Grass pea	*Lathyrus sativus*	Indian vetch, guaya	legume	Eastern	Extremely drought tolerant	[18]
Kodo millet	*Paspalum scrobiculatum*		cereal	western	tolerant to flooding	[17]
Little millet	*Panicum sumatrense*		cereal		Drought tolerant	[17]
Noug	*Guizotia abyssinica*	Niger seed	oil seed	Eastern & southern	High oil content	[19]
Okra	*Abelmoschus esculentus*		Leafy vegetable	West Africa	Tolerant to biotic stresses, fast growing	[20]
Pearl millet	*Pennisetum glaucum*		cereal	All regions	Drought tolerant	[21]
Plantain	Musa spp.		fruit	Eastern & western		[11]
Proso millet	*Panicum miliaceum*	Common millet	cereal		Drought tolerant	[17]
Quinoa	*Chenopodium quinoa*		cereal		High in protein content	[1]
Sesame	*Sesamum indicum*		Oil seed	All regions	oxidatively stable oil	[1]
Sweet potato	*Ipomoea batatas*		Root	All regions	rich in riboflavin and calcium	[1]
Tamarind	*Tamarindus indica*		fruit		Long storage time	[4]
Taro	*Colocasia esculenta*		Root and leaf vegetable	western		[22]
Tef	*Eragrostis tef*	teff	cereal	Horn of Africa	Tolerant to abiotic stresses; free of gluten	[23,24]
Vernonia	*Vernonia galamensis*	ironweed	industrial oilseed	East Africa	High oil content	[12]
Yam	*Dioscorea spp*		Root crop	Western and Eastern	Drought tolerant	[12]

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THE COMPARATIVE GENOMICS OF ORPHAN CROPS

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Abstract

The common ancestry of all flowering plants, reflected in the DNA sequences of those which have been sequenced to date, provides opportunities for 'translational biology' -- leveraging information and tools from major crops and botanical models in the study and improvement of many 'orphan crops' that are understudied at the genomic level although they are important sources of food , medicine, or other ecosystem services. We discuss some general approaches for reaping the benefits of translational genomics in orphan crops and offer a small sampling of examples of orphan crops that might benefit from this approach, also noting limitations of the translational approach in understanding unique features of particular plants. Translational genomics offers opportunities for many mutually-fruitful partnerships between African and non-African scientists, requiring ethical and responsible actions from both sides which acknowledge the global forces at play in the arena within which scientists practice.

Keywords: domestication, Nikolai Vavilov, DNA sequence, genetic map, translational genomics, medicine

Introduction

Flowering plants, known to botanists as angiosperms, are the Earth's dominant vegetation, sustaining humanity by providing 'ecosystem services' including oxygen, food, feed, fiber, fuel, medicines, spirits, erosion and flooding control, soil regeneration, urban cooling and green-space, wildlife habitat, and other benefits. Tracing to a common ancestor thought to have existed 140-180 million years ago [1, 2], about 250,000-400,000 angiosperm species are thought to exist [3, 4, 5], and a remarkable average of 2,350 new ones are discovered each year [6].

A tiny subset of about 200 angiosperms have become 'domesticated', over about the past 10,000 years literally co-evolving with humans to such a great degree that " changes in the (crop) population's genotypes ... makes them more useful ... and better adapted to human intervention" [7]. Domestication and ongoing improvement of crop plants ranks among the greatest of human achievements, permitting small numbers of people to feed much larger numbers, and freeing the remainder to pursue science and technology, arts and culture [8] Even after several centuries of progress, modern plant breeding is estimated to offer a 35% rate of return on public research investments [9].

Although often thought of as a Neolithic activity, crop domestication and improvement is an ongoing process driven by changing human needs and priorities, and constraints on agriculture. In particular, growing attention to the needs of Africa is re-awakening the merits of further improving native plants already reasonably well suited to local cultivation, and which have been subjected to degrees of domestication ranging from none to advanced. Even those African crops that have received some scientific plant breeding lag behind other leading crops -- for example, yields of sorghum, a staple for large populations in the African Sahel that is used for feed or ethanol (fuel) production on other continents, only gained a total of 6% from 1961-1963 to 1999-2001[10, 11], much less than maize, rice, and wheat and also far outstripped by population growth.

The sequencing and detailed functional analysis of the genomes of a few select botanical models opens new doors into comparative biology of flowering plants, with especially great potential benefits for improvement of many 'orphan crops' that are under-studied at the genomic level although they feed large populations already [12] and could feed many more people, in some cases with less inputs and at less cost than established crops.

Many orphan crops are also a first line of defense against illness. In South Africa, it is estimated that 60% of the indigenous people consult a traditional healer (sangoma) as a first attempt to get medical attention for a variety of illnesses [14]. Given that more than 50% of all drugs in clinical use in the world are derived from natural products or their derivatives [15], it is alarming that very little breeding or development of these plants has taken place even in developing countries, although a substantial part of their population are highly dependent on these genetic resources for basic healthcare. For example, *Artemisia afra* (wildeals in Afrikaans, lengana in Setswana) is widely used in South Africa for the treatment of a variety of ailments including, coughs, colds, influenza and also fever and malaria [15]. The isolation of the well known anti-malaria drug artemisinin from the Chinese equivalent *Artemisia annua* highlights the potential application of these plants in agricultural biotechnology [16]. Encouragingly, South Africa's Department of Science and Technology has adopted the 'farmer to pharma' strategy which recommends the cultivation of genetically engineered crops for the production of biopharmaceuticals [17]. Artimisin, could very well be one such biopharmaceutical.

Among 27 orphan crops collectively planted to 250 million ha/yr and yielding US$100 billion/yr farm gate value in the developing world, only 4 (barley, sorghum, cassava, and sunflower) had appreciable numbers of sequences (>10,000) in Genbank as of 11 Feb 2005 [12]. Pearl millet and tef are prime examples of such orphan crops having utmost importance in feeding millions of people [18a, 18b], yet with limited resources in Genbank. Sorghum recently became the first of these, and indeed the first plant of African origin, to have its genome fully sequenced [19]. Efficient methods to leverage genomic knowledge for botanical models in the study and improvement of orphan crops will play a central role in translation of genomic discovery research into increased agricultural development of indigenous medicinal and food crops by developing countries.

Shared ancestry of all flowering plants in the common language of DNA

Nearly a century ago, Nikolai Vavilov suggested an underlying commonality to exist in the hereditary information of diverse crop plants [20]. Today, based on many different lines of evidence we believe that all flowering plants trace to a common ancestor thought to have existed 140-180 million years ago [1, 2], with most modern lineages of flowering plants having been established by the mid-late Cretaceous period [21, 22]. While Vavilov's observations were based on similarities in the appearance (phenotype) of different plants, the transition to DNA-based plant genetics about 25 years ago has revealed commonality among the hereditary information of diverse crop (and other) plants at three levels:

i) Genetic and/or physical mapping. Measuring recombination and/or physical quantity of DNA between reference 'DNA markers' along the chromosomes of different species, shows common DNA markers to exhibit parallels in chromosomal affinities ('synteny') and orders along the chromosomes ('collinearity'). Very strong parallels in gene arrangement are generally found among different species within common genera (for example *Solanum tuberosum*, potato and *Solanum lycopersicon*, tomato, thought to be separated by only a few million years of evolution [23]); lesser but still clear parallels are common among divergent genera within a taxonomic family (for example, *Sorghum bicolor*, sorghum, and *Oryza sativa*, rice, members of the Poaceae family that are thought to be separated by ~50 million years of evolution [19]); and 'islands' of non-random similarity remain discernible even among clades such as monocots and eudicots that diverged early in angiosperm evolution [24, 25]. These parallels can be obscured to varying degrees in different lineages by polyploidy and associated gene loss, by structural rearrangement of chromosomes, and by duplication and/or transposition (movement) of individual genes or small groups of genes.

ii) Whole-genome sequences. Once a daunting task, requiring an international consortium and many tens of millions of dollars to sequence the first angiosperm genome [26], as of this writing primary descriptions of ten angiosperm genome sequences have been published, including the eudicots *Arabidopsis thaliana* [26], *Populus trichocarpa* (poplar, or black cottonwood) [27], *Vitis vinifera* (grapevine) [28, 29], *Carica papaya* (papaya) [30], *Cucumis sativus* [31] and *Glycine max* (soybean) [32]; and the monocots *Oryza sativa* (rice, including each of two subspecies) [33, 34, 35, 36], *Sorghum bicolor* (sorghum) [19], *Zea mays* (maize) [37], and *Brachypodium distachyon* [38]. Primary descriptions and/or sequencing are in progress for many more. Growing evidence shows that cytologically-distinguishable domains of chromosomes classically referred to as 'euchromatin' and 'heterochromatin' are also distinctive in their DNA content and evolutionary histories. Euchromatin is relatively rich in genes and DNA transposons, and relatively poor in rapidly-evolving elements such as retrotransposons and other repetitive DNA such as centromere-associated elements. As with genetic and/or physical maps, parallels among genomes in euchromatin range from near-identity of gene arrangements in the respective subspecies of rice to 'islands' of non-random similarity between monocots and eudicots. In contrast, heterochromatin is gene-poor, rich in repetitive DNA (indeed, accounting for most of the difference in physical size of some genomes) and is rapidly evolving in both structure and individual DNA elements, with little discernible synteny and/or colinearity among taxa [39]. Despite its rapid evolution in DNA content and order, the physical location of the heterochromatic domains of genomes appears to remain approximately constant for tens of millions of years [19, 32, 39]. Because heterochromatin is largely recalcitrant to recombination, it accounts for very little of genetic maps, often being reflected as clusters of DNA markers that are poorly resolved, although they may be physically very far apart.

iii) Individual genes and their sequences. Most flowering plants share most of their basic gene sets with one another. A recent comparison of Arabidopsis, poplar, grape, and papaya yielded estimates of 'ancestral gene number' varying from 10149 for *Carica* to 13043 for *Populus*, a range very similar to the 12,000-14,000 from a previous estimate based on an independent gene birth model [40]. Another independent analysis comparing OrthoMCL-defined gene families (identified based on overall conservation of inferred protein sequence – [41]) in an independent genome (sorghum), to those of *Arabidopsis*, rice and poplar, likewise suggested 11,502 ancestral angiosperm gene families represented in at least one contemporary grass and rosid genome [19]. However, duplication of individual genes and entire genomes has conferred substantial variations among lineages in 'copy number' of genes that share common ancestry, with actual gene numbers in most angiosperm genomes typically being 2-3x the number of ancestral gene families. Moreover, genes are classified into common gene families based on well-conserved features that are often important to their function – however, outside these features, and even within them, the sequence ('spelling') of individual family members can range from some with near-identity to others no longer recognizable as being related to one another.

'Translational genomics' – leveraging information and tools from major crops and botanical models in improvement of less-studied crops

Through the common 'language' of DNA, shared ancestry provides approaches by which to accelerate progress in improvement of indigenous 'orphan crops' by utilizing information from the growing set of botanical models and well-studied crops in several ways. Three broad categories of such approaches follow.

i) *Targeted assays of candidate genes or genomic regions*. The collective efforts of agricultural and life scientists worldwide have revealed the functions of an appreciable set of plant genes already, and progress is accelerating. Many genes function in similar ways in different plants, and the function of a gene in a botanical model is predictive of its function in crops that are separated by the model by tens, even hundreds of millions of years of evolutionary history. In a particularly prominent example, a wheat gene conferring reduced height and increased harvest index of wheat that contributed to the 'Green Revolution' is clearly recognizable as a homolog of an *Arabidopsis* gene conferring insensitivity to the plant hormone gibberrellin [42], notwithstanding that the wheat and *Arabidopsis* genes have experienced ~150 million years of independent evolutionary history. Hence, an attractive means for leveraging genomic information is analogy – recognition that a trait of interest in a less-studied crop is similar to a well-known trait in a well-studied plant, followed by targeted evaluation of candidate genes. For example, envision that one had identified in funde (*Digitaria exilis*, a drought tolerant West African grain crop that is taxonomically a member of the panicoid cereals) a strain with reduced height and increased harvest index. By crossing the new strain to a conventional strain and inbreeding the F_1 hybrid, one would expect to find some F_2 progeny with the conventional (tall, lower yielding) phenotype and others with the desired trait (shorter, higher yielding). One could very simply test the hypothesis that a funde homolog of the Green Revolution gene accounted for the new trait. Briefly, one would scrutinize the Green Revolution gene to identify feature of its DNA sequence that were common to a wide range of known cereal genes (for example, wheat, rice, sorghum, and maize) and tacitly assume that those 'conserved' features were likely to also be shared by funde. Experience has shown this assumption to be reasonable, especially when several candidate features can be targeted [43]. DNA primers for the now-ubiquitous 'polymerase chain reaction' (PCR) can be designed and applied to DNA from each member of the funde population, to test the hypothesis that an allele from the short, high yielding parent correlates very closely with the phenotype in the progeny. Identification of such a diagnostic marker might be of high value in permitting a breeder to identify plants that will exhibit a desired trait at the seedling stage, avoiding the need to grow undesirable plants to maturity (saving time and space), or to distinguish true-breeding homozygotes from heterozygotes, cutting generations from the breeding cycle. This general approach could be applied in principle to quickly seek diagnostic markers for a wide range of additional traits, including other aspects of plant development (flowering time, for example), resistance or tolerance to biotic (diseases and pests) or abiotic (drought, soil conditions) stresses, grain quality, and others.

ii) *Whole-genome searches.* While functionally-known genes may explain some important mutations in less-studied crops, the majority of differences among plant genotypes within a species are thought to be determined by the collective effects of numerous genes, each one of which has only a small effect. Such genes, often referred to as quantitative trait loci, have been the subject of literally thousands of studies in plants and animals over the past two decades. While there is some predictive value of the locations of QTLs for some traits across taxa, QTL mapping most commonly involves searching an entire genome with DNA markers at closely-spaced intervals to identify genomic regions that make the largest contributions to the genetic control of a complex trait.

The fact that most flowering plants share most of their genes, and that gene sequences change relatively slowly because proper gene function is intolerant of many changes, provides a means to design the large numbers of pan-taxon DNA markers that are required for genome-wide surveys of plant genomes, even in plants for which no *de novo* sequence information exists. As for the single 'Green Revolution' gene above, one can computationally scrutinize tens of thousands of genes to identify subsets that have islands of sequence shared by a wide range of taxa. Such islands of conserved provide targets for designing synthetic oligonucleotide primers that can be used by the PCR to amplify corresponding sequences from a wide range of taxa, even those lacking sequence information. Such cross-utilization of genomic tools to study genetic diversity requires resolution of a fundamental conflict between the need to identify genomic sequences that are conserved (largely or wholly) across many divergent taxa, and the need to identify DNA-level differences that reflect diversity at its most elemental level. The relatively high level of conservation of the locations [44], but not the sequences of introns, provides a resolution to this dilemma. The identification of vast numbers of probable gene and intron locations in the sequences of botanical models is routine. 'Conserved intron scanning primers (CISP)' within relatively conserved exons located near exon-intron boundaries, can be used to scan introns for variation suitable for DNA marker identification. permitting systematic sampling of entire genomes for well-distributed markers, or targeted enrichment of particular regions containing a gene of interest. This approach has been particularly well studied in the monocots, using information from rice and sorghum to identify DNA markers useful in pearl millet and other less-studied cereals [43], as well as in non-cereal monocots [45]. Variations on the same method have been applied to many other plant families (legumes, crucifers, nightshades) and in principle are generally applicable to all flowering plants, as well as animals.

iii) Deductions about probable gene repertoire and function. Let us take the funde example a step further. Let us assume that the trait could NOT be accounted for by a homolog of the Gren Revolution gene. Accordingly, a whole-genome scan was done and the trait associated with several genetic markers on a different chromosome. The genetic markers all show correspondence a small region of same rice chromosome, that contains a different gene known to confer reduced height and increased harvest index. In other words, the initial candidate gene did not explain the phenotype but other candidates of known function from different regions of the genome may do so. As the number of genes for which functions are known increases, naturally the number of candidates to consider in such comparative studies does, too.

Limitations – Lineage specific genes

Plants are selected to become crops because of some unique feature(s) that attracted indigenous peoples, that were able to be improved by either conscious or unconscious selection. As such, each of our crops becomes a sort of botanical model for some extreme feature of plant growth and development. To understand and manipulate the features that make a crop unique, i.e. for which we cannot draw upon analogy to other plants, will require crop-specific enabling tools, technologies, and resources; in particular targeting genes that are substantially different from those of other organisms. How will we recognize the genes that confer these features, and how will we determine how they work?

A curious finding in virtually all eukaryotic genomes sequenced to date is 'lineage-specific' genes, for which an ortholog cannot be discerned in closely related species. Among 18447 'deduced ancestral loci' in Arabidopsis, poplar, grape, papaya and rice [46a], 3680 (20%) were specific to only one species. Among OrthoMCL gene families in sorghum, 3,983 (24%) appear to be absent from the dicot genomes sequenced to date; and 1,053 (6.4%) appear to be absent from the other monocot genome, rice. One might expect lineage-specific genes to be relatively frequent in angiosperms due to recurring polyploidization/diploidization cycles [46a] that do increase regulatory and may increase morphological complexity [46b]; and to transposition of gene segments that may evolve new genes [47, 48], albeit rarely [49]. Nonetheless, recent analyses of well-groomed mammalian genomes [50] report higher lineage-specific gene frequencies than found in sorghum versus rice.

Lineage-specific genes are a tantalizing target for early functional analysis because they may perhaps relate to features that differentiate closely related taxa, but caution is especially warranted in selecting such genes for analysis. Rapid gene evolution may be due to a lack of structural or functional constraint (indicating a *lack of important function*) or to strong positive selection for functional divergence (indicating *important new function*), possibilities that can be distinguished statistically [51, 52, 53, 54]. Genes under strong positive selection in *Drosophila*, mammals, and several other species are vital to reproductive success, cell-cell recognition, and cellular response to pathogens [e.g., 45, 55, 56]. However, identifying lineage-specific genes is inherently error-prone. Transposable-element associated genes have been a major contributor to inflated gene number estimates and false positive inferences of lineage-specific angiosperm genes [57]. Whole-genome shotgun sequencing approaches (often including many gaps), and occasional errors in accurate inferences of intron-exon boundaries, each may artifactually truncate or elongate gene models. Indeed, gene annotations of virtually all angiosperm genomes, even *Arabidopsis* which has been scrutinized by hundreds of scientists for nearly a decade, remain works in progress and different annotations of even one genome invariably differ. While much may be learned from functional analyses of lineage-specific genes, their careful manual annotation should precede investments in their analysis. Each additional genome available to study using comparative approaches further improves power to expose false-positive cases of apparent lineage-specific genes, for example by gross phylogenetic incongruities in inferred gene sets [19].

Exemplary opportunities to increase knowledge of African crops

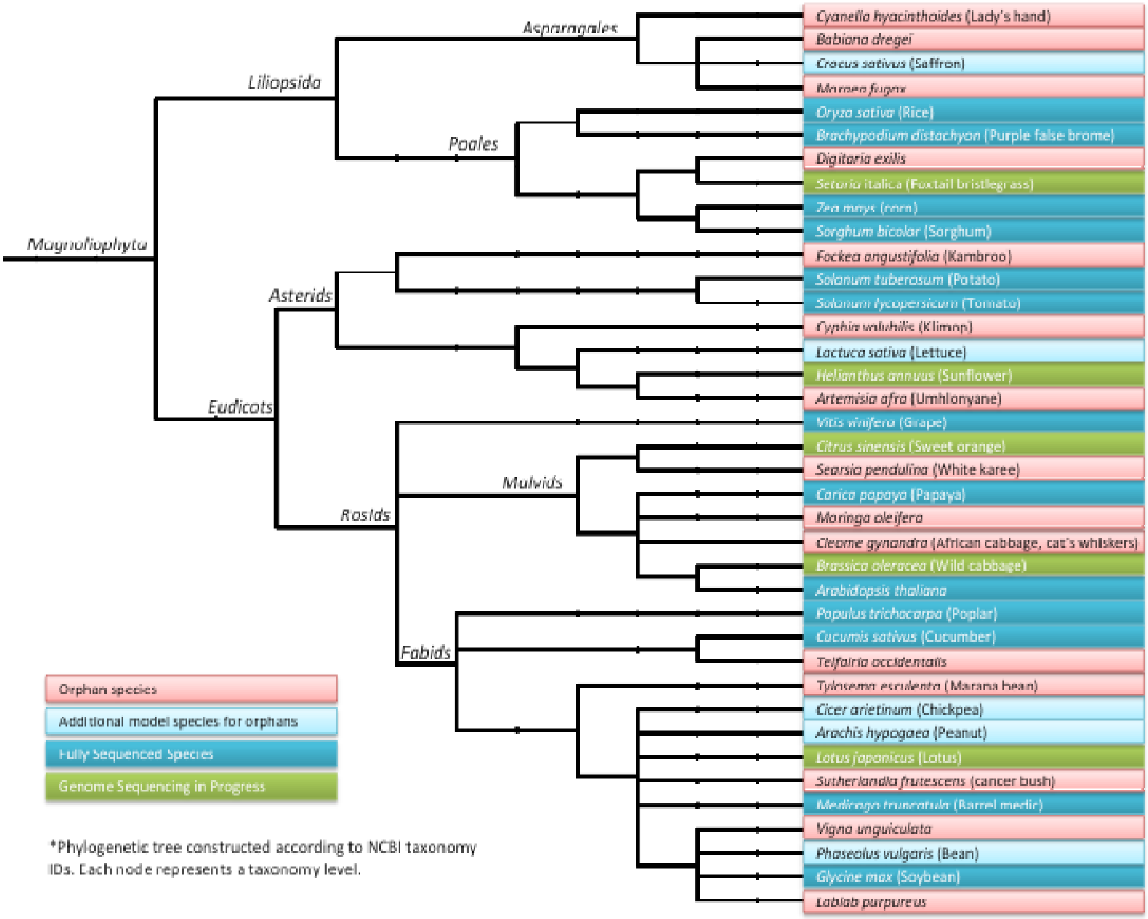
While a host of indigenous and introduced crops would benefit from comparative approaches to their improvement, below and in **Figure 1** we briefly offer a few exemplary cases. For example, *Cleome gynandra*, a leaf vegetable from South Africa, and *Moringa oleifera*, introduced from India but widely grown in Africa and referred to in a prominent US National Academies report [58] as "a sort of supermarket on a trunk", are both members of the plant order Brassicales which includes the fully-sequenced models *Arabidopsis thaliana, Carica papaya*, and *Brassica rapa* (turnip). In addition, extracts from the leaves of *Cleome* spp. have demonstrated insecticidal, repellent, antifeeding properties [59,

60a] displaying their potential use as additives in insect control agents [60b].

Digitaria exillis, noted above, is a panicoid cereal as are fully-sequenced sorghum and maize, and Setaria italica (Foxtail millet) for which sequencing is in progress. *Telfairia occidentalis, a leaf vegetable, is a member of the* Cucurbitaceae, closely related to the fully-sequenced cucumber.

Numerous nitrogen-fixing legumes are important indigenous crops, perhaps reflecting the relatively nitrogen-poor soils of much of Africa, including *Tylosema esculenta, Vigna unguiculata*, and Lablab purpureus*, all of which might derive considerable benefit from the fully-sequenced genome of Glycine (soybean), and expected*

Figure 1. Examples of orphan crops and their relationships to botanical models and/or other crops which have information that might be utilized for translational genomics.



*Phylogenetic tree constructed according to NCBI taxonomy IDs. Each node represents a taxonomy level.

sequences for Medicago (alfalfa), Lotus, Phaseolus (common bean), and Arachis (groundnut or peanut). The large seeds of *Tylosema esculanta* (marama bean in English, ombanui in Herero) are eaten in South Africa, Botswana and northern Namibia. This is seen as a significant crop for arid regions [61] with comparable protein content to existing crops. It is also being developed in Texas, Australia and Israel [15]. *Sutherlandia frutescens* (cancer bush in English, musa-pelo in Sesotho) is a widely used indigenous medicinal crop in South Africa, Botswana, Lesotho and Namibia [62]. *In vitro* studies indicate anticancer activity [63], and antidiabetic effects [64].

Study of *Searsia pendulina* (White karee), formerly *Rhus pendulina*, might benefit from analogy to the relatively small genome of members of the genus Citrus (orange). The fruits of *S. pendulina* are mixed with the gum of *Acacia karoo* to form a high energy (1202 kJ/100 g) confection [65]. Several other asterids, *Fockea angustifolia, Cyphia volubilis,* and *Artemisia afra* might benefit from a planned genome sequence for Helianthus (sunflower), and to a lesser degree the fully-sequenced genomes of more distantly-related tomato and potato.

Several corm-producing members of the Asparagales such as *Cyanella hyacinthoides* , *Moraea fugax* and *Babiana dregei* are highly regarded as staple foods in the drier parts of Southern Africa [65]. Other tuber species that are consumed during periods of drought include several *Fockea* species such as *F. angustifolia medulis* and to a lesser extent *F. camaru* and *crispa.* The watery, white flesh is consumed by inhabitants of the Kalahari desert when no surface water is available [15]. While these may benefit from the albeit limited EST resources available for the Asparagales taxon saffron crocus, the lack of genomic information for this clade points to the need for greater investment in its genomic characterization.

What else might be out there? (species and alleles)

About 250,000-400,000 angiosperm species are thought to exist [3, 4, 5], and a remarkable average of 2,350 new ones are discovered each year [6]. The vast majority of such newly-identified species are either confined to small areas, or are exceedingly rare, although one recent discovery in Eastern Ethiopia dominates the vegetation over at least 8000 km^2, an area nearly the size of the island of Crete [66]. This area has been seldom visited by botanists because of its inaccessibility and general unrest, and the newly-named plant, *Acacia fumosa*, does not seem to have any noteworthy uses, but it begs the question of what else might be found in such 'terra incognita' [67]. Even plants that lack obvious uses to researchers and non-native people may harbour sources of novel 'versions' (alleles) of genes with effects that are useful or informative, and which are ever-more accessible to discovery through genomics.

More immediate opportunities lie in the deeper exploration of the gene pools of indigenous African plants, and greater utilization of their full genetic potential. A few better-studied crops of African origin, such as sorghum, are well represented in the world's 'gene banks', and many of the extant alleles in the species will be discovered in due course by 'resequencing' projects. Nonetheless, there are various known gaps and probably additional unknown gaps in collections that warrant further sampling. Moreover, association of rare alleles with their functions would be expedited by access to greater numbers of genotypes containing the alleles. Many of the 'orphan' crops lack large-scale collections, or even any collections. Indigenous communities accumulate immeasurable knowledge of the various uses of the flora and fauna they have co-existed with for millennia, and the documentation and cataloging of such indigenous knowledge may lead to identification of new ecosystem services and their causal genes.

A further challenge is that restrictions on flow of germplasm due to intellectual property issues and the privatisation of agricultural biotechnology often hinder what could be very fruitful partnerships between African and non-African scientists to bring the full spectrum of biotechnologies to bear on these crops. Transfer of cutting edge/ frontier technologies which would empower researchers in developing countries to advance independently has often lagged transfer of germplasm from developing to developed countries [68]. With the privatization of agricultural biotechnology, scientists in technology rich (developed countries) and gene rich (developing countries) find themselves under the cooperation—comptition paradox [69]. Indigenous people have in the past cooperatively exchanged germplasm and information on plant use with researchers and managers, with little reciprocal exchange in technology that would allow the development of profitable products from such plants. The way forward requires ethical and responsible actions from both sides which acknowledge the global forces at play in the arena within which scientists practice.

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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.

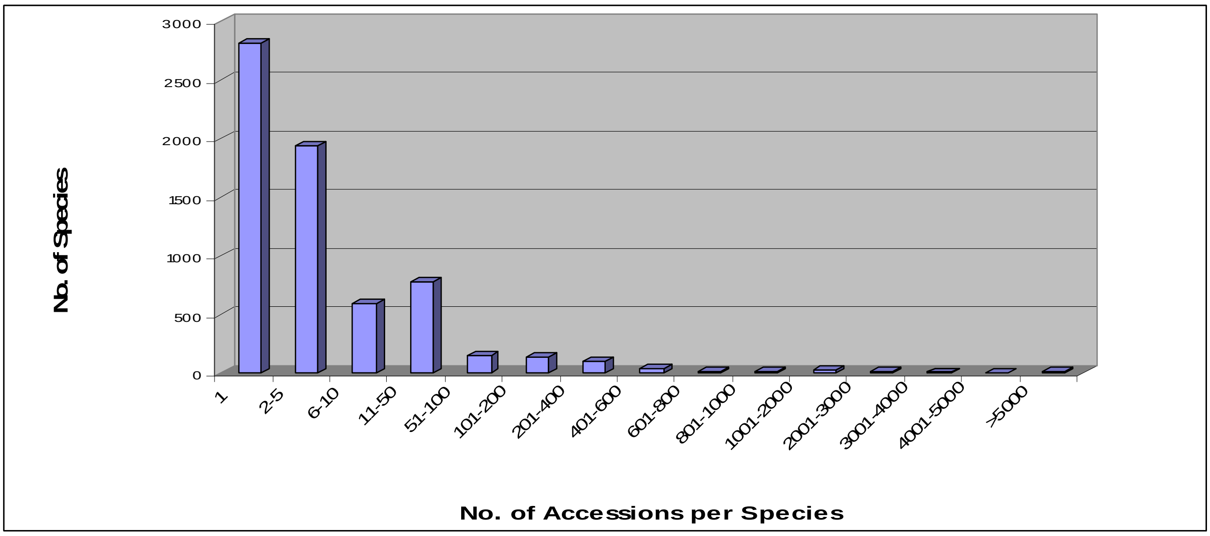
Orphan crops: Development Context

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 poorest 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 criterions 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 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]:

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

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 models 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 collatetraditional 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), *Irvingia 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 resistance 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 podborers 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 particular 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 sequences 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

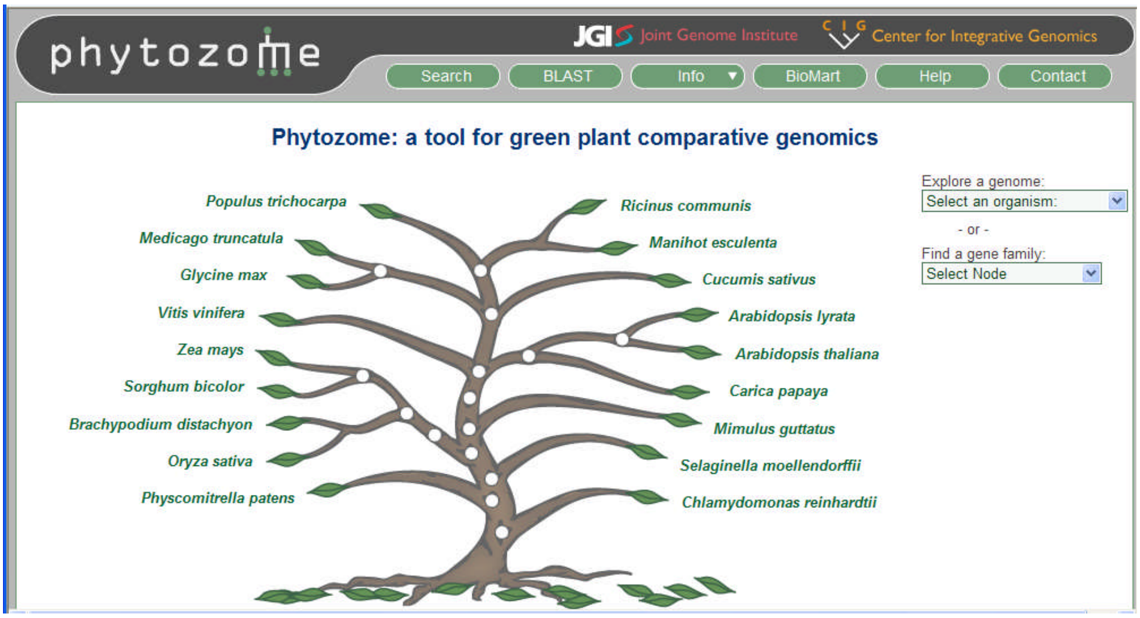
The completion of genome sequences of the model plant *Arabidopsis* and the first crop plant, rice, heralded the dawn of the genomics era. Following these landmark achievement, the research community is aggressively taking on the challenges of integrating molecular breeding into the existing breeding 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 and climate change range from exerting the power of science to break the yield barrier [35] to broadening the wheat-rice-maize dominated source of food by improving underutilized crops [14]. The aforementioned major crops have undergone substantial extensive genomic investigations. According to the NCBI-GenBank release note 176.0 (Feb. 2010), six plant species are among the 20 most sequenced organisms based on the number of entries and bases of nucleotide sequences in GenBank/ EMBL/DDBJ. The most sequenced plant species, maize (*Zea mays*), has 3.9 million nucleotide entries followed by rice (*Orzya sativa* Japonica group; 1.2 billion bases). In comparison, crops listed in **Table 1** have a fraction of the amount of genomic resources available for the major crops and model plants such as *Arabidopsis*. For instance, the number of entries

Table 1. Available genomic resources at the NCBI databases for selected orphan crops and alternative resources for comparative studies

Crops	Cowpea	Peanut	Grass Pea	Chick Peas	Tef	Finger millet	Coffee	Rape seed	Sunflower	Yam	Lentil	Banana Musa sp
Genus	Vigna	Arachis	Lathyrus	Cicer	Eragrostis	Eleusine	Coffea	Brassica	Helianthus	Dioscorea	Lens	Musa
Family	Fabaceae	Fabaceae	Fabaceae	Fabaceae	Poaceae	Poaceae	Rubiaceae	Brassicaceae	Asteraceae	Dioscoraceae	Fabaceae	Musaceae
Related species	Soybean/ Lotus	Soybean/ Lotus	Soybean/ Lotus	Soybean/ Lotus	Rice/ maize	Rice, Maize	Dicot	Arabidopsis	Lettuce	Monocots	Soybean/ Lotus	Monocots
Resources	LIS	LIS	LIS	LIS	Multiple	Multiple	GPD	TAIR*	Compositdb	PlantGDB	LIS	PlantGDB
Nucleotide	507	1,767	25	934	564	188	680	10,817	9,448	683	184	4,210
Nucleotide EST	187,483	87,002	178	34,208	2,816	1,927	43,619	643,943	133,682	31	9,513	31,268
Nucleotide GSS	54,123	9,347		50,853	40		3,875	102,619	573		485	7,186
Protein	365	966	22	756	6	65	453	9,711	5,575	622	127	2,510
Structure	8	28	1			3		7	5		5	6
Genome Sequences				1			1	2	1	1		
Genome Projects	1			1	1	1	1	2	1	2	1	1
Popset	32	16	6	6	5	8	17	8	197	54	2	34
3D Domains	29	124	4			4		10	5		36	10
GEO Datasets	5	3	11	14				22	2		9	3
UniGene	15,740	11,909						27,139	12,216			
UniSTS	75	203	5	54	4	42	17	284	1,627	4		69
PubMed Central	645	443	27	217	24	34	142	1,514	693		20	265
Gene				108			140	106	138		213	
Taxonomy	11	3	1	1	1	3	1	3	1		4	77

Figure 1. Version 5.0 of Phytozome comprises twenty genomes (sequenced at the Joint Genome Institute and other institutions) the phylogenetic relationships of the species to facilitate comparative genomic studies.



Source: www.phytozome.net

for the multi species Genus *Dioscorea*, which consists of such important cultivated yam species as water yam (*D. alata*, 31 sequences), yellow yam (*D. cayenensis*, 6 sequences), and white yam (*D. rotundata*, 3 sequences) is less than a thousand GenBank sequences in total. The paucity of genomic resources considerably hampers the application of marker assisted breeding in orphan crops. However, advances in technology and ultra high throughput genotyping technologies are changing the landscape of genomic research.

The list of completed genome sequences is publicly available for several crops and tree plants at the Entrez Genome database (http://www.ncbi.nlm.nih.gov/genomes/PLANTS/PlantList.html) and at the Phytozome web site (www.phytozome.net). **Figure 1** displays the list of crops with complete draft genome in the database of Phytozome. The resources cover different families of green plants representing cereals, legumes, fruits, vegetable, root crop, and tree plants, the majority of these being completed in the past few years. Mega initiatives such as "The 1000 Plant/Animal Genomes De novo Sequencing Project" of the Beijing Genomics Institute (BGI; http://www.genomics.cn/en/) is aiming to sequence 500 plant and 500 animal species.

Genomics for marker development and gene discovery

The new technologies furnished a new set of molecular markers that are amenable for high throughput discovery and genotyping [2]. Besides the number of completed and ongoing genome sequencing projects, numerous large-scale plant EST sequencing projects were launched to generate molecular data that can be used for marker development (http://www.ncbi.nlm.nih.gov/genomes/PLANTS/PlantList.html#IP_SEQ). As a result of these massive genomic data, there is a trend of shifting from the first generation DNA-based markers such as RFLP, RAPD, and AFLP towards functional or gene-targeted markers such as EST-derived SSRs and single nucleotide polymorphism (SNP) markers [1]. Preference for SNP is largely driven by the cost-effectiveness of its discovery, availability of high throughput genotyping technologies, and the range of applications for genetic and genomic studies [4]. One of the notable outcomes of developments in genomics is the rapid rate of discovery and characterization of novel genes. Advances in functional genomics have begun shedding light on the mechanism underlying physiological processes relevant to agricultural productivity thereby elucidating a repertoire of stress-induced genes and pathways, pathogen defense genes, nutritional quality traits such as pro-vitamin A carotenoids [21]. Knowledge of genes and pathways opened a new avenue of research for enhanced efficiency and effectiveness of breeding programs through development of gene targeted markers (GTM) and functional markers (FM) [1, 51]. The potential use of markers for germplasm management, trait conversion, and trait stacking and pyramiding are becoming widely accepted.

There are large number of orphan crops that are neglected by global research community and the private sector owing to their negligible or unknown importance outside their locality or region [3]. The CGIAR, a

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* (Table 1) Cassava not included because genome has been sequenced; General Plant Databases (NAR vol 38, 2010)

Legume: In addition to soybase, Legume Information center.

Cereals/grasses: Graingenes, gramene, plantGDB coalition of 15 research centers (www.cgiar.org), whose mission is to achieve sustainable food security and reduce poverty in developing countries through scientific research and research-related activities, strives to improve 19 crops of which 12 could be considered as orphan crops including millet, chickpea, lentil, pigeon pea, cassava, yam, cowpea, sweet potato, plantain and banana, coconut, and groundnut. Among these, the draft genome of cassava has been released recently triggering several projects in its wake (http://www.phytozome.net/cassava.php).

Below are some useful resources for plant genomes and comparative analysis

⇒ *GreenPhylDB* (http://greenphyl.cirad.fr/): a platform for full genome comparison of Arabidopsis and rice but also includes GOST (GreenPhyl Orthologs Search Tool) which assists the identification of orthologous and paralogous genes for any plant gene.

⇒ *PlantGDB* (http://www.plantgdb.org/): is a comprehensive resource for comparative plant genomics with tools and tutorials for downloading, comparing, and annotating sequences.

⇒ *The Genome On Line Database, GOLD* (http://www.genomesonline.org/): ar comprehensive access to information regarding complete and ongoing genome projects, as well as metagenomes and metadata. This tool allows viewing of species phylogenetically which is very important in orphan crops to find genomic-rich related plant.

⇒ *Phytozome* (http://www.phytozome.net/): Release version 5.0 of Phytozome features 20 sequenced and annotated plant genomes which have been clustered into gene families at fifteen evolutionarily significant nodes (see **Figure 1**). Phytozome provides various tools for similarity search and viewing as well as links to external resources.

With the new technologies, numerous genotypic data are being generated in short period of time. The challenge, however, is how to utilize such genomic data to improve the efficiency and effectiveness of crop improvement strategy. Broadly, molecular breeding comprises three components: phenotyping, genotyping, and data management. For under-researched crops, the limited genomic information currently available as well as innovative molecular tools discovered in other crops could be adapted and incorporated into the existing breeding programs through comparative genome analysis. The paucity of genomic resources in orphan crops could hamper the implementation of molecular breeding. Below are basic bioinformatics end-user tools that are accessible to scientists in developing countries and that could help reduce the gap in genomic knowledge and pave the way for the application of molecular breeding.

Bioinformatics

Bioinformatics can be viewed broadly as the development and application of computational tools to acquire, store, organize, retrieve, and analyze large amount of biological information. In the context of this review, bioinformatics refers to the search, evaluation, and utilization of biological computational tools and databases for candidate gene discovery and/or marker development. The spectacular advances in genome sequencing and the subsequent generation of large amount of biological data triggered the development of tools for data management, visualization, integration, analysis, modeling, and prediction [38]. At the moment, the number of scientists involved in bioinformatics are too few to meet the increasing demand for tools and methods to make sense out of the mounting data. On the other hand, substantial number of biological scientists is not in a position to utilize the existing tools due to lack of information on the existence of such tools or the function of the tools. A number of recent books [34, 51] and review articles [31, 36, 38] are testimony to the growing importance of bioinformatics skill. Rhee *et al*. [38] described basic and vital areas of bioinformatics such as sequence analysis, transcriptomics, proteomics, ontology, and databases. More specific presentation of a set of tools and databases relevant to weed science was provided by Larrinua *et al*. [24]. More recently, Armstead *et al*. [3] discussed the challenges and opportunities of using bioinformatics in the improvement of orphan crops, represented by three forage crops. The focus of the present review is limited to orphan food crops of Sub-Saharan Africa (SSA).

The role of bioinformatics as a pivotal tool for molecular breeding is growing steadily, particularly in identification of nucleotide variants associated with key traits [25]. The first step towards variant discovery is the mining of data in public databases. Subsequently the retrieved data would be subjected to compare nucleotides, perform similarity search, deduce protein sequences, and understand the function of the protein. Many users are either unaware of the presence of myriads databases and tools or intimidated by the idea of getting into such bioinformatics research. Here, I provide highlights of the relevant databases and end-user tools and services that can be employed in the breeding of orphan crops with limited genomic resources.

In silico Marker development

Availability of nucleotide sequences is the prerequisite for the application of marker-assisted breeding. In the past two decades, numerous labs were engaged in generating molecular markers such as RFLP probes, AFLP, SSR, and SNP using laborious and capital intensive protocols. This was not affordable by many institutions in the developing countries. Even when funds are provided by charity organization/donors, the lack of skilled personnel and infrastructure hampers the introduction of molecular techniques. Nowadays, genome sequences and associated functional genomics studies have become the primary source of genomic resources for comparative genomics. Experts in data mining are able to perform *in silico* research to develop molecular markers from public databases using a combination of search and computational techniques [2, 40].

The most popular contemporary sequence-based markers are SSR and SNP.

SSR: Since its advent in mid 1980 [47], SSRs have been used in a variety of applications and crops. Variability is generated when a sequence is amplified by a pair of primers flanking a mono-, di-, tri-, or tetra-nucleotide repeats due to variable number of repeats in different individuals. For small scale number of sequences, manual designing of primers is possible. For large number of sequences, manual prediction is not only cumbersome and time consuming but also error-prone. Several software packages were developed to identify SSRs and design flanking primers including FastPCR [22] and Repeatfinder [49].

SNP: The free Dictionary (http://encyclopedia.thefreedictionary.com/Single-Nucleotide+Polymorphism) defines SNP as "A single nucleotide polymorphism (SNP, pronounced *snip*), is a DNA sequence variation occurring when a single nucleotide - A, T, C, or G - in the genome (or other shared sequence) differs between members of a species (or between paired chromosomes in an individual). For example, two sequenced DNA fragments from different individuals, AAG**C**CTA to AAG**C**TTA, contain a difference in a single nucleotide. In this case we say that there are two *alleles*: C and T (bold font). Almost all common SNPs have only two alleles. For a variation to be considered as SNP, it must occur in at least 1% of the population".

The following sections introduce a novice bioinformatics user how to discover nucleotide variations such as SSR and SNP and convert them to molecular markers for gene-tagging, linkage mapping, diversity analysis and other applications. More information on high throughput marker discovery and genotyping can be obtained [2].

Basic skills in bioinformatics

Besides sequence-based markers such as SSR and SNP, researchers working with RAPD and AFLP, may require some basic skills in bioinformatics in such a situation as a RAPD and AFLP fragment/band cosegregating with an interesting trait and the investigator wants to convert this fragment into a PCR-based, single-locus specific genetic markers such as sequence characterized amplified region (SCAR) [33] or cleaved amplified polymorphic sites (CAPS) [23]. Such conversion entails basic bioinformatics end-user skills such as sequence editing, similarity search, primer design, among others. In this section, a brief guide and example of web-services and open source software is outlined.

Choosing databases and web servers

It is challenging for biologists to choose the right tool amid the explosive growth of web sites and tools. It is absolutely important that users distinguish between personal web sites and reliable peer-reviewed and up-to-date resources. The challenge is how to choose from the multitude of databases and tools available. A good starting point could be the collection of molecular biology databases published in the journal Nucleic Acid Research, the latest being volume 38, Database issue (http://www.oxfordjournals.org/nar/database/c/). The most recent update of molecular biology databases feature over a thousand databases of which several hundreds are on plants [12]. Two world renowned organizations, NCBI (The National Center of Biotechnology Information) and EMBL (European Molecular Biology Laboratory), provide access to a comprehensive and integrated collection of biological data worldwide. NCBI [43] maintains many database resources including primary nucleotide and protein sequences, derived databases, bibliography, books, software, and tutorials. GenBank, the nucleotide sequence database of NCBI [6], comprises nucleotide sequences for more than 300,000 organisms, submitted by individual laboratories and batch submissions from large-scale sequencing projects. Two other public databases with whom GenBank daily exchanges data with are the EMBL Nucleotide Sequence Database in Europe and the DNA Data Bank of Japan (DDBJ). Entrez, the query and retrieval system at NCBI can be used to access several linked and integrated databases including DNA and protein sequence databases along with taxonomy, genome, mapping, protein structure and domain information, and the biomedical journal literature via PubMed. Equivalent comprehensive collection of databases and tools can also be found at the European Bioinformatics Institute (EBI) [7] web site (http://www.ebi.ac.uk/) including SRS, data integration platform for easy access to large amount of biological data across 100s of databases. While the primary nucleotide sequence databases are identical with GenBank, the data retrieval system or the user interfaces are different. Users' choice of databases is a matter of preference or ease of learning the tools. Furthermore, the Online Bioinformatics Resources Collection (OBRC) which contains annotations and links for 2681 bioinformatics databases and software tools have compiled 148 plant specific databases [10]. (http://www.hsls.pitt.edu/guides/genetics/obrc/plant). Other systems with integrated querying are BioMart [44] and PLAZA [37].

Sequence retrieval and manipulation

Finding sequences in one of the above public databases is basically the same. Searches begin with keywords, accession number, gene name, species name, etc. The Entrez search engine at NCBI, in addition to retrieving sequences, returns pre-computed lists of data elements such as related sequences, gene, protein, taxonomy, and others. Search can be performed in all databases or restricted to nucleotide in the drop down menu. The result can be displayed in different format or downloaded. The most common download format is FASTA format. Description of FASTA format at NCBI is as follows:

A sequence in FASTA format begins with a single-line description, followed by lines of sequence data. The description line is distinguished from the sequence data by a greater-than (">") symbol in the first column.

It is recommended that all lines of text be shorter than 80 characters in length. An example sequence in FASTA format is:

```
>gi|532319|pir|TVFV2E|TVFV2E envelope protein
ELRLRYCAPAGFALLKCNDADYDGFKTNCSNVSVVHCTNLMNTTVTTGLLLNGSYSENR
T
QIWQKHRTSNDSALILLNKHYNLTVTCKRPGNKTVLPVTIMAGLVFHSQKYNLRLRQAWC
HFPSNWKGAWKEVKEEIVNLPKERYRGTNDPKRIFFQRQWGDPETANLWFNCHGEFFY
CK
MDWFLNYLNNLTVDADHNECKNTSGTKSGNKRAPGPCVQRTYVACHIRSVIIWLETISKK
TYAPPREGHLECTSTVTGMTVELNYIPKNRTNVTLSPQIESIWAAELDRYKLVEITPIGF
APTEVRRYTGGHERQKRVPFVXXXXXXXXXXXXXXXXXXXXXXVQSQHLLAGILQQQKNL
LAAVEAQQQMLKLTIWGVK
```

It should be noted that FASTA is just one of several formats that is accepted by many sequence analysis software. However, many of the software have a choice of input format and also allow exporting sequences in various formats. Further details on sequence format can be found in one of the resources listed below.

Sequence alignment

Sequence alignment is the prerequisite of virtually all forms of sequence analysis ranging from search, to assembly, and to phylogenetics. Various algorithms have been developed to produce optimal alignment, a topic which is beyond the scope of this review. It is suffice to know that many softwares have been developed to perform nucleotide or protein sequence alignments. Two examples of widely used open access softwares, namely BioEdit [19], and MEGA [46], are freely downloaded and installed with easy-to-understand user's manual. A pair of sequences or multiple sequences saved, for example in FASTA format, can be used as an input. However, sequence alignment can also be done on the web at one of the resources listed in this review (e.g. EMBL-EBI) using the ClustalW program or other methods.

Phylogenetics

Phylogenetic analysis is the basis of taxonomical and evolutionary studies. In the context of this paper, phylogenetic analysis is performed to cluster multiple sequences based on genetic distances. This is a broad topic and a subject of 100s of articles and books. A deluge of tools and web services can also be found online (e.g. http://evolution.genetics.washington.edu/phylip/software.html). For beginners, stand alone programs such as MEGA can do excellent job of phylogeny tree construction. In addition, web services such as EMBL-EBI provide similar tools.

Similarity search

Sequence comparison is essential for understanding evolutionary relationship between genes. The most common and widely used similarity search tool is BLAST (Best Local Alignment Search Tool [53]. BLAST is a set of programs used to compare a nucleotide or protein query sequence to all of the available sequence databases. NCBI and EBI provide many different types of BLAST. Information on how to access BLAST services on WWW, choosing the right type of BLAST, interpreting BLAST results, how to do batch BLAST jobs, and others can be found at NCBI-BLAST home page (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Primer design

There are several applications in which primer designing is required for marker development. Such cases include, but not limited to, retrieved sequences containing simple sequence repeats suitable for SSR marker development. Orphan crops lack sequence information in which comparative genomics approaches such as homologous sequences are used to design degenerate primers, or re-sequence the gene of interest. The most widely used program for primer designing is PRIMER 3.0 (http://frodo.wi.mit.edu/primer3/) with several versions of web interface. The web-site provides user-friendly web interface and user manual describing the underlying principle of the program.

Advanced Skills

The major areas of high-end bioinformatics include the development of databases and algorithms for multiple sequence alignment, analysis and annotation of various types of microarray platforms, high-density oligonucleotide chips, variety of mass spectrometry, and diverse platforms of next generation sequencing. Computer savvy researchers who aspire to become bioinformatics tool developer should consider learning a scripting language program such as Perl (community web site: http://www.cpan.org/). Some genomics tasks such as discovery of SNPs or SSR in thousands of sequences, filtering sequences with the target motif and designing assay reagent (e.g. primer), filtering the result of BLAST, and annotation of thousands of EST sequences is a daunting job. Programming skill allows automation of such large scale and complex jobs.

Comparative Genomics

Comparative and functional genomics tools greatly facilitate the transfer of knowledge from thoroughly-studied model plants to orphan crops. Discovery of genes involved in flowering in model plants such as *Arabidopsis* have been successfully utilized to identify homologous genes in garlic [39] and in cauliflower [41]. Comparative genomic analysis tools have been used to investigate functional diversification and evolutionary mechanisms of plant genes [27, 55]. Most importantly, these tools provide an insight into the biochemical mechanism underlying economically important traits such as lignin biosynthesis for biofuel researchers [52], the cellulose synthase superfamily [54], improvement of the balance of essential amino acids and starch quality and quantity [15]. In the cases of orphan crops, where sequence information is meager or lacking, a host of comparative genomics techniques can be employed to tap into the benefit of genomics advances [17]. In groundnut, for instance, several hundred SNPs were identified in the Conserved Cross-Legume Orthologs (unpublished data).

Investigators could identify curated databases of the genus, family, or other category to which the crop in question belongs. **Table 1** provides an example of crops and relevant databases and potential information that can be obtained. Such databases may be very useful for scientists looking for markers, QTL information, etc. However, many of the other tools and databases could be searched for conserved genes such as NBS-LRR disease resistance genes, pVAC, drought tolerance, etc.

Identification of candidate disease resistance genes could be used as an illustration of the successful application of comparative genomics. Methods of enriching the repertoire of gene level knowledge in orphan crops can take approaches such as the identification of resistance gene analogs (RGAs) for identification of genes involved in plant defense [30]. This technique capitalizes on the presence of conserved regions of resistance genes for designing degenerate primers and isolating resistance gene homologues from different plant genomes using the polymerase chain reaction (PCR). This homology-based approach has led to the identification of thousands of partial sequences of NBS-LRR genes in a wide array of plant species [5, 8, 9, 18, 48]. In general, understanding the structure, localization, function, variation, and evolution of resistance genes will provide the basis for devising an efficient breeding strategy for disease resistance [20, 45]. The RGA techniques can easily serve as an entry point to bioinformatics in which beginners can retrieve sequences, design primers, amplify candidate R gene regions, and characterize it by similarity search and other sequence manipulation tools. The database 'PRGdb' [42] provides a manually curated database of well characterized and candidate plant disease resistance genes belonging to nearly two hundred plant species. Users can download reference genes of interest to design degenerate primers to amplify homologous genes in their species of interest or simply follow the various links provided for further information on domains, motifs, bibliography.

Capacity building opportunities

A recent article on mobilizing science to break yield barrier, emphasized the role of emerging technologies could play in improving agricultural productivity in Sub-Saharan Africa and South Asia [36]. It also advocates investing in human resources and briefly discussed the effort of CGIAR and the donor community towards training young scientists in developing economies. Bioinformatics has established itself as the cornerstone of modern molecular biology research. It is vital to initiate various forms of training, through non-credit courses and workshops, as well as degree awarding academic programs [28] to keep developing countries scientists abreast of current technologies. Below are listed some avenues for training:

Free Online courses and tutorials

In the field of bioinformatics it is not uncommon to find free online courses such as one offered by S*Star, an alliance of eight universities, spanning five continents (http://s-star.org). Webinars on specific topic or analysis method are also available from the private sector. However, the most important resources to get started are tutorials provided by worldwide renowned institutions such as NCBI and EBI. Open access journals published by Public Library of Science (e.g. PLoS computational biology) and Biomed Central (e.g. BMC Bioinformatics) are a good source of full text articles as well as numerous tools and hyperlinks.

NCBI: The NCBI handbook, one of the 257 free online books available on the BookShelf, provides information about the various databases and tools available at the site (http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=handbook).

2Can Support portal at EBI: offers a number of tutorials on different topics. http://www.ebi.ac.uk/2can/tutorials/index.html

Nucleic Acid Research, volume 38, Database issue contains list of curated databases as well as open access full text articles.

Tips on online search engines

While this seems trivial, nowadays, using the most popular search engines such as Google and Yahoo for any information could be frustrating and often unlikely to furnish the needed information. Filtering through the results can be a daunting task if appropriate search strategy is not used. Too often, the results could be unwanted aggressive marketing web sites with flashy popups or even indecent websites which have no relevance to the query word. While many web sites provide site-specific search engine, the following alternatives help for effective search of scientific terms; i) *Google Scholar*; ii) *Scirus* (www.scirus.com) is a comprehensive scientific research tools with options for filtering data and refining search results; iii) *Wikipedia* is an online free-content encyclopedia that anyone can edit and contribute to. Even though the quality of information is not good enough to be cited, it can help as a stepping stone through the links to primary sources when available (http://en.wikipedia.org/wiki/Main_Page); and iv) *Bioforums* such as Protocol-online.org, Biotechnique's Protocol wiki allow posting of questions in a specific subjects where members provide useful information thoes are hard to find otherwise.

Courses and workshops

African scientists should be on the look out for trainings by the CGIAR centers such as International Institute of Tropical Agriculture (IITA, www.iita.org), BecA-ILRI (http://hub.africabiosciences.org/), generation challenge program (GCP, http://www.generationcp.org/), and other centers (www.cigar.org). Bioinformatics trainings tailored for plant breeding had been organized by the International Centre for Advanced Mediterranean Agronomic Studies, in Zaragoza, Spain (http://www.iamz.ciheam.org/ingles/cursos09-10/) with broad spectrum of topics covering the most common bioinformatics tools relevant to plant breeders. In Africa, the West African Biotechnology Workshop Series of Nigeria facilitates such trainings (http://www.wabws.org/workshops.htm) in collaboration with international research centers such as IITA and advanced labs in North America. Scientists in advanced labs such as Ontario Institute for Cancer Research collaborate with African institutions to offer bioinformatics education to many African young scientists (http://www.oicr.on.ca/Portalnews/Vol2_Issue4/africa.htm). Aside from the South African National Bioinformatics Institute (SANBI, http://www.sanbi.ac.za/), the author has no knowledge of African university offering undergraduate or graduate degree, a certificate, or other degrees with emphasis on bioinformatics. It is high time for African academic institutions to incorporate bioinformatics in their curriculum.

International institutions

The Generation Challenge Program (GCP, www.generationcp.org) provides various learning materials those are relevant to molecular breeding in its ‘capacity building corner’ (http://mbp.generationcp.org/). The Global Partnership Initiative for Plant Breeding (GIPB) at the Food and Agriculture Organization (FAO) strives to promote capacity building in main-stream plant breeding in Sub Saharan Africa (SSA) and in other continents. Young scientists trained in main-stream plant breeding programs should be equipped with complementary bioinformatics skill simultaneously for successful application of molecular plant breeding. Furthermore, in the electronic and computer age, applied bioinformatics can greatly appeal to young breeders with a knack for computer.

Conclusion

Advances in the genotyping technology has accelerated the growth of bioinformatics as evidenced by recent increase of publications in existing journals (e.g. Nucleic Acids Research vol 37, Web server Issue and vol 38, Database Issue), dozens of dedicated bioinformatics-specific journals (e.g. Bioinformatics, Briefings in Bioinformatics, BMC-Bioinformatics, PLoS Computational Biology), and books [11, 34, 51]. In appreciation of the role of bioinformatics in life science research, a number of research institutions in developing countries have embarked on the development of their computational biology capacity including China [50], and Mexico [32]. However, there is no substantial effort to develop computational capacity in Africa. The aim of this review is to urge individual scientists to consider capacity building in bioinformatics, on one hand, and to implore policy makers and national institutions to devise a strategy to benefit from these technological advances. It may not seem reasonable to promote bioinformatics capacity enhancement when only a handful of ill-equipped biotech laboratories in developing countries, particularly in SSA, are struggling to generate molecular data. However, it should be noted that several start-up companies are offering affordable genotyping services that orphan plant breeders, with limited resources, can tap into for innovative breeding approach to accelerate the process of variety development.

The myriads problems in agricultural production in developing countries offer extensive avenue for research. The new molecular technologies are revolutionizing crop improvement. Advances in genomics technologies and the associated computational resources are consistently evolving towards cost-effectiveness and accessibility thereby increasing the potential to be adopted by resource-poor countries. The rapidly growing and expanding advances in information and communication technology (ICT) such as World Wide Web greatly facilitate accessibility of these scientific advances. Bioinformatics is one of the remarkable achievements of this century that scientists in developing countries can mobilize to leapfrog agricultural productivity. However, these resources are largely unknown to scientists in low income nations. The field of genomics has become a vast, information-intensive discipline, sparking the development of numerous databases and tools in its wake. The frustratingly large number and variety of databases and tools calls for end-user support in identifying appropriate and reliable resources [28].

The rapid growth of sequencing and genotyping technology and the parallel growth of bioinformatics and online biological resources further broaden crop improvement strategies for well-studied and under-studied crops alike. Nowadays, genome sequencing of an organism does not entail large sum of money and long time. The rate limiting step is rather, mining the genome to unravel the genes and pathways underlying economically important traits. This entails a strong team of multidisciplinary genomics and informatics scientists. Africa has to go long way to build the critical mass of scientists dedicated to improvement of orphan crops. With the plethora of existing and emerging web resources, the sky is the limit for scientists. African governments, institutions, and policy makers should gear up to accelerate the development of bio-computational human resources if African agriculture is to benefit from the current ‘omics’ boom. In the interim, strong partnerships with advanced research institutions around the world should be fostered to leverage genomics and bioinformatics for accelerated improvement of Africa’s neglected crops.

African bioinformatics scientists, however small in number, should join forces to mobilize funds to help create shared resources and expertise and build synergy. Regionally organized professional groups will have the opportunity to play a policy advocacy role to enhance government funding and also to liaise with ARIs.

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THE IMPROVEMENT OF AFRICAN ORPHAN CROPS THROUGH TILLING

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Abstract

People in African countries mostly depend on special staple crops – orphan crops – that are particularly important for their food security, nutrition and income. These crops are better adapted to local soil and climatic conditions as well as to the agro-ecology and socio-economic conditions in developing countries. However major challenges in orphan crops are low productivity, low nutrition and the production of toxic substances. Conventional breeding methods often do not overcome these problems and transgenic methods can not be applied due to several reasons. Alternative molecular methods like TILLING (Targeting Induced Local Lesion IN Genomes) are needed from which orphan crops may benefit. TILLING is a general, easy, non-transgenic and low-cost reverse genetic method to identify single base pair changes in genes of choice. The technique was first developed for the model plant *Arabidopsis* but successfully adopted to other species including crop plants. The procedure of TILLING comprises: classical mutagenesis, development of a non-chimeric population, preparation of a germplasm stock, DNA extraction and sample pooling, population screening for induced mutations as well as validation and evaluation of candidates. Here, we present a general overview of the TILLING method with special focus on crops and give an example how the technique can also be applied easily to orphan crops. We discuss our experience on tef (*Eragrostis tef*), one of the understudied crops of Africa.

Key words: Orphan crops, TILLING, EcoTILLING, mutagenesis, *Eragrostis tef*

Need for orphan crops improvement

Feeding the ever-increasing population of Africa will be a challenge in the future. Most of the population of the continent depend as food source or income generation on so called 'orphan crops' which are mainly unknown outside their countries or have at least no economic importance. However, these crops are particularly important for food security, nutrition and income to resource-poor farmers and consumers in developing countries. In addition, orphan crops are much better adapted to the often difficult local soil and climatic conditions. Although large number of orphan crops are known to exist on the continent, the major ones are cereals [e.g., finger millet (*Eleusine coracana*), tef (*Eragrostis tef*) and fonio (*Digitaria* spp)], legumes [cowpea (*Vigna unguiculata*), bambara groundnut (*Vigna subterranea*) and grass pea (*Lathyrus sativus*)] and root crops [cassava (*Manihot esculenta*), yam (*Dioscorea* spp.) and enset (*Ensete ventricosum*)]. Despite their importance in adapting to the adverse agro-ecological conditions, orphan crops have also several limitations. Some of the prominent bottlenecks related to these crops are low productivity (e.g. in tef), poor in essential nutrients (cassava and enset) or production of toxic substances (cassava and grass pea; for review [1]). Another challenge is that breeders of orphan crops are mostly depend on the conventional breeding techniques particularly on selection which fail to improve some valuable traits in these crops. Modern biotechnological techniques including the transgenic approach are not yet employed for orphan crops due to negative perception in most African countries and the lack of biosafety regulations.

TILLING has high potential to improve orphan crops

Various types of crop improvement techniques are known to exist. Broadly they are grouped into two although sometimes there is no clear distinction between these two: i) *conventional techniques* that mainly include various types of selection methods and introgressions or hybridizations, ii) biotechnological or modern techniques that include transgenic and non-transgenic techniques. Among non-transgenic methods TILLING (Targeting Induced Local Lesion IN Genomes) becomes recently popular and is extensively implemented for major crops and to a certain level to orphan crops. TILLING is a general, easy, non-transgenic and low-cost reverse genetic method which uses traditional mutagenesis followed by high-throughput mutation detection. It identifies single base pair changes in targeted genes and can be applied to every organism independent of the genome size, reproductive system, generation time and polyploidy level [2, 3, 4, 5, 6, 7]. In contrast to forward genetic screenings where the mutants are first selected based on the phenotype reverse genetic approaches refer to the targeted discovery of mutations in genes known by their sequence [5].

TILLING was first developed and established in the model plant *Arabidopsis thaliana* [2] but was later successfully adopted to numerous animal and plant species. The technique is so far implemented in crops such as pea, soybean, maize, barley, rice, wheat, sorghum and in the orphan crop tef [8, 9, 10]. Thus, since its first description in the year 2000 [2] TILLING gained a lot of popularity [9]. TILLING can also be useful in inducing a wider genetic diversity in the genomes of domesticated species. Classical breeding approaches such as domestication and selection are facing the problem of limited genetic diversity in adapted lines since much of the genetic variation available in wild crop progenitors has been lost. TILLING can introduce genetic variation directly to elite germplasms without the need to acquire variation from exotic cultivars avoiding the introduction of agriculturally undesirable traits. In this case, several backcrosses to the parent cultivar remove unlinked mutations and result in a novel allele in the parental background [11, 12].

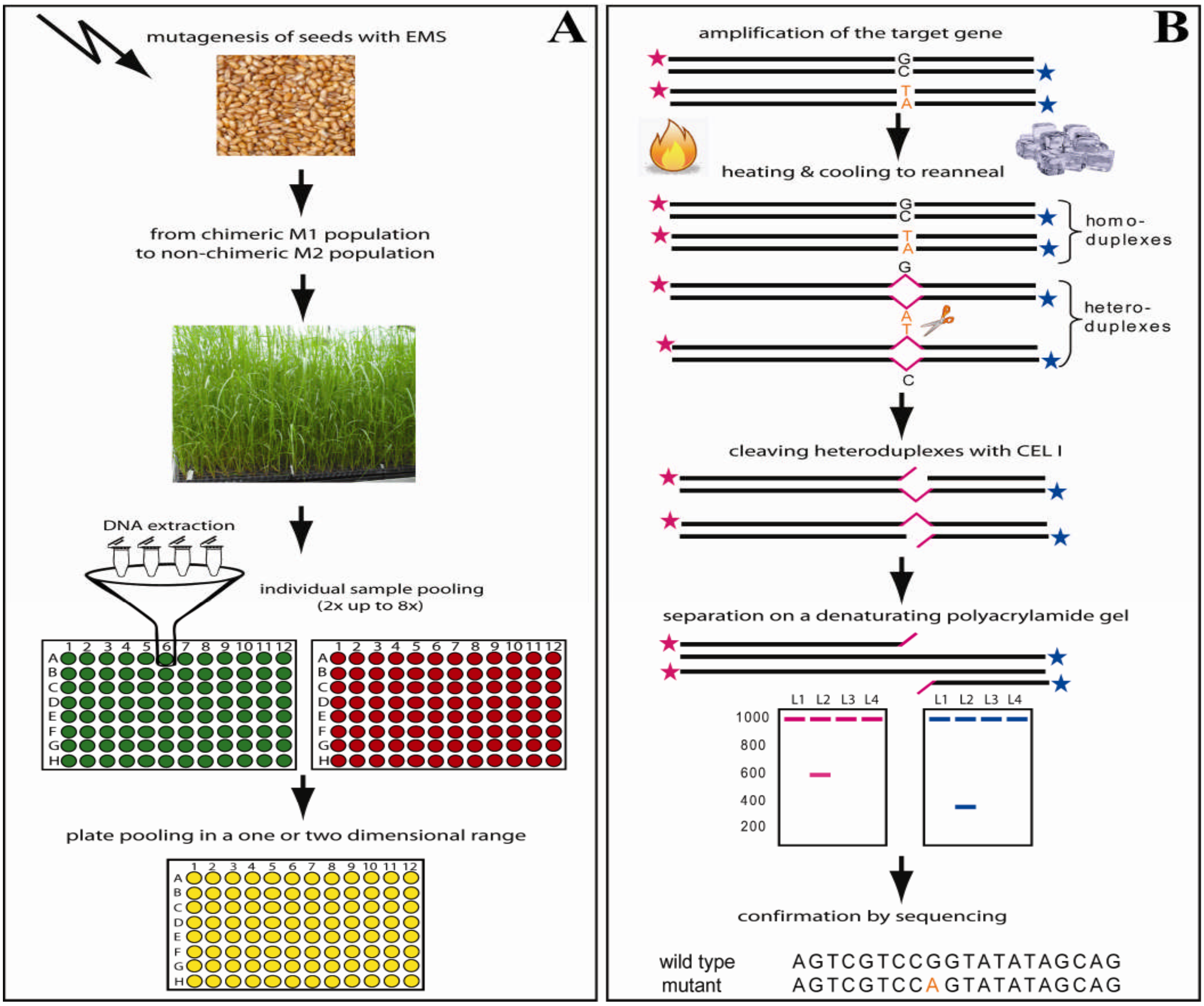
A general overview of the TILLING method is given in **Fig. 1.** Till et al. [13] present a simple and efficient protocol of the technique. In general the technique of TILLING comprises the following main steps: i) mutagenesis, ii) development of a non-chimeric population, iii) preparation of a germplasm stock, iv) DNA extraction and sample pooling, v) screening of the population for induced mutations as well as validation and vi) evaluation of the candidates [7, 9].

In addition, TILLING can also be applied to detect naturally occurring single base changes known as SNP (Single Nucleotide Polymorphism) that correspond to the randomly induced mutations. This adapted method is known as EcoTILLING [14].

Mutagenesis is the crucial step in TILLING process

The starting point of TILLING is the mutagenesis of seeds or pollen to induce single nucleotide changes [11]. Conventional chemical mutagenesis has a long history in crop breeding and the broad experience simplifies its application [4, 7, 9, 12, 15]. The technique of TILLING can be

Fig. 1 shows the diagrammed TILLING procedure. Panel A) starts with the beginning of the TILLING process, the mutagenesis of the seeds with EMS. Subsequently the seeds are grown to chimeric M1 plants. These plants are selfed and a single seed of each plant is used to establish the screening population (M2 population). M3 seeds are used for further investigation. Tissue samples from M2 plants are used for the DNA extraction. DNA samples are normalized to assure equal concentrations and subsequently pooled 2x up to 8x in a one- or two dimensional range. The example above shows 4x pooling in a two dimensional range. After preparation of the DNA samples the amplification of specific target genes follows with specific labelled and unlabelled primer combinations (Panel B). The amplified DNA strands are then denatured by heating and slowly cooled down to allow heteroduplex formation. Mismatches that occur in heteroduplex molecules are cleaved with the enzyme CEL I. Finally the purified PCR products are separated on denaturing polyacrylamide gels for mutation detection. Cleaved PCR products are visible each time in one of the two channels of a LI-COR DNA analyzer and sum up to the full-length fragment size. The last step shows the confirmation of candidates by sequencing and phenotypic examination (not shown).



applied to nearly all species even to orphan crops that lack well developed genetic tools [16]. In addition since no exogenous DNA is introduced into the plant, the technique is considered as non-transgenic and the products are exempted from regulatory restrictions that are imposed on the transgenic products [11, 15, 16].

A number of mutagens are used to create mutations in different organisms. Broadly the mutagens are grouped into two: i) *chemical mutagens* including ethyl methanesulfonate (EMS), sodium azide, N-methyl-N-nitrosourea (MNU), methyl methanesulfonate (MMS), hydrogen fluoride (HF) and hydroxylamine, and ii) *physical mutagens* such as gamma- and x-ray. Mutations are randomly induced and target virtually every gene depending on the mutation density [8, 17]. The advantage of chemical mutagenesis is that it creates an allelic series of mutations. Three types of mutations are recovered from chemical mutagenesis: i) *truncation or nonsense mutation:* where a single base pair change coverts an amino acid codon into a stop codon, ii) *missense mutation*: in which a single base pair change alters the amino acid encoded by a particular codon and can be distinct into conservative and non-conservative changes and iii) *silent mutation*: where a single base pair does not alter the amino acid encoded by a particular codon [2]. EMS is the most commonly used mutagen especially for TILLING experiments because it induces point mutations [15]. It specifically creates a G:C to A:T transitions since it alkylates G residues which then pair with T instead of C [7, 8]. On average, in the genome mutagenized with EMS, the following mutation rates are expected: 3% truncations, 50% missense and 48% silent mutations [16].

The allelic series of induced mutations can potentially confer to various phenotypes that range from subtle to strong. Mutations in the coding region of the gene might alter plant metabolism and maybe the effective level of a gene product that might be useful for breeding. In addition, splice site mutations that inhibit proper intron splicing, partial loss-of-function as well as novel-function alleles may occur [5, 9, 11].

The most important point to be considered while making mutagenesis is to balance between the mutation density and a feasible germination rate that is also linked to low sterility of the plants after mutagenesis [8, 18, 19]. Therefore, pilot studies need to be made before embarking large-scale mutagenesis in order to find the right mutagen, optimum concentration and proper handling of the chemical [9, 19]. In general, optimizing the concentration of the mutagen is difficult for diploid species since these plants have lower tolerance towards mutagens; hence increases the amount of mutagenized population to be screened [8]. On the other hand, polyploids show a higher tolerance due to complementation of essential genes by homeologous copies; therefore, expected mutations could be revealed from smaller sized populations [8]. However, in polyploid species genetic buffering makes it less likely that recessive mutations show a phenotype. Therefore, it may be necessary to identify mutations in each homeologous copy of the targeted gene and bring these together by crossing [8].

The mutation rate is estimated as the total number of mutations scored divided by the total number of base pairs screened, i.e. amplicon size x screened individuals [16]. According to Weil [10] one mutation per 500 kb or less is regarded as optimal. The highest mutation density was obtained from two polyploid wheats [18] i.e., one mutation per 25 kb in hexaploid wheat and one mutation per 40 kb in tetraploid wheat as compared to one mutation per 500 kb in maize and rice [7].

Detailed procedures of the TILLING method

Although some minor procedural differences are reported from various labs depending on the nature of plants and availability of resources, most TILLING experiments apply the following basic four steps.

1. Mutagenesis

For the majority of plant species, with the exception of maize, seeds are used as source of mutagenesis [7, 15]. Due to the multicellular stage of embryos in seeds, the first generation of mutagenized plants (defined as M1 population) is typically chimeric; i.e., different cells make different genotypes [9]. Hence, M1 plants are selfed and a single seed from each M1 plant is used to establish the M2 population. In comparison to the M1 plants, plants growing from M2 seeds are uniform, do not segregate in their cells and the induced changes are stable and heritable [15, 16]. Tissue samples are collected for DNA extraction from individual M2 plants while the seeds are harvested and long time stored from the M3 population [4].

2. DNA sampling

Genomic DNA is extracted from the tissues collected from individual M2 lines and subsequently normalized to achieve identical DNA concentrations for all samples. This will be followed by pooling of DNA samples from 2x up to 8x in a one- or two dimensional range. In addition to saving the cost and time of the screening, pooling also facilitates the detection of potential mutations [9, 15]. Two dimensional pooling has additional advantage in avoiding false positives since candidate mutants are visualized at two independent sites [9].

3. PCR amplification

PCR amplification is performed using a set of Infra-red dye (IRD) labelled specific primers for the gene of choice. The gene of choice refers to the gene that regulates the traits of interest. Even though prior information on the genome sequence is not required for TILLING, the presence of full-length genomic sequence for the gene of interest improves the chance of success and accelerates the development of suitable targets especially in designing effective primers [4, see also 8]. Specificity of the primers is important especially when various members of gene families are needed to be amplified and particularly in polyploid species [9, 11, 18]. Specificity of primers can be improved in polyploid species focusing on more divergent regions particularly in the intron region [12, 18]. Another approach to tackle the problem of gene copies in polyploid species is to pre-treat the genomic DNA prior to PCR amplification with a restriction enzyme that removes only one copy [16].

4. Mutation detection

PCR products amplified using fluorescent labelled primers are separated on a denaturating polyacrylamide gel for detection of mutations. In order to increase the efficiency of PCR amplification, unlabelled primers are added in the same PCR reaction with the labelled ones. The length of the amplified products could range between 0.3 to 1.6 kb [11]. However, earlier studies reported that it is difficult to detect mutations at the ends of fragments and normally 200 bp have to be excluded from the analysis; 100 bp from each side [19].

The PCR amplification is followed by the heteroduplex formation step where the amplified products are first denatured and then slowly cooled. In this step, heteroduplex molecules are formed due to the mismatches that occur when wild type and mutant DNA anneal [3, 6]. While at the beginning detection of single base pair differences were done using denaturating HPLC (DHPLC; [2]) nowadays single-strand cleavage is used to detect mutations [4, 6, 15]. The mismatches or heteroduplexes are recognized and cleaved by single-strand specific nucleases that are members of the S1 nuclease family such as CEL I and mung bean nuclease [7, 20]. CEL I is isolated from celery leaves and it is the most preferred enzyme for mutation detection in TILLING projects [13, 20]. The CEL I enzyme cleaves to the 3' side of mismatches and loop outs in heteroduplexes while leaving duplexes intact [4]. The CEL I digested products are purified (e.g. using Sephadex ® purification; [13]). Finally products are resolved on denaturating polyacrylamide gels. LI-COR DNA analyzer is the standard system for most TILLING projects.

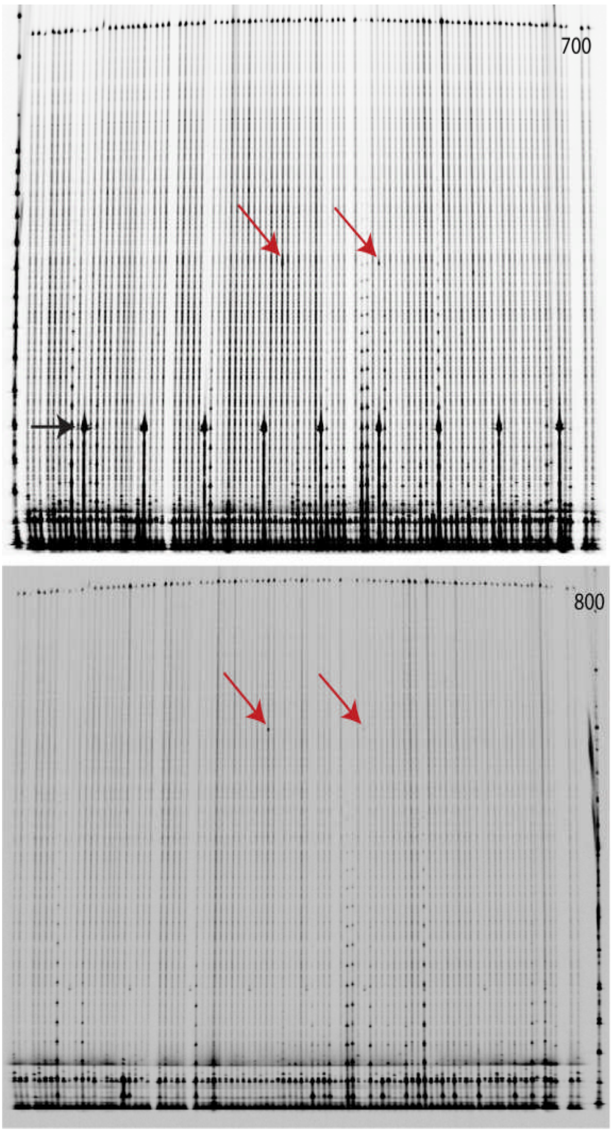
Since forward and reverse primers are labelled with different infrared dyes, cleaved products are visible in both channels of a LI-COR machine (**Fig. 2**). Confirmation for the right mutation detection is made when the two cleaved products observed in different channels sum up to the original PCR fragment size. Another important advantage of the mismatch cleavage is that it pinpoints to the location of the polymorphism making confirmation by sequencing quite efficient [6, 15].

Variations in mutation detection can also be done, for example, using capillary electrophoresis [6, 8] and recently agarose gels [8, 21].

Web-based tools applied in TILLING

Several web-based and freely available programs facilitate various operations in TILLING procedure. Some of the widely used tools are indicated below.

Fig. 2. Discovery of induced mutations in *Eragrostis tef* by TILLING. Individual DNA samples of mutated *E. tef* plants (M_2 population) were pooled 4x in a two dimensional range. The target region was amplified using a specific pair of infra-red labelled (IRDye 700 and 800) and unlabelled primers. After heteroduplex formation, samples were digested with the CEL I enzyme purified from celery juice. Mismatches were cleaved and samples were separated on a denaturating polyacrylamide gel after Sephadex purification. Gel electrophoresis was performed using a LI-COR DNA analyzer. For each gel run, two images were produced, one for DNA labelled with IRDye 700 (top) and one for IRDye 800 (bottom). The red arrows indicate cleaved PCR products and the molecular weight of the respective cleaved fragments detected in the 700 and 800 channel of the LI-COR analyzer sum up to the molecular weight of the full-length PCR product size. Due to the two dimensional pooling the two detected mutations refer to a single plant. The black arrow indicates a labelled 200 bp fragment that was applied to the polyacrylamide gel every 10th lane to facilitate the lane scoring. The analysis was



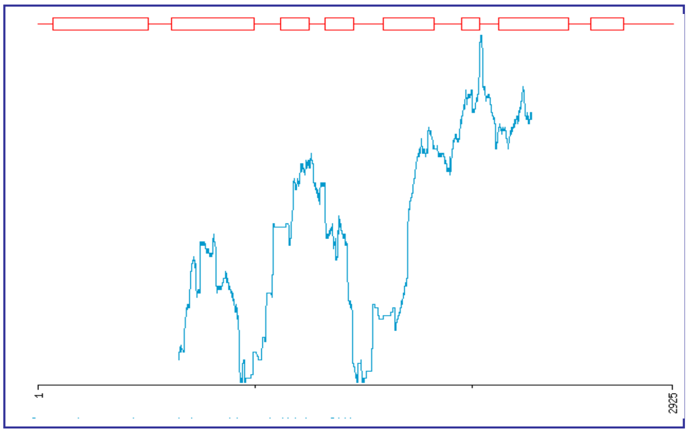
- *CODDLE* (Codons Optimized to Detect Deleterious Lesions; http://www.proweb.org/coddle/; accessed April 2010) allows to target a functional domain or the domain which is likely to be the most sensible to amino-acid substitutions (**Fig. 3**). The use of this program increases the probability of: i) detecting deleterious mutation in the gene of interest, and ii) obtaining regions with high frequency of stop codons and those which are evolutionary conserved hence useful for providing an allelic series [17].
- *GelBuddy* (http://www.proweb.org/gelbuddy/installissues.html; accessed April 2010) is used to automate band calling in the electrophoretic gels [13].
- *PARSESNP* (Project Aligned Related Sequences and Evaluate SNPs; http://www.proweb.org/parsesnp/; accessed April 2010) is useful in revealing the changes in the nucleotide and amino-acid sequences as well as documenting any restriction endonuclease site that have been altered [6].

SIFT (http://sift.jcvi.org/; accessed April 2010) is useful in predicting whether the change in the amino-acid has deleterious effect on the protein [4, 22].

Application of TILLING to the orphan crop improvement

To date, the technique of TILLING and/or EcoTILLING is applied to few orphan crops including cassava, banana and tef [9]. The cassava and banana projects are based at the Joint FAO/IAEA Program in Vienna while the Tef TILLING and EcoTILLING Projects are hosted by the Institute of Plant Sciences, University of Bern, Switzerland. Tef is a major cereal crop from Ethiopia that is closely related to finger millet. Similar to other orphan crops, tef adapts to diverse climatic and soil conditions and also tolerates many pests and diseases. In addition to its nutritional advantages, seeds of tef are also free of gluten for which a high number of people are allergic. However, the productivity of tef is limited mainly due to the prevalence of lodging. The tef plant has a tall and tender stem which is susceptible to damage by wind and rain. Therefore, developing lodging resistant semi-dwarf tef cultivars is the main goal of the Tef TILLING and EcoTILLING Projects. About 7000 EMS mutagenized M2 population plants and 500 accessions are generated for the TILLING and EcoTILLING, respectively. Genes known to control plant height in major crop species including the so called 'Green Revolution genes' of rice and wheat are used as a target. The current identification of a number of genes affecting plant height from major cereals crops including wheat, rice and maize (for review [23]) facilitate TILLING of plant height genes in tef. In addition, the information from the Tef Genome Sequencing Initiative (see this issue [24]) improves the application of TILLING in tef. Full-length genomic clones for two genes were isolated from tef. The two genes are homologs of the DWARF 4 gene [25] and the HIGH TILLERING and DWARF 1 gene from rice, respectively (HTD1, [26]).

Fig. 3. CODDLE analysis of the tef HTD1 gene (EtHTD1). In the above example the CODDLE program uses the genomic and cDNA sequence of the EtHTD1 gene to determine exons which bring nonsense and missense mutations when mutagenized by EMS. Since COODLE selected exons 5, 6 and 7 as high potential regions for the two types of mutations, our screening using TILLING focused on this part of the HTD1 gene. CODDLE is freely available at http://www.proweb.org/coddle/



One of the major obstacle in our Tef TILLING Project is related to the polyploidy nature of tef, as the species is allotetraploid, that is, each gene exists in two copies. In order to detect intended mutations, only a single copy should be amplified. This particular polyploidy problem is overcome by designing at least one of the two primers from the unique intron region in order to amplify a single copy at a time.

The detailed procedure we adopted for our Tef TILLING project is briefly indicated below:

- *DNA extraction* using the Machery-Nagel NucleoSpin® 96 Plant extraction kit
- *DNA normalization* to 5 ng and 4x pooling in a two dimensional range
- *Amplification of target genes* (single step PCR using GoTaq polymerase; Promega); PCR products vary between 770 bp-1140 bp
- *Heteroduplex formation and digestion* of mismatches with the CEL I enzyme
- *Separation on denaturating polyacrylamide gels* (LI-COR DNA analyzer)
- *Detection and sequencing of the candidates* as well as phenotypic confirmation

So far, by screening 3264 mutants using the DWARF 4 gene, four candidates were recovered. Similarly, by screening 4224 plants for the HTD 1 gene, 12 candidates were obtained. These initial results show that the frequency of mutation in tef for the two genes is one mutation per 465 kb for DWARF 4 and one mutation per 348 kb for HTD1. The next steps are to further investigate the phenotype of the candidate lines and to screen more mutants using other plant height-related genes.

Summary and future perspectives

TILLING is a general, easy and non-transgenic reverse genetic method that was first described for *Arabidopsis thaliana*. Quite rapidly the method was adopted to several plant and animal species including widely cultivated crops such as rice, wheat and maize. In addition, there is a growing interest to apply TILLING to orphan crops that lack well developed genetic tools. The technique is proved to be efficient in obtaining desirable mutant lines of agronomic importance. However, before new cultivars will be released to the farming community a number of backcrosses (at least four) should be done to remove undesirable traits [9, 11].

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IMPORTANCE OF TISSUE CULTURE FOR ORPHAN CROPS

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Abstract

Tissue culture is one of the most basic biotechnological tools available in sub-Saharan Africa (SSA), and its applications are varied and vast. The technique has contributed tremendously to the safeguarding, improvement and distribution of orphan crops, especially the vegetatively produced crops. As a tool, it has been a driver for biotechnological advances made in orphan crops, both for research as well as commercial purposes. Tissue culture is also a vehicle to most efficiently deliver important biotechnological products such as genetically modified orphan crops. Commercial micropropagation, despite potential pitfalls, is essential tool to distribute crops such as cassava and banana to smallholder farmers in SSA.

Keywords: micropropagation, orphan crop, tissue culture

1. Introduction

In sub-Saharan Africa, several orphan crops are essential for food security and income generation of smallholder farmers, ensuring their livelihood. Orphan crops can be organized as fruit crops [including banana and plantain (*Musa* spp.)], root and tuber crops [cassava (*Manihot esculenta*), sweetpotato (*Ipomoea batatas*), yam (*Dioscorea* sp.), enset (*Ensete* spp.), taro (*Colocasia esculenta*) and *Plectranthus* spp.], cereals [pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana*), foxtail millet (*Setaria italica*), fonio (*Digitaria* spp.) and tef (*Eragrostis tef*)], legumes [cowpea (*Vigna unguiculata*), groundnut (*Arachis hypogaea*), grass pea (*Lathyrus sativus*) and bambara groundnut (*Vigna subterranea*)], and oilseed crops [sesame (*Sesamum indicum*) and noug (*Guizotia abyssinica*)].

Some of these orphan crops are relatively better researched than others, and it is therefore dangerous to lump them all into the same category. For example, research into bananas on the continent has been implemented by the International Institute of Tropical Agriculture (IITA) and the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) since decades, and has covered in depth not only upstream issues (pests and diseases, conventional breeding, etc.) but also downstream ones (market pathways, product diversification, etc.). It is equally encouraging to notice that The Alliance for a Green Revolution in Africa (AGRA), a joint venture between the Rockefeller Foundation and the Bill and Melinda Gates Foundation, is taking a proactive interest in some orphan crops like cassava and cowpea [1]. However, all crops have in common that they are largely ignored by the international community and are almost solely investigated by Africa-based international organizations and their national partners.

A large discrepancy exists between the potential role of these crops in improving food security and livelihoods, and the low levels of private or public investment they have received [1, 2]. The reason why these orphan crops receive relatively less attention in sub-Saharan Africa compared to, for example, maize (*Zea mays*) and rice (*Oryza sativa*) is unknown and surprising. For example, sorghum and pearl millet are more important than rice and wheat, both in area and in contribution to diet. Cassava is the third most important source of calories in Africa [3]. In Uganda, the average annual per capita consumption of banana is estimated at 207 kg [4]. Furthermore, many of these orphan crops, such as cassava, are known for their hardiness and tolerance to adverse environmental conditions such as infertile soils [5]. Finally, many of these orphan crops, cultivated mainly in Africa, are less vulnerable to price fluctuations on international markets and act as safe havens when international prices increase.

2. Tissue culture as a research tool for orphan crops

Tissue culture is a wide concept, and involves the culture or maintenance of plant cells or organs in sterile *in vitro* conditions. The technique has been applied to only a number of orphan crops, and most applications included the exchange of breeding material and the production of disease-free germplasm through micropropagation [6]. Nevertheless, applications of tissue culture are varied and vast, and examples from orphan crops are highlighted below.

2.1. Germplasm conservation

Orphan crops are very diverse in terms of their genetic, agroclimatic, and economic niches [7]. This diversity needs to be captured, and tissue culture techniques such as cryopreservation are essential for the conservation of large gene pools of especially vegetatively propagated crops. At IITA, an extensive *in vitro* genebank of cassava and yam is maintained for worldwide distribution [8]. Such genebanks function as a foundation for conventional breeding. As a matter of fact, molecular marker characterization of genetic diversity from genebanks appear to be the most widely used biotechnological technique on orphan crops [6].

2.2. Recombinant proteins and biopharmaceuticals

Plant cells can be cultured commercially inside liquid culture in large bioreactors as a source of secondary products like recombinant proteins or biopharmaceuticals. Examples of such technology among orphan crops are few, although, based on their intrinsic diversity and unexplored potential, the production of secondary metabolites from orphan crops could be worthwhile. For example, Jin *et al*. [9] used the hairy root technique to mass produce recombinant phytase from sesame.

2.3. Conventional breeding

Tissue culture is very important for breeding of clonally propagated orphan crops because it allows movement of sufficient quantities of pathogen-tested plants for safe multi-locational screening, not only internationally but also within large countries.

Several specialized tissue culture techniques exist that are fundamental to breeding efforts in orphan crops. *Anther culture* (androgenesis) generates haploid plants from microspores, and significantly speeds up crosses between distantly related species. If barriers are present that prevent development of interspecific crosses, it is sometimes possible to aseptically culture the plantlets from the embryo in a technique called *embryo rescue*. For example, embryo rescue is an essential technique, used for decades, in banana breeding. Another, more complicated method to cross distantly related species is by *protoplast fusion*. Tissue culture techniques are also being used for production of *dihaploid plants* to reduce breeding cycle by obtaining pure line for further improvement in crops like tef [10]. Finally, using *somaclonal variation* and *in vitro* mutagenesis, plant breeders have actively used tissue culture systems to create variability in their breeding programs.

One of the more recent promising applications of tissue culture is that it allows plant breeders to screen for advantageous characters in cells rather than plants, thereby greatly reducing time and costs. Studying cells opens the way for genomics, and the related fields of proteomics and metabolomics. Ngara et al. [11] produced cell cultures of sorghum (*Sorghum bicolor*), which provided them with a continuous supply of experimental units.

2.4. Recombinant DNA technology

Although genetic transformation can be performed on explants, protoplasts or cell suspensions obtained through tissue culture are usually the starting material for genetic transformation. Somatic embryogenesis followed by regeneration of transgenic plants is often a central component in genetic modification. For example, most transformation protocols for banana use cell suspensions, although systems are being developed that are based on organogenesis from meristematic tissues [12].

Plant regeneration is a prerequisite for successful transformation. However, this tissue culture step is sometimes the most difficult and limiting step in the development of genetic engineering technology for orphan crops such as sorghum and millet [13]. O'Kennedy *et al*. [14] established an improved regeneration system for pearl millet using immature zygotic embryo explants. The lack of an efficient regeneration system had also slowed the improvement of cowpea, an orphan legume crop. However, Diallo *et al*. [15] reported an efficient regeneration method from cotyledonary node explants, which is different from that of other *Vigna* spp.

Plant generation systems are often highly genotype-dependent and therefore problematic especially in orphan crops due to their genetic divergence. Successful millet tissue cultures show strong genotype dependency and rapidly lose their morphogenic capacity after subculturing [13]. In cassava, an efficient regeneration system based on embryogenesis has been developed but it is, however, limited to a few cultivars. Development of regeneration systems that are genotype-independent are important for orphan crops.

Another challenge is the optimization of gene delivery system for orphan crops. Although efficient transformation techniques have established for model crops, the same method can not be applied to other crops including orphan crops. Hence, optimum transformation method has to be investigated for each crop type. This might require special facilities and significantly increase the cost and time of study. Although, cowpea is known to be recalcitrant to transformation, efficient DNA delivery techniques has been recently developed [16].

3. Commercial tissue culture for orphan crops

3.1. Meristem culture: virus elimination

Meristem culture is a technique used to eliminate viruses from virosed plants based on propagation from meristematic tissues. Virus elimination techniques have been used for the last 20 years in South Africa for the production of disease-free planting material for sweet potato. All commercial plantings of sweet potato in South Africa use these types of matrials in order to suppress the level of disease present in the field [17].

3.2. Micropropagation: mass production

Micropropagation uses meristem and shoot culture on stock plants to rapidly produce large numbers of clonal plantlets that are pest- and disease-free. Somatic embryogenesis, in conjunction with genetic transformation, can also be used for mass production. For many plants, healthy seeds are easily produced in great numbers, rendering micropropagation not applicable. However, micropropagation is particularly important for sterile plants (i.e., in the absence of seeds or pollinators to produce seeds), that have low-germinating seeds, do not produce enough seeds or produce seeds that cannot be stored (recalcitrant seeds).

Many of Africa's orphan crops, such as cassava, banana, sweet potato and yam, are vegetatively propagated [8, 18, 19] and are consequently perfect candidates for commercial micropropagation. Since orphan crops are genetically diverse, new cultivars adapted to local conditions can be constantly developed. Efficient distribution channels for these improved cultivars, however, remain a problem [2]. Micropropagation is ideal when it comes to rapidly upscale production and delivery large quantities of superior cultivars and as such a perfect distribution channel for these improved yet underutilized cultivars [6].

3.3. The delicate balance between private and public sector

By 1992, very few countries in sub-Saharan Africa had reached the take-off stage for large-scale micropropagation of important crops. However, several countries had established biotechnological centers of regional or international character specializing in micropropagation of not only cash crops such as coffee (*Coffea* spp.) and vanilla (*Vanilla* spp.), but also orphan crops such as banana, cassava and cowpea [20].

Ultimately, micropropagation for mass production should be carried by the private sector, which fills a niche in sub-Saharan Africa by focusing on commercial production of orphan crops. In West Africa, some successful public-private partnerships have been set up for this purpose. In 1986, CIRAD set up the subsidiary Vitropic (Saint-Mathieu-de-Tréviers, France) to produce disease-free banana plants [19]. Especially banana micropropagation has seen a rapid commercial growth in some countries in East Africa, with a handful of small- and medium-scale enterprises collectively producing in excess of more than 1.5 million banana plants per year in Burundi, Kenya and Uganda. Although the entry barrier is steep, the “tissue culture business” is very lucrative for the entrepreneur who engages in it.

In several countries in Africa, however, tissue culture orphan crops continue to be commercially produced by both the private and public sector, often with their roles blurred. In Zimbabwe, for example, both academic institutions and private organizations have been actively involved in supplying tissue-cultured planting materials to smallholder sweet potato farmers [21]. In Uganda, Kenya and Burundi, private companies compete with universities and research organizations in production of banana. On the other hand, researchable issues are sometimes too lightly transferred to the private sector, under the label of “public-private partnerships” and in vogue with some of the donor’s current perceptions. Roles for each should be clearly defined, so that donors and governments can engage in more efficient use of taxpayer money.

The burden for crop improvement will continue to solely fall onto the shoulders of the public sector. Sometimes, the public sector can play a temporary but essential role in micropropagating orphan crops with little commercial value even for local tissue culture laboratories. In South Africa, the Agricultural Research Council (ARC) micropropagated Livingstone potato (*Plectranthus esculentus*), a popular semi-domesticated orphan crop once part of the diet of rural communities but whose planting material became neglected. Tissue culture was used to rapidly produce plantlets and reintroduce them to the Northern Province to benefit resource-poor farmers [22]. Another temporary role of the public sector focuses on the development of proper tissue culture protocols (e.g. optimal growth media, reduction of off-types or reduction of secondary metabolites during multiplication).

3.4. Dangers and bottlenecks of commercial tissue culture

One of the biggest dangers for sustainable commercial tissue culture is the lack of phytosanitary and quarantine conditions, in the form of certified standards, codes, protocols and laws that are regionally harmonized. Such conditions are especially important to avoid spread of viruses, which are easily transmitted through tissue culture. In the case of banana, viruses such as Banana Bunchy Top Virus (BBTV) and Banana Streak Virus (BSV) are widely distributed on the continent [23, 24] yet implementation of harmonized virus indexing schemes are largely absent in East Africa, despite the fact that tissue culture bananas are being moved across borders in ever increasing quantities. Based on the experience from East Africa, where the private sector is outpacing implementations from public phytosanitary and quarantine institutions, it is clear that the capacity to use tissue culture to generate clean planting material must be developed in tandem with efficient virus indexing mechanisms. Elements of such mechanisms include rapid diagnostic kits for detection during import inspections, certification of nursery-propagated materials, establishment of independent institutes that set and implement standards, and properly trained personnel [25, 26].

Physical infrastructure is expensive, while human capacity is often lacking. The costs of establishing a tissue culture laboratory in sub-Saharan Africa is relatively high, since most of the equipment and chemicals are imported at elevated costs [25]. In addition, water and electricity supply is sometimes erratic, further elevating the cost. These elevated production costs are ultimately off-loaded onto costumers.

A danger for a healthy commercial tissue culture sector is the lack of sustainable market pathways to deliver the plants to the farmer. In Burundi, tissue culture plants are bought from the private sector but given for free to farmers by donors. This temporary solution is not sustainable and reduces demand and flow of improved seeds, fertilizers, tools and pesticides [27]. In some cases, commercial supply is trailing demand, which can be caused by private sector players focusing on large orders from donors, rather than small ones from farmers. In Zimbabwe, farmers increased yields and economic returns when growing tissue culture sweet potato compared to conventional material, but they were also constrained by an inadequate supply of improved planting material [21]. To make tissue culture systems sustainable, they often involve propagators as distribution points between the tissue culture laboratories and the farmers [25].

In sub-Saharan Africa, distributing planting material alone will not ensure a good crop. Whereas commercial farmers are skilled in juggling the inputs and effort needed to produce and make profit from crops, small-scale farmers are constrained by factors such as lack of land, capital, access to technology and good marketing infrastructure [2]. Hence, efficient distribution systems need to deliver the tissue cultured plants as part of an agronomic package, including training and access to micro-credit. Most of the current grants awarded by The Alliance for a Green Revolution in Africa (AGRA) address seed development programs [28]. A strong focus is on policy support and market development, and AGRA has set aside significant funds for creating conducive environments for agro-business development, including market development of tissue culture banana in East Africa.

Finally, for reasons of convenience, cost reduction and uniformity, biotechnology involves the passage of germplasm through narrow genetic bottlenecks. This is especially the case for commercial micropropagation [6, 29] and an aggravated danger for orphan crops that are heralded as resilient partly because of their genetic variability.

4. Tissue culture as a driver and a vehicle for biotechnology

4.1. Tissue culture as a driver

Tissue culture is a driver for biotechnology in two ways. First, as is demonstrated above, it lays at the basis of most other biotechnological technologies, from conventional breeding to genetic engineering. Although many countries in sub-Saharan Africa are becoming less hostile to genetically modified foods and many believe that transgenic technologies offer the key to unlocking the full potential of crops like cassava and banana [30], several scientific, legal, economic and political barriers exist to their widespread acceptance. As a result, genetically modified orphan crops, as opposed to tissue cultured ones, are still relatively a long way from routine use by smallholder farmers [8, 31].

Second, simple tissue culture techniques are the initial stepping stone for development of more advanced biotechnological research capacity in Africa because they are the easiest to implement. By focusing on tissue culture, the skills necessary to maintain and manage a biotechnology laboratory can be developed. The second phase is the application of more advanced biotechnological tools, such as molecular marker applications, ultimately leading the way for the third phase, which is the development of capacity to produce transgenic plants [18, 19]. Tissue culture capacity seems to be well present across sub-Saharan Africa. For instance, a recent survey conducted in 12 sub-Saharan countries indicated that national programs in all countries implement tissue culture, but only three of them apply genetic engineering [32].

4.2. Tissue culture as a vehicle

In the vegetatively propagated orphan crops, transgenic technologies might have the significant impact [30]. However, successful application of advanced biotechnologies is conditional on connecting the science to downstream delivery efforts [3]. Unfortunately, many of the laboratories that have the capacity to produce transgenic plants still lack the ability to commercialize the product or ensure that these plants reach the end user, i.e. the African farmer. To bridge this gap, it is necessary to form partnerships with either seed companies, producer organizations or government institutions who can ensure that the sophisticated technology be delivered in the most well known and accepted technology known to farmers: the seed [33]. In the case of the vegetatively produced orphan crops, this often translates in a vibrant commercial micropropagation sector, as detailed above.

5. The need for local capacity

In the future, the genetic and biotechnological improvement of orphan crops is confined to local and specialized research at specific crop centers within Africa [19]. In sub-Saharan Africa, there are many research organizations and universities with well established biotechnology facilities [8]. While some of the technologies from the west might be appropriate, many of the solutions in the future need to come from African research laboratories focused on African constraints [2]. Insights and tools with practical utility for orphan crops can be obtained from research obtained using model species and major crops [3]. However, local capacity in developing countries will still need to be built to address specific local problems [8, 33, 34]. Advanced tissue culture systems such as embryogenesis need to be adapted to suit germplasm, which requires time and resources. In the case of cassava, for example, it is important that development of tissue culture systems required for the transformation of specific cassava varieties be carried out within the respective cassava-growing regions.

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SIGNIFICANCE OF GENOME SEQUENCING FOR AFRICAN ORPHAN CROPS: THE CASE OF TEF

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Abstract

Knowledge on genome sequence is of paramount importance for the application of modern genetic improvement programs. A genome sequence can provide a better understanding of the tolerance mechanisms conferring adaptability to adverse agronomic conditions. In order to unleash the potential of genetic improvement, we have initiated the Genome Sequencing Project on Tef (*Eragrostis tef*), an African under-researched crop. Tef is a small grain cereal widely cultivated in the Horn of Africa, particularly in Ethiopia. The crop is tolerant to several biotic and abiotic stresses. Tef performs better than other cereals in poorly drained vertisols, dominant in the highlands of Ethiopia. Despite its importance, tef suffered from a lack of genomic research and is commonly classified as an 'orphan crop'. The first five runs made using the 454 Next Generation Sequencing Platform generated each about 400Mbp. After assembly the sequences are 244 Mbp with an average size of 610 bp. In order to validate the quality of the sequences, we analysed the phylogenetic relationship between the so called 'Green Revolution' gene from major crops and the orthologous tef gene. The results from the pilot sequencing showed promising results and call for the completion of sequencing the whole genome of tef at reasonable cost.

Keywords: Genome sequencing, Next Generation Sequencing (NGS), *Eragrostis tef*, gibberellic acid insensitive, shotgun sequencing, paired-end sequencing

Introduction

Under-utilized or under-researched crops, commonly referred as 'orphan crops' are those crop plants that for historical and economical reasons did not benefit from intensive breeding programs. These plants did not benefit from the genetic improvements due to Green Revolution that dramatically increased cereal production. Genetic improvements have been hindered by a lack of research investments. Molecular tools like genetic maps, mutant collections, molecular markers, DNA libraries, and genomic sequences were so far not affordable. With the advent of the second generation sequencing platforms based on nanotechnologies, the generation of sequence information become cheaper. The number of organisms whose genome has been or is being sequenced is rapidly growing. In this new scenario, also crop plants with a marginal economical importance can profit from these new technologies (see **Table 1**). At least two factors influence the choice of species being sequenced: i) *genome size*: due to technical limitations in most cases organisms with smaller genome size are chosen for sequencing, and ii) *genetic distance*: the phylogenetic distance from previously sequenced organisms is also important for ease of transfer of information on genome structure and function from a well-characterized species to the related but less studied species.

Importance of tef in the economy of developing country

Tef is a staple food in East Africa particularly in Ethiopia where it is annually cultivated on about 2.5 million hectares of land [11]. The tef plant possesses a number of advantages: i) the crop performs better than other major cereals under extreme environmental conditions such as drought and water-logging; ii) the tef flour does not contain gluten, making it particularly suitable in the diet of celiac consumers; iii) the seeds are less attacked by storage pests, hence, can be stored for long time without losing viability; and iv) the crop requires small agronomical inputs, making it particularly suitable for extensive and sustainable agriculture [12]. Tef is therefore important in maintaining food security in rapidly evolving environmental conditions, by allowing its cultivation in adverse regions where high input crops would normally fail.

Nevertheless, tef presents a major noteworthy disadvantage: its yield is very low. In Ethiopia, where tef is extensively grown, the national average yield is only 0.9 t/ha as compared to 1.7 t/ha for wheat [13]. The major yield limiting factor is in tef lodging, i.e. the permanent displacement of the stem from the upright position. Tef has a tall and weak stem that falls on the ground due to wind and rain. The application of nitrogen fertilizers also aggravates lodging. The lodged plants produce inferior yield both in terms of quantity and quality. Because of its very local cultivation and marginal economical relevance, Green Revolution was not implemented on this particular crop. The majority of research on tef has been so far undertaken in Ethiopia, using mainly conventional breeding techniques. Up to date, the Ethiopian research institutes released 17 improved cultivars to farmers, seven of which are from hybridization while the remaining ten from selection [14].

Need for sequencing the tef genome

Whole genome sequencing is particularly important for the improvement of the orphan crops such as tef for which few genetic and genomic resources are available. Tef is an allotetraploid species ($2n = 4x = 40$). To

Table 1. Genome size and the status of genome sequencing for crop plants

Common name	Scientific name	Polyploidy level	Genome size	Current state of work
Apple	*Malus domestica*	2n=2x=34	750 Mb	Genome published [1]
Barrel medic	*Medicago truncatula*	2n=2x=16	550 Mb	Genome published [2]
Canola	*Brassica napus*	2n=2x=38	1129Mb	Completed
Cassava	*Manihot esculenta*	2n=2x=36	760 Mb	Completed
Cotton	*Gossypium raimondii*	2n=2x=26	880 Mb	In progress
Cucumber	*Cucumus sativis*	2n=2x=14	367 Mb	Genome published [3]
Grape	*Vitis vinifera*	2n=2x=38	500 Mb	In progress
Maize	*Zea mays*	2n=2x=20	2600 Mb	Genome published [4]
Papaya	*Carica papya*	2n=2x=18	372 Mb	Genome published [5]
Peach	*Prunus persica*	2n=2x=16	270 Mb	Incomplete
Poplar	*Populus trichocarpa*	2n=2x=38	480 Mb	Genome published [6]
Potato	*Solanum tuberosum*	2n=4x=48	840 Mb	Completed
Rice	*Oryza sativa*	2n=2x=24	430 Mb	Genome published [7, 8]
Sorghum	*Sorghum bicolor*	2n=2x=20	736 Mb	Genome published [9,10]
Soybean	*Glycine max*	2n=2x=40	1115 Mb	Completed
Tomato	*Solanum lycopersicum*	2n=2x=24	950 Mb	Completed

date the two diploid ancestors that originated its genome by hybridization remain uncertain [15]. Despite its ploidy level, tef maintains a medium genome size (730Mbp) [16], almost the same size as sorghum [10]. However, tef is allotetraploid whereas sorghum is diploid, suggesting a double gene density in tef. Phylogenetically, sorghum is also the closest sequenced genome to tef and represents a template for comparative studies. Partial transcriptome sequence information, consisting of an EST collection of 3603 sequences has been developed and used to construct a genetic map derived from an interspecific cross (*Eragrostis tef* x *E. pilosa*) [17,18]. From this work, a QTL analysis on 22 yield-related and morphological traits has been performed [19]. Given the available molecular tools and the new cost-effective sequencing technologies, sequencing the gene-dense genome of tef has become not only feasible but also a compelling necessity. Some of the specific advantages of sequencing the tef genome are i) sequence information for any gene of interest will be available, including promoter and terminator regions, hence avoiding the need of predicting gene sequences by homology to well-characterized model plants like rice and sorghum; ii) being tef a tetraploid, the complete genome sequence will facilitate the isolation of a specific gene, while distinguishing between the two possible orthologous copies. This is of special interest during the implementation of TILLING (Targeting Induced Local Lesions In Genomes) techniques [20], where the presence of an orthologous variant could be erroneously interpreted as a mutant; iii) genomic sequence information will provide a template for the development of genetic markers such as Single Nucleotide Polymorphisms (SNPs) and Simple Sequence Repeats (SSRs). These high-throughput molecular markers are more and more employed for marker-assisted breeding, for the construction of high density genetic maps and for linkage disequilibrium studies on diverse germplasm; molecular applications such as gene cloning, gene overexpression, or down-regulation of genes of choice will be achieved with minimum effort and finally; iv) tef is tolerant to many adverse climatic and soils conditions, especially to water-logging. The understanding of the molecular basis of tolerance mechanisms gained from the genome sequence of this unique plant can possibly be transferred to other economically important crops with higher input requirements.

The Tef Genome Sequencing Initiative

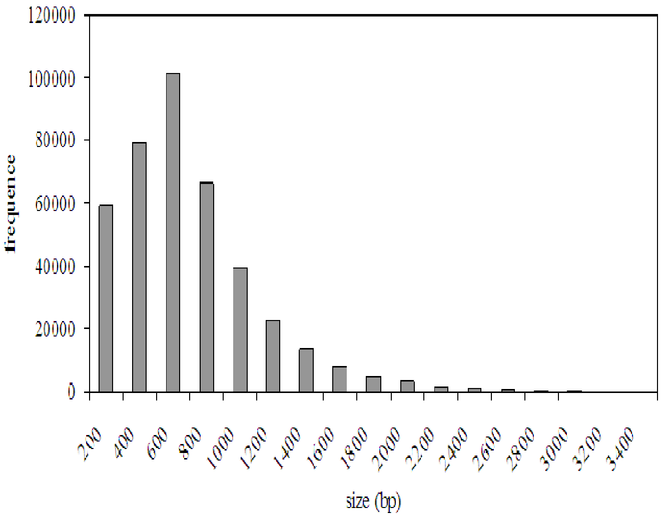
The Tef Genome Sequencing initiative began at the end of 2009. The early maturing and wide adaptable tef cultivar DZ-Cr-37 was selected for the sequencing. The main objectives of the tef sequencing initiative are, i) to sequence, analyze and annotate the tef genome, and ii) to make publicly available all generated information. The strategies to be used in sequencing the tef genome are the following: i) fragment sequencing up to 10x coverage of the genome; ii) paired-end sequencing of libraries with different insert sizes. This strategy will help to overcome assembly problems related to polyploidy; iii) BAC sequencing will be done to improve the final assembly if necessary.

The Pilot Sequencing Project

In order to investigate the quality of the sequencing obtained from the Next Generation Sequencing (NGS), we run a pilot project using 5 runs of the Roche 454 GS-FLX Platform [21] producing a total of 245 Mbp reads. These were clustered and assembled using the Newbler software from Roche, with the default settings of 98% similarity and 50 bp of overlap. The assembly consists of 402 304 contigs with a minimum length 100 bp. Of these, 214 750 contigs were longer than 500 bp, the biggest contig has currently a length of 17 200 bp (see **Fig. 1** for the sequence length distribution of the assembly).

A blastx search [22] with a threshold of e^{-10} was performed on tef contigs to identify sequences with homology to the proteome of sorghum (version sbi.1.4, 34496 loci [10]). The low stringency threshold of e^{-10} was adopted to compensate for partial and fragmented tef sequences. Under these blast parameters, 92% of the sorghum loci had a hit in our tef contigs with an e^{-10}. Nevertheless, the low stringency threshold used can lead to misidentification of paralogous and orthologous gene copies [23]. To exclude multiple hits

Fig 1. Frequency distribution of the tef contigs after assembly

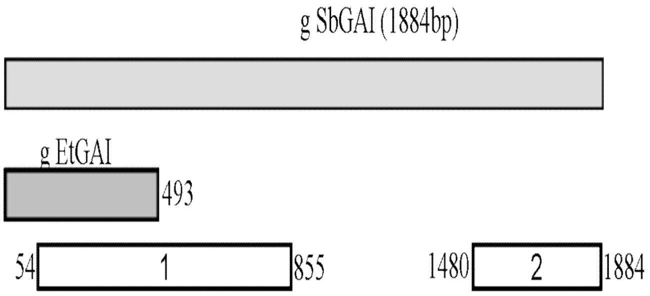


to non-orthologous gene family members, we repeated the blast search using a subset of 5796 sorghum genes with low homology to any other gene in the genome. This set consists of only those genes that do not have any other match in the sorghum genome with a blastn threshold of e^{-10}. Within this 'non-redundant' subset only 55% of the genes had a hit in the tef database.

Due to our interest to obtain semi-dwarf tef lines that are tolerant to lodging, we used sequences from the pilot sequencing to analyze the so called Green Revolution genes that boosted the yield of wheat and rice in the 1960s and 70s. As case study we focused on the Gibberellic Acid Insensitive (*GAI,* [24]) gene from the model plant Arabidopsis. The corresponding genes in crops species are Reduced height (Rht) in wheat, Dwarf 8 (d8) in maize, and Slender 1 (SLR1) in rice. The mutants of this particular gene in the indicated species are affected in size and do not respond to the exogenous application of gibberellic acid ([25 to 29]). In order to validate the quality of the sequencing, the orthologous copy of the GAI gene was identified from the tef contigs. Tef amplicons of genomic and coding DNA of the GAI gene were obtained with specific primers (EtGAI_S1: 5'- ATGGAAGCGCGAGTACCAAG -3' and EtGAI_AS1: 5'- ACCACCGGTAAGGAGATCG -3') designed on the contigs generated within this pilot sequencing project. Sequences of the orthologous gene from related grasses were obtained from the NCBI database [30] (sorghum: "XM_002466549", sugarcane: "DQ062091", maize: "AJ242530", pearl millet: "FJ011693.1" and rice: "AB262980"). The alignment of these sequences with the contigs of tef was done using ClustalW [31]. Then it was manually refined and pairwise comparison were confirmed with BLAST [22]. A genetic tree was generated with the program MEGA 4.1 [32], using the neighbour joining method under Jukes-Cantor model with a bootstrap value of 1000 replications.

Fig 2 shows the current state of work for GAI homology in tef. From the sequences made so far, two tef contigs with the size of 730 and 398 bp having homology to the 5' and 3' regions of the GAI gene, respectively, were obtained. By using primers designed from the first contig, we amplified a single amplicon (490bp) showing a high homology with the tef contig (99%) proving the usefulness of using the contigs to design specific primers. The small differences observed between the sequences of the amplicon and the contig could be explained by either having amplified the other allelic copy of GAI homolog gene as tef is an allotetraploid species or might be due to errors present in the sequencing or in the assembly. Moreover, a similar phylogenetic tree [33] was obtained for the region of GAI homolog genes corresponding to the amplified sequence of tef (**Fig. 3 and 4**, for the homology percent and the phylogenic tree, respectively). No sequence of this gene was available in the genbank database [30] for finger millet (*Eleusine coracana*), a closer species to tef. The cluster comprising the two sequences of tef (the contig and amplicon) were closest to pearl millet, followed by a cluster formed by maize, sorghum and sugarcane.

Fig 2. Alignment of tef contigs to genomic sorghum sequence. The numbers correspond to the genomic position in sorghum. The numbers in the boxes correspond to the two contigs.

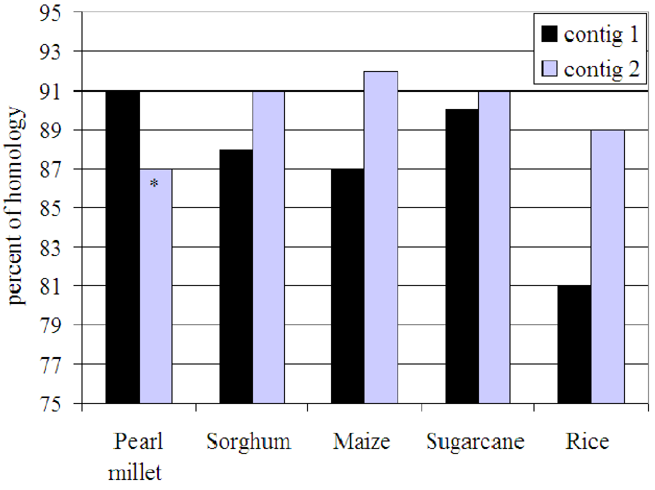


The prospects of genome sequencing on orphan crops

Recent progress in the development of genome-scale data set for several crop species offer important new possibilities for crop improvement. This will enable breeders and biotechnologists to more rapidly and precisely target genes that underlie key agronomic traits, and with such knowledge to develop molecular assays that are both relevant and of appropriate scale for breeding application. The main target will be abiotic and biotic stresses limiting crop productivity [34].

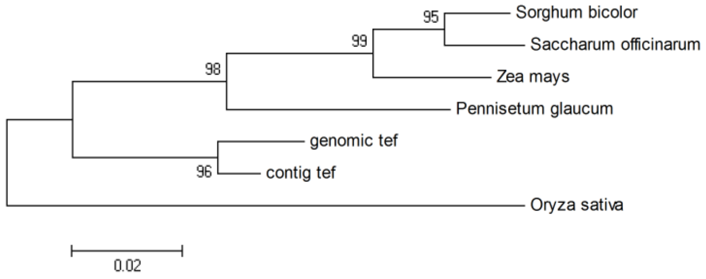
In the present study, the identification and PCR amplification of a gene using primers designed on a contig has shown the utility of the sequence information that has so far been generated, although the status of the assembly is still largely fragmented and incomplete. The unfinished status of the genome sequence resulted in a number of limitations including, i) most genes are partial and in many cases difficult to identify the orthologous copies of each tef sub-genome as tef is an allotetraploid; ii) the identification of gene family members is hindered by the fragmented nature of the current assembly and; iii) several genes present in sorghum and rice could not be identified in tef. Given the available data, it is not possible to determine whether these genes are indeed not present in tef or they have not been

Fig 3. Percent of homology between the two tef contigs and homologs of GAI gene of sorghum, maize, sugarcane, pearl millet and rice. Accessions of full length sequences (except for pearl millet) were obtained from NCBI and indicated in



* As partial sequence, only 250bp were compared.

Fig 4. Phylogenic analysis of GAI genes. Numbers above the branches are bootstrap values.



sequenced yet.

In conclusion, we have described the first genome sequence initiative on the under-studied African crop tef. This is of paramount importance for the genetic improvement of this staple crop. As tef is tolerant to abiotic and biotic stresses, the information from the sequencing will shed light on the molecular mechanisms conferring tolerance to a variety of stresses. This information may be transferred to other crops that are less adapted to adverse growing conditions. More investments have to be devolved to the genetic improvement of African neglected crops. Marginal environmental conditions expose African low input agricultural systems to a higher risk than high input modern agriculture. African enduring crops deserve more investments to stabilize future food production especially in the current scenario of climate change.

Acknowledgments

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PROGRESS IN PREVENTION OF TOXICO-NUTRITIONAL NEURODEGENERATIONS

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Abstract

Grass pea (*Lathyrus sativus*) is a survival food during drought in Ethiopia and the Indian Subcontinent, producing the cheapest dietary protein and saving thousands of lives. It also is a mixed blessing as the cause of the crippling neurolathyrism after prolonged over-consumption. Cassava (*Manihot esculenta*) is a protein-poor root crop that is the staple food for over half a billion people in Africa, South America and Southeast Asia, and the cheapest source of dietary carbohydrates. Over-consumption of cassava roots in a monotonous diet can cause konzo, with clinical symptoms indistinguishable from neurolathyrism. The prominent features of both diseases are sudden onset of symmetric spastic paraparesis of the calf muscles and scissor gate. The common feature of grass pea seed and cassava roots is the low content of the essential sulphur containing amino acids methionine and cysteine. The focus of breeding has been the reduction of the neuro-excitatory amino acid b-ODAP (b-N-oxalyl-L-a,b-diaminopropionic acid) in grass pea and the reduction of the cyanogenic glucosides in cassava. Developing varieties with higher content in methionine and cysteine and a better balanced diet may be more relevant in improving nutrition without jeopardizing the tolerance for biotic and abiotic stress of these crops.

Keywords: *Lathyrus sativus*, *Manihot esculenta*, oxidative stress, methionine deficiency, drought tolerance, spastic paraparesis

Introduction

High dietary dependence on grass pea (*Lathyrus sativus* L.) and cassava (*Manihot esculenta* C.) with neurotoxic potential has been implicated in the aetiology of neurolathyrism and konzo, respectively. Those two neurological diseases are clinically very similar but the food plants causing these diseases are very different, belonging to different plant families. Overconsumption of the protein-rich seeds of grass pea is linked to the incidence of neurolathyrism, and overconsumption of insufficiently processed carbohydrate rich roots of cassava is linked to the occurrence of konzo. Identification of a neuroactive non-protein amino acid β-ODAP in grass pea seed and the presence of cyanogenic glucosides in cassava roots did not bring the aetiology of these diseases much closer. Both neurolathyrism and konzo are characterized by spastic paraparesis of the legs, leading to scissor gate with various degrees of incapacitation, up to bedridden or crawler stage.

Reliance on grass pea or on insufficiently processed bitter cassava as staple foods, combined with a diet poor in methionine, has resulted in outbreaks of neurolathyrism and konzo. Epidemiology of the two diseases is more similar as both occur mainly among isolated very poor remote rural people with very little or no formal education, living as subsistence farmers on drought prone or poor soil conditions. Droughts and other determinants of food insecurity lead them to consumption of either grass pea seeds or short-cut processed bitter cassava roots as almost exclusive staple food for extended periods. Individual susceptibility to the toxic effects of these plants has been suggested to vary with subject age, gender, nutritional status, ingested quantity of the toxin and quality of the diet.

Grass pea (*Lathyrus sativus* L., tribe *Vicieae*, family *Fabaceae*)

As a crop for human consumption grass pea has survived since the Neolithicum. The centre of biodiversity is the Eastern Mediterranean and Ethiopia. In many European countries it was a popular crop in the Middle Ages and it has a specific name in all European languages [1]. It is still grown by traditional farmers in small amounts in many European countries. Of the over 150 species of the genus *Lathyrus*, very few can be crossed by simple means. Self-pollination is the rule with limited out-crossing due to male sterility. The crop grows on marginal land and is tolerant to biological and environmental stresses better than other legumes [2]. It is also the most efficient nitrogen fixer compared to other food legume crops [3] and thus improves the fertility of the soil for subsequent crops. This property is even exploited by Ethiopian farmers to increase the economic value of land. **Figure 1** shows the cropping system in north-western of Ethiopia where grass pea is extensively cultivated. Grass pea is being used not only for human consumption but also as green manure, as fodder, as feed and for grazing. Grass pea seed has high protein content (25.6-28.4 %) and produces the cheapest protein available for the poor and is the legume consumed in largest quantities especially during periods of drought and famine when other crops fail [2]. No other legume seed is consumed as a staple food like grass pea.

Prolonged over-consumption of this protein-rich seed in a monotonous diet can cause neurolathyrism, the degeneration of upper motorneurones and the irreversible paralyzing of the legs in up to 6% of the population: A free non-protein amino acid b-ODAP (b-N-oxalyl-L-a,b-diaminopropionic acid) with neuro-excitatory properties is blamed as causal agent. This metabolite was identified in 1964 [4] and during the

Figure 1. Bird's eye view of Northern Ethiopia in December. Dark green patches are Lathyrus sativus, pale green is chick pea (Cicer arietinum), the cereals have dried out

following two generations, research to improve the dietary value of grass pea was focused on the reduction of b-ODAP. This multifunctional metabolite [5] is however highly affected by the environment. Varieties developed in Canada with very low levels of b-ODAP loose this trait when planted in the iron-rich soil (e.g. Vertisol) of volcanic origin in the highlands of Ethiopia [6].

Epidemiological surveys have now indicated risk factors and protective factors for neurolathyrism [7, 8]. These should indicate the directions for improvement of the nutritional quality of grass pea and for the prevention of neurolathyrism. Among the various culinary preparations based on grass pea seed, those that are consumed without any addition of condiments seem to be more risky than others requiring a lengthy preparation. Especially fermentation is beneficial as it can reduce the content of b-ODAP by 90% and improves the balance of essential amino acids that are deficient in unprocessed grass pea [9, 10]. Consumption of roasted seeds as snacks was found to be highly correlated with the incidence of lathyrism. This was even more significant for the consumption of the unripe green seeds picked from the plants by young boys tending the fields [8]. Young men are more affected by lathyrism than women, the ratio being about 3:1. Risk factors for the incidence of neurolathyrism include heavy physical labour and fever which are also involved in oxidative stresses that is often mentioned in the aetiology of neurodegenerative diseases [11, 12]. Recently identified protective factors are the addition of onion, ginger or garlic to the preparations, or the addition of one third cereals rich in sulphur amino acids to the grass pea [8, Bernard Senny, Pers. comm.]. In the coastal areas of Bangladesh where fish is also eaten, no case of neurolathyrism is reported, although the consumption grass pea is at similar level to the inland. Supplementing methionine to an exclusive raw grass pea seed diet fed to young broiler chicks confirmed that methionine significantly improved the growth and prevented the neurological symptoms compared to the control chicks without methionine addition in the feed [13].

Neurolathyrism is a disease of the subsistence farmers in remote rural areas. Although grass pea seed is a popular commodity sold on the markets in the cities, the occurrence of neurolathyrism in cities is rare and mostly limited to beggars who moved from rural areas to the city under socio-economic pressure after developing neurolathyrism. The presence of antioxidants or higher levels of the sulphur amino acids in the diet may protect against oxidative stress, that ultimately can lead to apoptosis and death of motor neurons. The statistical link of the incidence of neurolathyrism with illiteracy and poverty supports our believe that lathyrism is an easily preventable disease. During the latest epidemic of neurolathyrism in Bangladesh in the 1970s, the price of grass pea was lower than any other available foodstuff [14]. Recently the price of grass pea is higher than the price of rice and no victims of neurolathyrism reported.

Because of this stigma that consumption of grass pea can cause an irreversible crippling, the market value is lower than for other legumes such as lentil (*Lens culinaris*) or chick pea (*Cicer arietinum*). The poor who cannot afford the more expensive commodities are the main consumers of grass pea. This adds an additional stigma to grass pea as being the food for the very poor. Apparently, this reputation was different when the plant got its Greek name, meaning something exciting, because of its reputation as an aphrodisiac. Also during the era of the Egyptian Pharaoh's, grass pea had a better reputation as it was present in the pyramids as royal funeral offerings [15]. During the Second World War, grass pea was the food given to horses in the Russian cavalry. When the Russian army retreated from the Ukrainian town Vapniarca, the horse food was left behind and when the German army made a forced labour camp for male Jewish prisoners in the town, the 'horse food' was given to the inmates in this camp. On a diet of 400 g boiled grass pea and 200 g of barley bread per day, the estimated intake of b-ODAP by the inmates was about 0.5 to 1 g per day. After two months on this diet, the prisoners started developing the clinical symptoms of neurolathyrism and within four months 60 % of the prisoners had developed neurolathyrism in various stages [16]. When comparing this with the daily intake of Bangladeshi farm labourers, who claim to consume at least one kg of grass pea seed (containing some 5 g of b-ODAP) per day without any symptoms, there seems to be no correlation between intake of b-ODAP and the incidence of neurolathyrism. This lack of correlation also makes it impossible to determine a threshold toxic level for b-ODAP. Other factors must have sensitised the prisoners to the grass pea toxicity. These aggravating factors might be heavy physical labour, under-nutrition or mal-nutrition of the prisoners in Vapniarca. Stress was recently identified as an aggravating factor for the incidence of neurolathyrism in experimental rats [17]. Although neurolathyrism can cripple for life, it does not affect longevity or cognitive factors [18].

Interestingly, the metabolite b-ODAP in grass pea held responsible for neurolathyrism is also present in the longevity promoting Ginseng root (*Panax ginseng*) where it is named dencichine in Chinese herbal medicine and considered as a haemostatic agent [19, 20]. b-ODAP is a multifunctional plant metabolite for which over 30 different physiological or biochemical activities have been described in the plant or in animals [5]. Remarkable activities are the facilitation of the uptake of zinc ions by the plant during zinc deficiency [21] and the protection of enzymes of photosynthesis against high light intensity [22]. Besides the well known excitation of the AMPA-receptors on the neurons it also affects other receptors and transporters in brain cells [23] [24] and Ca^{2+} homeostasis in neuronal cells [25].

The prolonged consumption of a diet deficient in methionine and cysteine can deplete glutathione from the body, one of our main metabolic defences against oxidative stress. Any additional stress such as heavy physical labour, micronutrient imbalance [26] or the neuro-excitation by b-ODAP can contribute to this oxidative stress and jeopardize the integrity of motor neurons.

Cassava (*Manihot esculenta* Cranz, Family *Euphorbiaceae*)

The root crop cassava originated in South America and was already used as food plant for 5000 years when it came to Africa in the 17th century. It became the typical staple food crop of Africa, feeding over half a billion people. It is estimated that in D.R. Congo about 60% of dietary energy is derived from cassava roots [27]. It is a popular crop also in South America and Southeast Asia, where it may be the cheapest source of dietary carbohydrates. It is being used for human consumption under many traditional preparations from tapioca in 'pearl soup' to chips and the thick porridge ‘luku’ or ‘fufu’ that is the staple food in many African countries. Besides being used for human consumption it has also many industrial applications, and is even considered an economic source of bio-fuel. No other member of the *Euphorbiaceae* plant family is consumed in such quantities as cassava. Cassava is a very reliable crop that is tolerant to drought. The underground roots can retain their nutritional value even when the above ground parts are dry. A changing climate with increasing atmospheric CO_2 can however negatively affect the productivity of the plant [28].

Cassava is a perennial plant that is rarely left to develop into flowering stage because the young leaves are used as vegetable. Propagation is by vegetative multiplication using cuttings. The underground roots are harvested any time of the year, peeled and soaked in ponds or slow running streams for retting. During the retting processing, the integrity of the root cells is broken and the enzyme linamarase, stored in the cytoplasm, comes into contact with the cyanogenic glucoside linamarine that in intact cells is stored in the vacuole and separated from the linamarase enzyme. Linamarine was first described in linseed (*Linum usitatissimum*). The enzymatic breakdown will release the volatile hydrogen cyanide, a highly toxic gas interfering with respiration. An insufficient period of retting will result in residual cyanogens and higher intake of cyanide than the maximum tolerated level of 10 mg per kg of cassava flour [27]. To some extent the human body can detoxify the residual cyanide into the less toxic thiocyanate that is excreted with the urine. However, when the body is depleted of methionine this process is interrupted and cyanate is produced instead of thiocyanate. The resulting cyanate has neuro-excitatory activity that in turn can again contribute to oxidative stress [29].

Over-consumption of cassava roots in a monotonous diet can cause an irreversible crippling disease konzo. Insufficient processing which leaves residual cyanogens (mainly the glucosinolate linamarin) is blamed for this disease with clinical symptoms indistinguishable from neurolathyrism. The popular processing of cassava in the rural villages includes retting during three nights (60-70 hrs), drying in the sun, pounding and adding to boiling water to make a stiff paste. This is consumed with sauce, made from available cooked vegetables that often are the young fresh cassava leaves. The roots are very poor in protein (2.4 % of dry weight) and this can be balanced by using the protein-rich young leaves (28.6 %). But both roots and leaves are poor in methionine and cysteine [30]. This deficiency of sulphur containing amino acids was suspected to be part of the aetiology of konzo as these amino acids promote cyanide detoxification [31]. In villages where no other crops are available, long periods of monotonous consumption of cassava diets can lead to a crippling disease characterised by sudden onset of symmetric spastic paraparesis and scissor gate. The disease is called ‘konzo’ by the rural people in the Kwango area of Bandundu province, D.R. Congo, meaning ‘bound legs’. Epidemiological surveys did not include studies of the complete diet, but observations in the field showed that villages where corn is mixed with the cassava have a much lower incidence of konzo. Also the consumption of onion seems to have a protective effect [Bernard Senny, pers. comm.]. Higher incidences occur during the dry season when other food is available in low amount [31]. Increased incidence also occurs after merchants come to the village to buy processed cassava roots and the villagers sell their ready-to-eat food. The next two days they are forced to eat insufficiently processed roots containing higher levels of cyanogens and are at higher risk of developing konzo. In urban areas the people eat the same staple cassava preparations with no incidence of konzo. The reason for this may be the better selection for quality and availability of other food ingredients in the market.

Similarities between neurolathyrism and konzo and possible worst case scenario

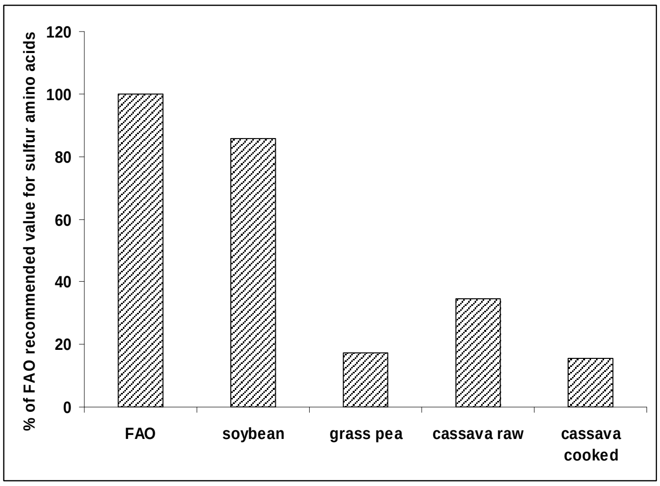
The two crops grass pea and cassava are cheap crops requiring minimal inputs and considered a reliable food supply during drought. The prominent similarities of the two crops are the resistance to biotic and abiotic stress, the tolerance to drought and the easy cultivation on marginal soil. Both crops are the food for the poor and when impoverished by environmental disaster such as drought or by military conflict these crops become the only available or affordable food. Both diseases neurolathyrism and konzo can be considered neglected orphan diseases of the remote rural areas and are virtually absent from urban areas [29, 32]. Both diseases occurred in areas where drought, poverty, illiteracy and malnutrition are prevalent and both are under-reported. The reason for under-reporting may be due to the remoteness of the locations, the illiteracy and poverty of the victims giving them little or no socio-economic or political voice, and the fact that both diseases are not infectious or transmissible.

As described above, both neurolathyrism and konzo are characterised by sudden onset of symmetric spastic paraparesis and scissor gate, after a prolonged period of monotonous unbalanced diet containing mainly the protein-rich grass pea or the protein-poor cassava roots. A common risk factor is heavy physical labour, while a common protective factor is the consumption of onion or cereals. In both cases there is a significant link with poverty and illiteracy. Remote areas with subsistence farming are more vulnerable especially during periods of drought. In urban areas where grass pea or cassava roots are available both diseases are virtually absent. Both diets of prolonged monotonous consumption of grass pea seeds or cassava roots may induce an increased oxidative stress due to the deficiency of the essential amino acids methionine and cysteine (**Figure 2**), and the physiological effects of a neuro-excitatory amino acid b-ODAP in grass pea or a methionine depleting detoxification process of cyanide after consuming cassava. For both diseases, a number of epidemiological risk factors can be explained as aggravating this oxidative stress while protective factors can be explained as counteracting oxidative stress.

The one unexplained difference between konzo and neurolathyrism is the higher incidence of konzo among women at childbearing age, while young boys are more vulnerable to neurolathyrism [33]. In some cases the physical effort of childbirth can trigger the onset of konzo, which gives rise to dramatic situations when the young mother can no longer get up from the bed to nurse the child [Lieve Van Wijmeersch, pers. comm.].

As of now, there is no geographical overlap of the production of grass pea and cassava. Considering the present interest in drought tolerant crops, demographic pressure and climate change conditions may be responsible for the increasing cultivation and consumption of both grass pea and cassava. There is a real risk that these crops spread and overlap. While the exact aetiology of neurolathyrism and konzo is not completely understood, the result of over-consumption of either grass pea or cassava in a diet poor in the essential sulphur amino acids methionine and cysteine is so similar that, when poor consumers would start mixing a cheap protein-rich crop with a cheap carbohydrate-rich crop that both have the same deficiency in essential amino acids could be predicted as a 'worst case scenario'. Education and dietary information on the risks of these crops would be extremely important in order to prevent such a worst case scenario. Also the consumption of cassava together with other legume seeds such as cowpea as is the case in D.R. Congo is not advised, because only soybean has an acceptable level of methionine and cysteine albeit not enough to balance the deficiency in cassava. Three approaches can reduce the incidence of these two disorders: i) better diversification of the diet, ii) development by plant breeding or transgenic technology of new cultivars with low toxin content and iii) development of new cultivars with higher content of methionine/cysteine and of micronutrients.

Figure 2. Grass pea seed and cassava leaf preparations (raw and cooked) are both deficient in methonine and cysteine



Conclusion

The obvious road for making grass pea and cassava based diets more healthy is the improvement of the amino acid balance and the addition of antioxidant-rich condiments into the diet. Improving the nutritional quality of grass pea and cassava crops by increasing the content of sulphur amino acids methionine and cysteine may ultimately be the cheapest solution for the subsistence farmers surviving on those crops in environments where other crops are less productive, provided that unreasonably expensive bio-safety regulations are adapted to the real needs of the poor. The drought tolerance of these two ancient crops, that have retained their popularity during several thousand years, can make them even more important for a future affected by global warming and water shortages. Under certain conditions of over-consumption and malnutrition, both crops can have deleterious and irreversible effects on the consumer. However, while a single meal of unprocessed bitter cassava can be suicidal, large amounts of grass pea are needed to be consumed as a monotonous diet during several months to have a 6% chance of developing neurolathyrism. Because no other legume is consumed in such quantities it is not possible to predict whether a monotonous diet of an alternate legume would be safer than grass pea. A practical and cheap animal model for neurolathyrism or konzo does not exist but is urgently needed. Without such a model it is not possible to guarantee the safety of new varieties of grass pea with lower levels of b-ODAP or new varieties of cassava with lower levels of linamarine.

Very recently, a workshop on the two diseases was held in Ghent University (Belgium) with the aim to be an interface between researchers of neurolathyrism and konzo in facilitating the identification of common ground, stimulating communication between researchers of different areas, and preventing the two diseases through integrated approaches. One consensus was that both diseases are the clinical symptoms of abject poverty and malnutrition and are neglected by the authorities.

Main recommendations from the workshop on neurolathyrism and konzo

Because of their ability to perform well in marginal soils and under harsh agro-climatic conditions, cassava (*Manihot esculenta*) and grass pea (*Lathyrus sativus*) offer ample opportunities for sustainable agriculture and food security for the poorest of the poor in the context of climate change. These crops have been associated with health problems of konzo, tropical ataxic neuropathy (TAN) and neurolathyrism, which are irreversible neurodegenerations, that need to be addressed. The poorest populations of subsistence farmers mostly depend on these crops for their food and nutritional security in years of environmental extremities and are most at risk of this incurable but preventable paralysis of the lower limbs. A two-day "International Workshop on Toxico-Nutritional Neurodegenerations – Konzo and Lathyrism" was organized at Ghent University, Belgium on 21 and 22 September 2009 in which 55 scientists from five continents presented 18 oral presentations and 27 posters, highlighting various aspects of these two important crops and their health problems related to overconsumption. During the panel and round-table discussions, the following recommendations were made:

1. There is an **urgent need to better understand the etiology of these two diseases** with very similar epidemiology and the same clinical symptoms, but from consuming completely different food (konzo from cassava roots and neurolathyrism from grass pea seeds). There is a need to explore the molecular pathways of both diseases and to find the common biochemical and neurobiological events. From this common ground, effective prevention strategies can be designed.

2. We **need to more correctly evaluate the nutritional quality of these two most hardy lifesaver crops** and their contribution to food security as well as to dispel the misgivings about the toxico-nutritional problems (konzo and neurolathyrism) associated with these crops. The presence of toxins (neuro-active amino acids and nitriles) as well as the deficiency of essential nutrients need to be considered. Especially the sulfur amino acids that are deficient in both crops need special consideration. With genetic enhancement of the crops and optimized postharvest processing, the nutritional status of the populations depending on these crops can be much improved.

3. It is crucial to understand the genetics of the **unique traits (drought tolerance, disease resistance, and high nitrogen fixation** in the case of grass pea) of these lifesaving crops for harvesting the benefit of molecular tools. For the **genetic improvement** of these crops, research efforts should be directed towards better nutritional quality, higher yield and resistance to key diseases, drought and high temperature and also better nitrogen fixing ability or fertilizing. This requires creating and strengthening of plant breeding and molecular research at national and international levels involving grass pea and cassava . **These traits are of global importance and can also benefit other crops.**

4. Research on better **agronomic practices** and the environmental effect (drought, CO_2) on the toxin content and the nutritional quality are required for improving their adaptation to various crop production systems and new niches. Introduction of cassava and grass pea into new niches needs to be accompanied by knowledge transfer in agronomy, postharvest, processing, risks and opportunities. Introduction of both crops in the same cropping systems and niches might have a synergistic effect on the incidence of konzo and/of neurolathyrism and needs preliminary studies with experimental animals.

5. There is an urgent need to standardize the **food processing methods** and food preparations for lowering toxins in cassava roots and grass pea seeds for safe consumption. Fermentation and bio-fortification of cassava and grass pea foods need a focused research for optimized nutrition and a better balanced diet.

6. **Multi-disciplinary efforts** involving nutritionists, toxicologists, neuropathologists, biochemists, plant scientists and social scientists are required for solving these global problems of konzo and neurolathyrism.

7. **Konzo and Neurolathyrism are seen as clinical symptoms of abject poverty and malnutrition.** Making konzo and neurolathyrism **reportable diseases** can make emergency food aid more effective and will help to direct agricultural development to the most neglected and the really needy. Prevention of malnutrition through diversification of diets, alleviation of poverty and improved education needs to be **effectively communicated** among the different stakeholders in order to give cassava and grass pea the right place in a healthy diet. The poorest of the poor subsistence farmers who survive on grass pea and cassava as a source of staple food and an insurance crop should be identified and mapped. Involvement of policy makers, education institutions, NGOs, self-help groups and different stakeholders in communicating about the benefits and risks of cassava and grass pea needs to be ensured. Local knowledge associated with these two orphan crops needs to be documented for the benefit of society. This has to be coupled with rehabilitation programs for the affected people.

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AFRICAN YAM BEAN: A CROP WITH FOOD SECURITY POTENTIALS FOR AFRICA

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Abstract

The references to African yam bean (AYB) in the African culture is proof that it had sustained livelihoods in the past. The wide diversity within the crop's germplasm, its striking nutritional capacity as described in scientific reports, and its genetic stability across a wide range of environments with appreciable yields are reliable evidence of the need to foster the crop for its economic importance in Africa's food security. Like every other crop with low or no research attention, awareness of African yam bean is poor. It is mostly appreciated by the elderly farmers and rural women. The continual availability of the genetic resources of the crop is threatened. Moreover, the cultivation of the crop may further decline due to neglect and underutilization. Wide exploration of AYB's genetic resources in Africa will provide assurance of its future genetic improvement.

Key words: African yam bean, genetic resources, food security, underutilized, diversity

Introduction

Sastrapradja [1] asserted that biodiversity is obviously not equally distributed on the earth; the tropical belt holds more diversity than other regions of the world. Africa has huge underexploited plant genetic resources with potentials for meeting some of her micronutrient needs; however, many food components are available but yet undiscovered in the wilds of Africa [2]. Africa is richly endowed with a very high diversity of plants in wild and cultivated forms. Thousands of them, though not commercialized, somehow adequately sustained rural livelihood for ages.

The Green Revolution has been implicated in overturning the traditional African food culture and entrenching a few crops for full commercial production, lately coupled with nutrient fortification. The global dependence on these few crops has largely affected the utilization of others which have become neglected. Their production has lessened and the diversity of their germplasm is at serious risk of genetic erosion. The depletion of crop genetic resources in Africa may have drastic consequences on hunger and malnutrition.

Underutilized crops are indigenous, relatively common in specific areas, available, accessible, well-adapted, easy and cheap to produce, and culturally linked to the people who use them traditionally [2, 3, 4, 5]. Their cultivation and utilization usually draw on indigenous knowledge. The ecotypes and landraces of these species are cultivated less than in the past. They are rarely found in urban markets. They cannot compete with crops which now dominate the world's food. They are hardly represented in *ex situ* genebanks, so efforts to characterize them depend on the limited available and loosely-representative diversity [6]. They are therefore usually ignored by policy makers probably because their economic value is not apparent [7] and hence are excluded from the development agenda by research institutions. However, underutilized crops are important as household food and their contribution to food security is unquestionably significant [2, 8].

African yam bean (AYB)

African yam bean (*Sphenostylis stenocarpa*), is the most economically important among the seven species of *Sphenostylis* [9] and it is one of the most important tuberous legumes. The domestication, cultivation, and distribution of the crop are very evident in the tropics of Africa [3, 9, 10, 11] where it had been reported to exhibit very high diversity. There is no record of any other center of diversity for the crop beyond tropical Africa. It should not be confused with the *Pachyrhizus* spp. which in many places in the literature are referred to as "yam bean", Mexican yam bean, Jicama, etc. *Pachyrhizus* spp. is a more popular tuberous legume that is common in the tropics of Asia and Southern America.

The botanical profile and morphology of African yam bean

AYB belongs to the family Fabaceae, sub-family Papilionoideae, tribe Phaseoleae, sub-tribe Phaseolinae, and genus *Sphenostylis* [3, 12]. The crop has twining vigorous vines, which could be green or pigmented red. The vines twine clockwise around the stakes or climb other supports to a height of about 3 meters or more. The leaves are compound trifoliate. The large pink and purple flowers are admirable and attractive ornamentals. Pods are usually linear, housing about 20 seeds. These vary in size, shape, colour pattern, etc.

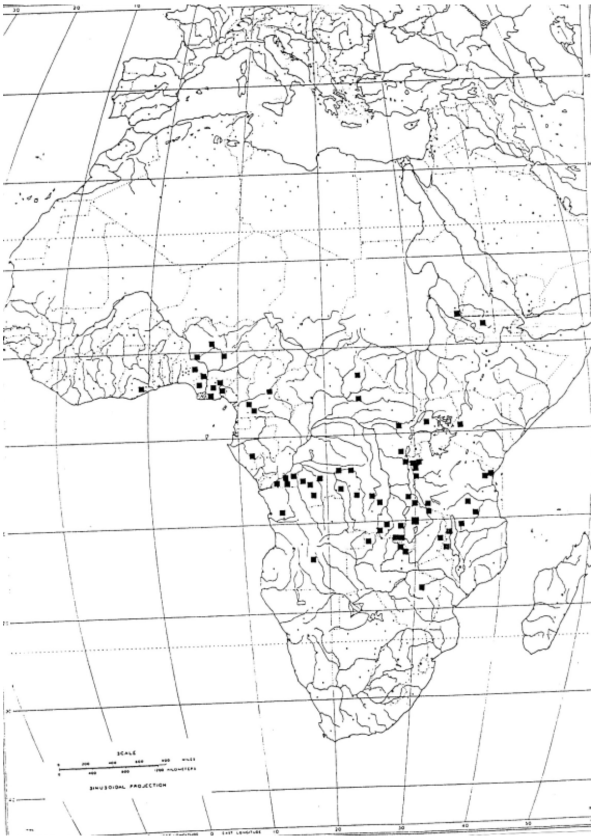
Eco-geographical distribution of AYB

The origins of AYB as indicated by GRIN [13] includes the following countries within the tropical regions of Africa: Chad and Ethiopia (Northeast tropical Africa); Kenya, Tanzania and Uganda (East tropical Africa); Burundi, Central African Republic and Democratic Republic of Congo (West-Central tropical Africa); Côte d'Ivoire, Ghana, Guinea, Mali, Niger, Nigeria, and Togo (West tropical Africa); Angola, Malawi, Zambia, and Zimbabwe (South tropical Africa). The centre of diversity of AYB is only within Africa (See **Figure 1**). Nigeria is very significant for AYB production [9] where extensive cultivation had been reported in the eastern [14] western, and southern [15] areas of Nigeria.

Domestication, cultivation, and the cultural place of AYB

AYB is rarely planted as a sole crop in Ghana and Nigeria; it is mostly interplanted with yam [3, 16]. The seeds and tubers (**Plates 1** and **2**) are the two organs of economic importance, providing food for humans and livestock. However, there is a cultural and regional preference for each: West Africans prefer the seeds to the tubers while the tubers are highly relished as food among East and Central Africans, especially among the Bandudus, the Shabas, and the tribe at Kinshasha in Democratic Republic of Congo [9, 17]. This exceptionally nutritious pulse [18] has a very significant link with African sociocultural life. For instance, the Avatimes in Ghana prepare a special meal from it during the celebration of the puberty rites of adolescent girls [16]. Likewise a special meal from it features during the marriage ceremony among the Ekitis in Nigeria [9]. Different forms of local recipes are prepared from the crop to meet the dietary needs of the people.

Figure 1. The Distribution of African yam bean in Africa



(Source: Potter, D. & Doyle, J. J. 1992 [44]).

Plate 1. Diversity in colour, colour pattern, structure, texture, brilliance etc. of African yam bean seeds

Plate 2. Tuberous root of an accession of African yam bean

Potentials of African yam bean

1. Food and nutrition

The economic potentials of AYB are immense. Apart from the production of two major food substances, the value of the protein in both tubers and seeds is comparatively higher than what could be obtained from most tuberous and leguminous crops [3, 17]. The protein in the tuber of AYB is more than twice the protein in sweetpotato (*Ipomea batatas*) or Irish potato (*Solanum tuberosum*) [19] and higher than those in yam and cassava [20]. Moreover, the amino acid values in AYB seeds are higher than those in pigeon pea, cowpea, and bambara groundnut [21]. Protein content is up to 19% in the tuber and 29% in seed grain The content of crude protein in AYB seeds is lower than that in soybean, but the amino acid spectrum indicated that the level of most of the essential amino acids especially lysine, methionine, histidine, and iso-leucine in AYB is higher than those in other legumes including soybean [14, 19, 22, 23, 24]. Generally, the amino acid profile of AYB (**Table 1**) compares favorably with whole hens' eggs and most of them meet the daily requirement of the Food and Agriculture Organization (FAO) and World Health Organization (WHO) [25]. AYB is rich in minerals such as K, P, Mg, Ca, Fe, and Zn but low in Na and Cu [26, 27].

2. Insecticidal and medicinal usefulness

AYB as a crop is less susceptible to pests and diseases [28] compared with most legumes; this quality may undoubtedly be due to the inherent lectin in the seed of the crop. Omitogu *et al.* [29] advanced the prospect that the lectin in the seed of the crop is a promising source of a biologically potent insecticide against field and storage pests of legumes. Therefore, the inclusion of the lectin extract from AYB in the meal for three cowpea insect pests, namely, *Maruca vitrata, Callosobruchus maculatus,* and *Clavigralla tomentosicollis* gave a mortality rate greater than 80% after 10 days. The physiological system of *C. tomentosicollis* was found to be very vulnerable to the lectin in AYB [30]. In Togo, Ghana, and Nigeria, paste made from the seeds of AYB is used as a cure for stomach aches, and when the paste is mixed with water it is traditionally used for the treatment of acute drunkenness [31, 32]. Asuzu [32] reported that there might be pharmacological evidence for the use of AYB in treating such conditions.

3. Stable yield across wide environments

The seed yield of AYB can be as high as 3000kg/ha [3, 28]. The average seed/plant is between 100 and 200g and the tuber yield per plant is 0.5 kg [10, 19]. In different yield trials in Nigeria (IITA, Ibadan and Nsukka), the most productive accession in each case gave 1860 kg and 2000 kg of seeds/ha [19]. Coupled with high yield is vast adaptability to diverse edaphic conditions [33, 34]. AYB produces an appreciable yield (See **Plate 3**) more than most other pulses on poor soil and in a hot climate [26]. AYB has very high ability to fix nitrogen [35]; it is therefore an important crop which merits significant consideration for land reclamation.

Limitations in AYB

Over time, some conditions have negatively influenced the productivity and acceptability of the crop among cultivators, consumers, and research scientists. Notable among the list are, i) the characteristic hardness of the seed coat [36, 37] which makes a high demand on the cost and time of cooking, ii) the agronomic demand for stakes, the long maturation period [11, 19], and iii) the presence of antinutritional factors (ANF) or secondary metabolites [38]. The photoperiodic sensitivity of AYB [10] seems to compound the above disadvantages as it confines the cultivation and production of the crop to one season in the year. However, a concerted crop breeding research programme may overcome these problems.

The way forward

Diversification in the production and utilization of crops is an essentially important strategy to alleviate food insecurity [39]. This will entail producing different foods and producing enough of each food crop to reduce its price and increase its supply, availability, and consumption. Climate change is already having a strong impact on human societies and the natural world; this is expected to continue for decades to come [40]. Africa has been identified as one of the continent most vulnerable to climate change and variability [41]. An adequate strategy needs to be in place for food security in Africa. Food security, quality of life, and livelihood for billions of people in the present and future generations are guaranteed only by the availability of diversity in crop genetic resources. Diverse genetic crop resources provide the foundation on which crop improvement depends; moreover, they provide diversity and are sources of traits to improve yield and quality, resistance to diseases/pests, and adaptability to climatic changes. For instance, Klu *et al.* [16] had speculated that AYB was nearing extinction. The quantity and availability of AYB's germplasm may have been declining over time. However, the ability of the crop to adapt to diverse environments [33, 34] may have been responsible for its continual existence and survival. Intensive exploration and conservation of large amounts of germplasm are an utmost necessity for AYB and other indigenous African crops. The amino acid requirements can be met by the consumption of mixtures of legumes in large amounts [17, 42]. Apart from the use of soybean as an animal-alternative protein source, the exploitation of protein from other legumes is rare [43]. With the acknowledgment of the nutritional potentials of AYB, the crop may well contribute to solving food security problems in Africa if its genetic diversity is saved for future genetic improvement.

Plate 3. An African yam bean plant with mature pods ready for harvest

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Table 1. Essential Amino acid content of some tropical food and feeding stuffs (Mg/N). Source: [24].

Common names	Botanical names	Arginine	Histidine	Isoleucine	Leucine	Lysine	Phenyllanine	Tyrosine	Cystine	Methioninie	Threonine	Triptophan	Valine
Cowpea	*Vigna unguiculata*	444	194	256	456	394	325	190	106	119	238	60	325
Groundnut (whole)	*Arachis hypogaea*	775	150	250	438	319	325	220	81	88	244	70	313
Gnut protein (Arachin)	*Arachis hypoagea*	763	119	413	425	250	344	300	81	38	144	56	244
Gnut protein (Conarachin)	*Arachis hypogaea*	744	119	219	363	375	106	156	163	106	113	31	200
Soybean meal	*Glycine max*	519	175	306	488	406	306	200	94	94	244	81	319
Lima bean	*Phaseolus lunatus*	388	206	350	556	431	400	160	61	119	300	56	363
Bambara nut	*Vigna subterranea*	394	118	275	494	400	350	219	180	113	219	-	331
Field bean	*Dolichus lablab*	456	163	228	525	388	325	220	69	50	225	-	244
Common pea	*Pisum sativum*	-	-	350	520	460	320	250	80	80	240	70	350
Green gram seed	*Phaseolus aureus*	-	188	350	560	430	300	100	40	70	200	50	370
Pigeon pea	*Cajanus cajan*	419	213	238	475	438	544	210	75	94	213	30	313
Sunflower seed	*Helianthus annuus*	513	137	356	419	238	313	163	88	213	250	81	331
Geocarpa seed	*Kerstingiella geocarpa*	425	181	275	494	388	369	220	63	94	244	-	406
Stenocarpa seed	*Sphenostylis stenocarpa*	388	231	275	481	425	331	270	94	119	256	-	350

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Name of institute or organization	Role/involvement	Mission/objective	Relevance to African orphan crops	Country or region of activity	Head quarter	URL
AAC	Investment in SME	To improve the livelihoods of small-holder farmers in East Africa	Indirect or general	East Africa	Kampala, Uganda	http://www.aac.co.ke/web/
AATF	Technology transfer	To access and deliver affordable agricultural technologies for sustainable use by smallholders	Cassava, banana, cowpea	Sub-Saharan Africa	Kenya	http://www.aatf-africa.org/
ABNETA	Discussion platform	To discuss, support, develop or use biotechnology in support of agriculture in Africa	Indirect or general	Africa	Nairobi, Kenya	http://www.abneta.org/
ABSF	Communication, training, development	To create an innovative and enabling biotechnology environment in Africa through Education	Indirect or general	Africa	Kenya	http://www.absfafrica.org/
ABSPII	Promote agricultural biotechnology	To support the development of expertise in the areas of research, policy development, licensing, and outreach	banana	Africa, Asia	Cornell University, Ithaca, USA	http://www.absp2.cornell.edu/
ACIAR	Grant provision	To contribute to the aid program objectives of advancing Australia's national interest through poverty reduction and sustainable development	Indirect or general	Global	Canberra, Australia	http://www.aciar.gov.au/home
ACTS	Knowledge and technology transfer	To strengthen the capacity and policies of African countries and institutions to harness science and technology for sustainable development	Indirect or general	Africa	Nairobi, Kenya	http://www.acts.or.ke/
ADC	Grant provision	To reduce global poverty, ensure peace and human security and preserve the environment in an international framework	Indirect or general	Global	Vienna, Austria	http://www.entwicklung.at/en.html
Africa College	Research & capacity building	To improve the lives of millions who are exposed to food insecurity and suffer malnutrition	Indirect or general	Africa	University of Leeds, UK	http://www.africacollege.leeds.ac.uk/index.php
Africa Harvest	Technology transfer	To harness modern science and technologies to help Africa achieve food security, economic well-being and sustainable development	banana	Africa	Nairobi, Kenya	http://africaharvest.org/site/

Name of institute or organization	**Role/involvement**	**Mission/objective**	**Relevance to African orphan crops**	**Country or region of activity**	**Head quarter**	**URL**
Africa Rice Center	Research & development	to contribute to poverty alleviation and food security in Africa	African rice	Africa	Contonou, Benin	http://www.warda.org/
African Development Bank	Grant provision	to spur sustainable economic development and social progress in its regional member countries, thus contributing to poverty reduction	Indirect or general	Africa	Abidjan, Côte d'Ivoire	http://www.afdb.org/
African-crops.net	Information	to provide service in the areas of crop improvement, seed systems development, access to information and capacity building	African crops	Africa	Nairobi, Kenya	http://www.africancrops.net/index.htm
AfricaNUANCES	research	To increase the understanding of rural livelihoods and their relationships with the food security and sustainability	Indirect or general	East, West & Southern Africa	Wageningen, The Netherlands	http://www.africanuances.nl/
AGRA	Funding for capacity building	To integrate programs in seeds, soils, market access and policy in order to transform subsistence farming into sustainable and viable commercial activity	African crops	Africa	Nairobi, Kenya	http://www.agra-alliance.org/
Agriculture Review	Information	A journal targets agricultural industry in Eastern and Southern Africa	Indirect or general	Africa	Nairobi, Kenya	http://agriculturalreviewonline.com/index.html
AGRINATURA	research, training and capacity building	To contribute to achieving the Millennium Development Goals	Indirect or general	Global	Prague, Czech & Paris, France	http://www.agrinatura.eu/index.html
Agri-ProFocus	promote farmer entrepreneurship	to provide coherent and demand-driven support to enhance the capacity of producer organizations in developing farmer entrepreneurship within the context of poverty reduction	Indirect or general	Ethiopia, Kenya, Mozambique, Niger, Rwanda, Uganda & Zambia	Arnhem, The Netherlands	http://www.agri-profocus.nl/
Agriterra	Knowledge or information transfer	to promote economic activities in developing countries	Indirect or general	Global	Arnhem, Netherlands	http://www.agriterra.org/en/index

Name of institute or organization	Role/involvement	Mission/objective	Relevance to African orphan crops	Country or region of activity	Head quarter	URL
ASARECA	Strengthen NARS activity	To promote economic growth, fight poverty, reduce hunger and enhance resources through regional collective action in agricultural research for development, extension and agricultural training and education	Staple and non-staple crops	ECA countries	Entebbe, Uganda	http://www.asareca.org/
BeCA Hub	Research, training	To provide a common biosciences research platform, research-related services and capacity building and training opportunities	Indirect or general	East and Central Africa	Nairobi, Kenya,	http://hub.africabiosciences.org/
BIO-EARN	Capacity building	to build capacity in biotechnology in Ethiopia, Kenya, Tanzania and Uganda and promote appropriate research and related policies	Indigenous cereals, sweet potato	East Africa	Kampala, Uganda	http://www.bio-earn.org/index.htm
Bioversity International	Research	To conserve and use of agricultural biodiversity	Banana, plantain	Global	Rome, Italy	http://www.bioversityinternational.org/
BREAD	Grant provision	to include international partners in efforts to generate sustainable, science-based solutions to problems of smallholder agriculture in developing countries	Indirect or general	Global	USA	http://www.nsf.gov/funding/pgm_summ.jsp?pims_id=503403&org=NSF&from_org=DBI
BTC	Grant provision	To provides present and future generations with sufficient resources and support the initiatives of our partners to eliminate poverty	Indirect or general	Global	Brussels, Belgium	http://www.btcctb.org/what-btc
CAADP	Trust Fund	to eliminate hunger and reduce poverty through agriculture	Indirect or general	Africa	South Africa	http://www.nepad-caadp.net
CCRP	grants program	To improve food production and nutritional content of crops important to the developing world	Sweet potato, tef, finger millet, Pearl millet, Cowpea, Bambara groundnut	Global	McKnight Foundation, Cornell University, USA	http://mcknight.ccrp.cornell.edu/

Name of institute or organization	Role/involvement	Mission/objective	Relevance to African orphan crops	Country or region of activity	Head quarter	URL
CGIAR	Coordinate IARC	To achieve sustainable food security and reduce poverty in developing countries	Several CGIAR centers work on orphan crops	Global	Washington DC, USA	http://www.cgiar.org/
CIAT	Research	To improve crops and soil for the poor	Beans, cassava	Global	Cali, Colombia	www.ciat.cgiar.org
CIDA	Grant provision	to support people living in poverty	Indirect or general	Global	Quebec, Canada	http://www.acdi-cida.gc.ca/home
CIP	Research	to reduce poverty and achieve food security on a sustained basis in developing countries	Sweet potatoes	Global	Lima, Peru	http://www.cipotato.org/
CIRAD	Research & training	to generate and pass on new knowledge, support agricultural development and fuel the debate on the main global issues concerning agriculture	Banana, plantain, tree crops	Global	Paris & Montpellier, France	http://www.cirad.fr/en
Comparative Genomics	research	To study genetic diversity of orphan crops and transfer molecular technologies into developing world	Tef, finger millet, fox tail	Global	University of Georgia, Athens, USA	http://www.genetics.uga.edu/jlblab/
CORAF/WECARD	Research coordination	to improve, in a sustainable way, agricultural productivity, competitiveness and markets	Staple and non-staple crops	West and Central Africa	Dakar, Senegal	http://www.coraf.org/
Crops for the Future	Training & policy issues	to promote neglected and underutilized plant species as a contribution to humanity	underutilized crops	Global	Serdang, Malaysia	http://www.cropsforthefuture.org/
CTA	Information & communication	To disseminate knowledge and information	Indirect or general	ACP-EU	Wageningen, Netherlands	http://www.cta.int/
DAAD	Training & capacity building	To assist developing countries in establishing effective universities and advises decision makers on matters of cultural, education and development policy	Train African researchers	Global	Bonn, Germany	http://www.daad.de/en/index.html
DFID	Grant provision	to meet the many challenges of tackling world poverty	Indirect or general	Global	London, UK	http://www.dfid.gov.uk/
FAO	Development, Information systems	to raise levels of nutrition, improve agricultural productivity, better the lives of rural populations and contribute to the growth of the world economy	Indirect or general	Global	Rome, Italy	http://www.fao.org/

Name of institute or organization	Role/involvement	Mission/objective	Relevance to African orphan crops	Country or region of activity	Head quarter	URL
FARA	Agricultural research co-ordination	To enhance broad-based agricultural productivity, competitiveness and markets sustainably in Africa	Indirect or general	Africa	Accra, Ghana	http://www.fara-africa.org/
Gates Foundation	Grant for research & conservation	to help small farmers flourish on their farms and overcome hunger and poverty	Indirect or general	Global	Seattle, USA	http://www.gatesfoundation.org/Pages/home.aspx
Gatsby Charitable Foundation	Grant provision	To assist small-scale farmers to improve agricultural productivity and equipping modest enterprises to take the early steps towards industrialization	Indirect or general	UK and Africa	UK	http://www.gatsby.org.uk/index.html
GCP	Research & capacity building	To use genetic diversity and advanced plant science in improving crops for greater food security in the developing world	Tropical legumes	Global	Mexico	http://www.generationcp.org/index.php
GFAR	Discussion forum	To provide the mechanism and the opportunity that brings together the global agricultural research for development community to work coherently, inclusively and equitably, driven by the needs of the poor	Indirect or general	Global	Rome, Italy	http://www.egfar.org/egfar/website?contentId=-1&
GTZ	capacity development	To promote international cooperation which contributes to sustainable development throughout the world	Indirect or general	Global	Eschborn, Germany	http://www.gtz.de/en/index.htm
HarvestPlus	research	to reduce hidden hunger and provide micronutrients to billions of people directly through the staple foods that they eat	beans, cassava, maize, pearl millet, rice, sweet potato	Global	Washington DC, USA	http://www.harvestplus.org/
IAASTD	Information provision	to asses the impacts of past, present and future agricultural knowledge, science and technology on the reduction hunger and poverty, improvement of rural livelihoods and human health, and equitable, socially, environmentally and economically sustainable development	Indirect or general	Global	Washington DC, USA	http://www.agassessment.org/

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ICARDA	Research and training	to improve the welfare of poor people and alleviate poverty in dry areas of the developing world	lentil, barley and faba bean	Global	Aleppo, Syria	http://www.icarda.org
ICRA	capacity building	stimulates innovation by strengthening the abilities of people and organizations in the rural sector, research and education to collaborate and learn from each other	Indirect or general	Africa & Latin America	Wageningen, Netherlands	http://www.icra-edu.org/page.cfm?pageid=ardhome&loginas=anon_e
ICRISAT	Research	to help poor people to overcome hunger, poverty and a degraded environment in the dry tropics through better agriculture	Pearl millet, Pigeonpea, chickpea, small millets	Global	Patancheru, India	http://www.icrisat.org/index.htm
IDSA	training	to provide a service to scholarship seekers and scholarship providers.	Unspecific	Global	Pisa, Italy	http://www.scholarshipnet.info/
IFAD	funding	To ensure that poor rural people have better access to, and the skills and organization they need	Indirect or general	Global		http://www.ifad.org/
IFPRI	Policy research	to provide policy solutions that reduce poverty and end hunger and malnutrition	Indirect or general	Global	Washington, D.C., USA	http://www.ifpri.org/
IITA	Research & capacity building	to enhance food security and improve livelihoods in Africa through research-for-development	Cassava, yam, cowpea, banana, plantain	Global	Ibadan, Nigeria	http://www.iita.org/
INRA	Research & training	To carry-out mission-oriented research for high-quality and healthy foods, competitive and sustainable agriculture and a preserved and valorised environment	Indirect or general	Global	Paris, France	http://www.international.inra.fr/
IPBO	Training & research	to promote sustainable socio-economic development in developing and emerging economies	Banana, cassava, grass pea, sweet potato	Global	Gent University, Belgium	http://www.ugent.be/we/genetics/ipbo/en
IPK	Germplasm provision	Management, analysis and evolution of plant genetic resources	Many crops	Global	Gatersleben,Germany	http://www.ipk-gatersleben.de/Internet

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IPS	Research & technology transfer	To implement modern improvement techniques to the under-researched crops of Africa	tef	Africa	University of Bern, Switzerland	http://www.botany.unibe.ch/deve/research/projects/tefbiotech/index.htm
ISAAA AfriCenter	Development, & information provision	To contribute to poverty alleviation by increasing crop productivity and income generation, particularly for resource - challenged farmers in Africa, and to bring about a safer environment and more sustainable agriculture development	banana	Africa	Nairobi, Kenya	http://africenter.isaaa.org/Default.aspx
JICA	Grant provision	To reduce poverty through equitable growth, improve governance and achieve human security	Indirect or general	Global	Tokyo, Japan	http://www.jica.go.jp/english/
Joint FAO/IAEA Programme	Training, research & service provision	To assist the use nuclear techniques and related biotechnologies for developing improved strategies for sustainable food security	Indirect or general	Global	Vienna, Austria	http://www-naweb.iaea.org/nafa/index.html
KFPE	Grant provision	To promote research partnerships with developing and transition countries	Indirect or general	Global	Bern, Switzerland	http://www.kfpe.ch/
Kirkhouse Trust	Grant provision	To improve crop research for the developing world	Cowpea and common bean	West & East Africa	Oxfordshire, UK	http://www.kirkhousetrust.org/
Laboratory of Tropical Crop Improvement	Research and training	To improve the livelihood of subsistence farmers in the tropics through sustainable agriculture	Banana and plantain	Global	K.U.Leuven, Belgium	http://www.biw.kuleuven.be/DTP/TRO/_data/home.htm
MDG	Grant provision, development	To eradicate extreme poverty and hunger	Indirect or general	Global	New York, USA	http://www.un.org/millenniumgoals/index.shtml
NEPAD		to eradicate poverty, to place African countries on a path of sustainable growth and development, etc	Indirect or general	Africa	Johannesburg, South Africa	http://www.nepad.org/home/lang/en
New Agriculturist	Information provision		Indirect or general	Global	UK	http://www.new-ag.info/index.php
NewCROP	Information provision	To provide information on New Crops and Plant Products	Indirect or general	Global	Purdue University, USA	http://www.hort.purdue.edu/newcrop/default.html

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Noragric	Grant provision	*To generate and exchange knowledge and provide education in the area of agricultural development, livelihood security and natural resource management*	Indirect or general	Global	Ås, Norway	http://www.umb.no/noragric/article/institutional-cooperation-2
North-South Centre ETH	Research & capacity development	To promote research collaboration and capacity development in international development and cooperation covering the technical, natural, human and social sciences	Indirect or general	Global	Zurich, Switzerland	http://www.northsouth.ethz.ch/
PAEPARD II	knowledge sharing	To build joint African-European multi-stakeholder partnerships in agricultural research for development	Indirect or general	Global	Brussels, Belgium	http://paepard.blogspot.com/2010/02/first-paepard-ii-consortium-meeting.html
PGML	research		millet	global	University of Georgia, Athens, USA	http://www.plantgenome.uga.edu/
Rockefeller Foundation	Grant provision	To achieve the well-being of humanity through smart globalization	Indirect or general	Global	New York, USA	http://www.rockefellerfoundation.org/
Royal Tropical Institute	Research & training	to improve the livelihoods of vulnerable producers in developing countries	Indirect or general	Global	Amsterdam, Netherlands	http://www.kit.nl/
SADC	Policy & development	To promote sustainable and equitable economic growth and socio-economic development that will ensure poverty alleviation	Indirect or general	Southern Africa	Gaborone, Botswana	http://www.sadc.int/
SciDev.Net	Information provision	to achieve better-informed decisions by individuals and organizations in the developing world on science- and technology-related issues	Indirect or general	Global	London, UK	http://www.scidev.net/en/
SDC	Grant provision	To support farmers in the areas of production, marketing and sustainable use of resources in order to substantially contribute to poverty reduction	Indirect or general	Global	Bern, Switzerland	http://www.sdc.admin.ch/

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SFSA	Grant provision	To create value for resource-poor small farmers in developing countries through innovation in sustainable agriculture and the activation of value chains	Millet, tef	global	Basel, Switzerland	http://www.syngentafoundation.org/index.cfm?pageID=2
SIDA	Grant provision	to contribute towards improving the living conditions of the poor people	Indirect or general	Global	Sweden	http://www.sida.se/English/
SNSF	Grant provision	to support high-quality research projects concerning problems relevant to disadvantaged countries	Indirect or general	Global	Bern, Switzerland	http://www.snf.ch/E/Pages/default.aspx
SNV	Grant provision	to alleviate poverty by focusing on increasing people's income and employment opportunities in specific productive sectors, as well as improving their access to basic services including water and sanitation, education and renewable energy	Indirect or general	Global	The Hague, Netherlands	http://www.snvworld.org/en/Pages/default.aspx
TCRU	research	To develop genetically improved bambara groundnut	Bambara groundnut	Botswana, Namibia and Swaziland	University of Nottingham, UK	http://www.nottingham.ac.uk/tcru/
USAID	Grant provision	To support long-term and equitable economic growth	Indirect or general	global	Washington D.C., USA	http://www.usaid.gov/index.html
USDA-ARS	Germplasm provision	To provide germplasm to breeders throughout the world	Seeds of many orphan crops	global	Washington, D.C., USA	http://www.usda.gov/wps/portal/usdahome
World Bank	Grant provision	to fight poverty for lasting results and to help people help themselves and their environment by providing resources, sharing knowledge, building capacity and forging partnerships in the public and private sectors	Indirect or general	global	Washington, D.C., USA	http://www.worldbank.org/
ZEF	Research, training & capacity building	To find solutions to development-related issues	Coffee, biodiversity	Global	Bonn, Germany	http://www.zef.de/1558.html

Abbreviations and Acronyms used

AAC	African Agricultural Capital
ABNETA	Agricultural Biotechnology Network in Africa
ABSF	Africa Biotechnology Stakeholders Forum
ABSPII	Agricultural Biotechnology Support Project II
ACIAR	Australian Centre for International Agricultural Research
ACP-EU	African, Caribbean and Pacific countries (ACP) and European Union (EU)
ACTS	African Centre for Technology Studies
ADB	African Development Bank
ADC	Austrian Development Cooperation
AGRA	Alliance for a Green Revolution in Africa
ASARECA	Association for Strengthening Agricultural Research in Eastern and Central Africa
BAMLINK	Molecular, Environmental and Nutritional Evaluation of Bambara Groundnut for Food Production in Semi-Arid Africa and India
BecA	Biosciences eastern and central Africa. Countries included are, Burundi, Cameroon, Central Africa Republic, Congo Brazzaville, Democratic Republic of Congo, Equatorial Guinea, Eritrea, Ethiopia, Gabon, Kenya, Madagascar, Rwanda, Sao Tome and Principe, Somalia, Sudan, Tanzania, Uganda.
BREAD	Basic Research to Enable Agricultural Development
BTC	Belgian development agency
CAADP	Comprehensive Africa Agriculture Development Programme. It is the agricultural programme of the New Partnership for Africa's Development (NEPAD), which in turn is a programme of the African Union (AU).
CCRP	Collaborative Crop Research Program funded by the McKnight Foundation
CGIAR	Consultative Group on International Agricultural Research
CIAT	International Center for Tropical Agriculture (Centro Internacional de Agricultura Tropical)
CIDA	Canadian International Development Agency
CIP	International Potato Center (Centro Internacional de la Papa)
CIRAD	Agricultural Research Centre for International Development (Centre de Cooperation Internationale en recherche agromique pour le development, France)
CORAF/WECARD	West and Central African Council for Agricultural Research and Development.
CTA	Technical Centre for Agricultural and Rural Cooperation
DAAD	German Academic Exchange Service (Der Deutsche Akademische Austauschdienst)
DFID	Department for International Development
ECA	Eastern and Central African countries including Burundi, D. R. Congo, Eritrea, Ethiopia, Kenya, Madagascar, Rwanda, Sudan, Tanzania and Uganda
FAO	Food and Agriculture Organization of the United Nations
FARA	Forum for Agricultural Research in Africa
GCP	Generation Challenge Programme
GFAR	Global Forum on Agricultural Research
GFU	Global Facilitation Unit for Underutilized Species
GTZ	Deutsche Gesellschaft fur Technische Zusammenarbeit, Germany
IAASTD	International Assessment of Agricultural Knowledge, Science and Technology for Development
IAEA	International Atomic Energy Agency
IARCs	International agricultural research centers
ICARDA	International Center for Agricultural Research in the Dry Areas
ICRA	International Centre for development oriented Research in Agriculture, is an independent non-profit foundation, supported by the governments of France (MAE), the Netherlands

	(LNV), Switzerland (SDC) and the United Kingdom (DFID).
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
ICUC	International Centre for Underutilised Crops
IDSA	International Doctoral Scholarships in Agrobiodiversity
IFAD	International Fund for Agricultural Development
IFPRI	International Food Policy Research Institute
IITA	International Institute of Tropical Agriculture
INRA	the French National Institute for Agricultural Research
IPBO	Institute of Plant Biotechnology for developing Countries
IPK	Institute of Plant Genetics and Crop Plant Research, Germany
IPS	Institute of Plant Sciences, University of Bern, Switzerland
IRD	French public research institute working for the development of Southern countries, reporting to the Ministries responsible for research and overseas development.
ISAAA	International Service for the Acquisition of Agri-biotech Applications (ISAAA)
JICA	Japan International Cooperation Agency
KFPE	Commission for Research Partnership with Developing Countries
MDG	Millennium Development Goals
NEPAD	New Partnership for Africa's Development
NewCROP	Center for New Crops & Plant Products, Purdue University, USA
NUANCES	Nutrient Use in Animal and Cropping Systems: Efficiencies and Scales
PAEPARD	Platform for African–European Partnerships on Agricultural Research for Development
PGML	Plant Genome Mapping Laboratory
SADC	Southern African Development Community including Angola, Botswana, the Democratic Republic of Congo, Lesotho, Madagascar, Malawi, Mauritius, Mozambique, Namibia, Seychelles, South Africa, Swaziland, United Republic of Tanzania, Zambia and Zimbabwe
SciDev.Net	Science and Development Network
SDC	Swiss Development Cooperation
SFSA	Syngenta Foundation for Sustainable Agriculture
SIDA	Swedish International Development Cooperation Agency
SME	Small and medium size enterprise
SNSF	Swiss National Science Foundation
SNV	International development organization of Dutch
TCRU	Tropical Crops Research Unit at University of Nottingham, UK
USAID	United States Agency for International Development
USDA-ARS	United States Department of Agriculture - Agricultural Research Service
WARDA	West Africa Rice Development Association (also known as Africa Research Center)
ZEF	Center for Development Research (Zentrum für Entwicklungsforschung)